STUDIES ON EGYPTIAN HONEY AND ROYAL JELLY

BY

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List of Abbreviations

CL.	: *Clostridium*
°C	Degree Celsius:
µg/g	: Microgram/gram
10H2DA	: 10-hydroxy-trans-2-decenoic acid (royal jelly acid)
HDA	10-hydroxydecanoic acid:
(CCR-5)	C-C chemokine receptor type 5:
A/hr	: Ampere/ hour
AA-2G	: Ascorbic acid-2-O-α-gluciside
Ac- TSHR	: Anti-thyroid autoantibodies
ACE	: Angiotensin 1 conversion enzyme
ACTH	: Adrenal cortex to adrenocorticotrophic hormone
ADP	**Adenosine diphosphate :**
Al	: Aluminum

AMP	Adenosine Monophosphate :
ATP	Adenosine Triphosphate:
Bi	: Bismuth
BV	Bee Venom:
C3H/HeJ	: Types of mice
Ca	: Calcium
CA1	: Cornu Ammonis-1
CLD	: Chronic liver disease
CNS	: Central nervous system
Cr	Chromium:
CRP	: C reactive protein
Cu	Copper:
ERK	Extracellular Regulated Kinase:
ESR	: Erythrocyte Sedimentation Rate
EU	**: European Union**

Fe	: Iron
g/ml	Gram/milliliter :
GC	: Gas chromatography
HB	Hemoglobin:
HDL	: high density lipids
Hela cells	Cancer marker :
IFNγ	Interferon gamma:
IgE Si IgGI	: Ovalbumin induced spontaneous hypersenisibility
IL- 1β	Interleukin-1 beta:
IL-10	Interleukin- 10:
IL-12	Interleukin 12:
IL-2	Interleukin- 2:
IL-6	Interleukin 6:
JNK	Jun kinase:
K	: Potassium

Kbar	Kilo bar:
KDa	Kilo Dalton:
Kg	: Kilo gram
KJ	: Kilo joule
LD_{50}	Lethal dose:
LDH	: Lactate dehydrogen
LDL	: Low density lipids
LDLR	: Lipoprotein
MCF-7	: Mammary cancer cells proliferation
Mg	Magnesium:
mg/Kg	: Mille gram/ kilo gram
MIC	: Minimum inhibitory concentration
MJ	: Mega Joule
Mn	Manganese :
MRJP3	: Royal jelly factor

Na	: Sodium
Ni	: Nickel
P	: Phosphorous
PCR	: Polymerase chain reaction
PLA2	: Phosolipase A2
PRJP	: Royal Jelly Protein type
PT	: Prothrombim
PYY	: Peptide YY
RJ	Royal jelly:
RJP30	: Royal jelly proteic type 30
S	: Sulfur
Sb	: Antimony
SGPT	: Serum glutamic pyruvic transaminase, an enzyme
Sn	: Tin
SPC	: Standard plate count

SQLE	: Squalene epoxidase enzyme
SRBC	: Sheep Red Blood Cells
T-cell	T lymphocytes:
TGA	: Thermal gravimetric analysis
TGF-beta	: Transforming growth factor beta
TGF-β1	Transforming growth factor beta:
Th	: Thyroid hormone
Ti	Titanium:
TLR4	Toll-like receptor:
TNF-α	: Tumor necrosis factor alpha
VEGF	: Vascular endothelial growth factor
W	Tungsten:
W/V	: Weight/volume
Zn	Zink:
IR	: Isolated room

INTRODUCTION

I. INTRODUCTION

"For so work the honey-bee creatures that by a rule in nature teach the act of order to a peapled kingdom they have a king and officer of sorts" **(Shakespeare, King Henry V).**

Honey bees are social insects, which mean that they live together in large, well-organized family groups. Social insects are highly evolved insects that engage in a variety of complex tasks not practiced by the multitude of solitary insects. Communication, complex nest construction, environmental control, defense, and division of the labor are just some of the behaviors that honey bees have developed to exist successfully in social colonies. These fascinating behaviors make social insects in general, and honey bees in particular among the most fascinating creatures on earth.

A honey bee colony typically consists of three kinds of adult bees: workers, drones, and a queen.

The honey bee, *Apis mellifera*, has become tightly linked to human agriculture as one of the most important pollinators. The recent honey bee population decline has raised global concerns of a pollination crisis, yet honey bee research lags far behind in available research tools compared to other model organisms, limiting the pace we can hope to advance our knowledge of honey bee biology and improve bee health.

The best known primary products of beekeeping are honey and wax, but pollen, propolis, royal jelly, venom, queen, bees and their larvae are also marketable primary bee products.

While, most of these products can be consumed or used in the state in which they were produced by the bees, there are many additional uses where these products from only a part of all ingredients of another products. Because of the quality and sometimes almost mystical reputation and characteristics of most primary bee products, their addition to other products usually enhances the value or quality of these secondary products. For this reason, the secondary products, which partially, or wholly, can be made up of primary bee products according to **Kreell (1996**). Many of the primary beekeeping products do not have a market until they are added to more commonly used, value added products. Even the value of the primary products may increase if good use is made of them in other products, thereby increasing the profitability of many beekeeping operations.

In some cases, the traditional and early technological uses of primary bee products have been replaced by other (often synthetic products) because of better availability, lower cost and /or easier processing. But in regard to food or health products, there are no synthetic substances which can substitute for wide variety of characteristics of primary bee products. Only when it comes to highly specialized applications and conditions, will synthetics sometimes outperform these unique and versatile products. In that sense, all products containing one or several of primary bee products are value added products **Kreell (1996**). Furthermore, the combination of several bee products synergistically increases their beneficial significance beyond their individual biological values.

Since monetary resources are limited in many societies, the additional value cannot always be obtained in the form of higher prices, but show itself in the form of preferred purchases. For the same reasons though, some products may not be able to compete against cheaper synthetic products. In such cases, the added value and cost may make a product unsuitable, unless other markets can be found.

Honey is the sweet, viscous fluid produced by honey bees (*Apis mellifera)* using the nectar of flowers. In general, the composition of honey contains approximately 70-80% sugar, mainly from fructose and glucose. Water, minerals, vitamins, traces of protein, and other substances such as antioxidants make up the remainder. Ancient Egyptians, Assyrians, Chinese, Romans and Greeks have traditionally used honey as a medical remedy, for the management of wound healing, skin ailments and various gastrointestinal diseases according to **Bogdanov *et al.*(2008).**

Modern research has shown that honey may possess anti-inflammatory activity and stimulate immune responses within a wound. The therapeutic importance of certain types of honey has been attributed to its antibacterial agents and, in some countries, approved for the market as a therapeutic product. Medihoney® and Active Manuka® honey can be currently are purchased as wound healing medicates in Australia and New Zealand **(Sulaiman *et al.*, 2012).**

In Canada, the majority of honey production comes from clover, alfalfa and canola, which is primary, produced in the Prairie Provinces. Honey products may benefit from using the

new labeling options to advertise their health benefit to gain entry into this niche market place. **(CNHP) January 1st, (2004)** so if a honey product was to have any health claims, it must be marketed under these NHP regulations. The regulations require each product to apply for a license, which will result in a natural health product number. New natural health products cannot be marketed in Canada without first receiving a product license. A critical summary of all the evidence and how it supports the health claim requested must be submitted to Health Canada for approval. The ability to communicate the health benefit to specific types of honey may also lead to greater consumer awareness of the benefits of local alberta products.

Honey also exhibits antimicrobial, antiviral, antiparasitic, antimutagenic and anti-inflammatory properties, according to **Bogdanov (2006).**

The European honey bee, *Apis mellifera* L. (Hymenoptera:Apidea) is economically important, primarily because of its role in the pollination of many agricultural and horticultural crops. In addition, honey, wax, venom, propolis and royal jelly are marketable products. **Carreck and Williams (1998)** reviewed the economic value of honey bees and reported that "adding the value of honey and bees wax to that of pollination, the total value of honey bees (in the UK) can be estimated as £ 153.6 M. Using the estimate of 200 000 colonies, the annual value to the UK economy of each honey bee colony is therefore about £800".

The benefits of honey pollination are usually either increased yield and fruit size, for example, in strawberries, or an

increase in the earliness and uniformity of seed set in crops such as oilseed rape **.** In recent decades, intensive agricultural practices have led to habitat destruction and increased pesticide use, resulting in a significant reduction in both the numbers and species diversity of wild bees and other beneficial insects. There is now an increased dependence on managed pollinators in UK. Honey bees also play an important role in the maintenance of biodiversity through the pollination of wild flowers and these in turn provide a food source for many small mammal and bird species, either through herb ivory or by providing prey **(Felsner *et al.*, 2004).**

In a series of test in Russia a Russian preparation Ap-iton 25was tested in highly trained sportsmen. The tests were carried out at the beginning and after 21 days, the intake of RJ was 4 pills sublingually daily,each one containing 369 mg lactose-glucose absorbed RJ, in total corresponding to about 1.2 g dry RJ daily, a control group took placebo. In the tests the resilience of sportsmen was tested in a treadmill, until the sportsmen rejected a further load increase, the load being changed every minute. The endurance of the sportsmen who took the RJ supplement was significantly better than the controls after 10 and 21 day of the test and remained significant 5 days after the sportsmen stopped taking the supplement. It is known that in physical performance the endurance decreases because of an increase of blood lipid hydroperoxides. An antioxidant as RJ should theretically inhibit the building of the lipid peroxidaton. Indeed, there was a highly significant difference between the test and the control groups regarding this parameter, on the 10^{th} and 21^{st} day

of training, this difference persisted 5 days after stopping supplementation. It is known that immunity decreases upon persistent physical strain. This results in a decrease of immunogloblines IgA, IgG and IgM. The humoral immunity was tested by measuring these parameters in the blood of the test persons. The IgA concentration in comparison with the initial values was higher than the controls after 21 days, while the change of the other two parameters was not significantly changed. The number of leucocytes, lymphocytes, T lymphocytes, T-helpers and T-suppressers in the blood are a function of the cell immunity. All of hese parameters were significantly higher in the test sportsmen after 21 days, when the values were compared to the initial ones. This preparation is sold in Russia and is accepted as a sport supplement by the Russian Antidoping Agency, **(http://www.bee-hexagon.net (2012).**

A 45% ethanol solution containing 2.4 g native RJ in 100 ml was tested in a sport performance of 17-20 year old female students, who took 3 times 10 drops (approx. 100 mgRJ). Following tests were carried out: measurement of body mass, performance of Stange's breath holding test, measurement of the viso-motoric reaction, hanging on the bars, 30 m runs with maximal speed, measurement of heart rate afterwards, then a 5 minute step test with a step height of 30 cm, 30 climbs per minute followed by a 5 minute rest. There was a significant increase of the reaction of the organism to hypoxia, as measured by the Stange test and an improvement of the viso-motoric reaction. **Bogdanov (2011a).**

The general pharmacological effects of bee venom and venom extracts on a range of physiological parameters of the central nervous system, digestive, cardiovascular and respiratory systems in rodents. A single clinical dose of bee venom was taken as 5 µg/kg when administered by intradermal or subcutaneous route to human patients. They administered bee venom to mice, rats and rabbits in doses up to 200-fold the effective clinical dose (i.e. 1,000 µg/kg). Using a variety of indicators the results of this study showed that treatment with whole bee venom (at a dose 200 times the recommended clinical dose) did not produce any significant effect on the central nervous system (as determined by general behavior, sleep induction time and duration, spontaneous activity, motor function, body temperature, or drug-induced convulsions). Bee venom was a potent antinociceptive agent without the side effects associated with many narcotic drugs. Bee venom treatment did not affect motor activity, intestinal peristaltic function or gastric function. Additionally, bee venom did not alter blood pressure and heart rate in rats nor respiratory rates in rabbits **(Kim *et al.*, 2004).**

Manuka honey with added bee venom appears to have no acute or sub-acute toxic effects in both liquid and freeze-dried forms. Animals gained weight, were observed to behave normally, and showed no signs of change in internal organ form or function**. (Regulation (EC) No 258/97 of the European Parliament and the Councilof 27th January 1997 concerning novel foods and novel food ingredients).**

Aim of investigation

The essence of the investigation is to highlight the importance of applying lyophilization to bee products.

The major aim of this study is to apply the art of lyophilization to royal jelly and bee venom; and to preserve them by being dissolved in bee honey. Lyophilization was carried out by a lyophilization device created by the student, the perform of this study, by implementing simple available material.

The study included the following:

1 – The lyophilization of bee venom and royal jelly.

2 – The physical and chemical properties of bee venom before and after lyophilization.

3 – The physical and chemical properties of royal jelly before and after lyophilization.

4 – The physical and chemical properties of honey.

5 – The effect of adding the lyophilized royal jelly in nurturing of infants who are not breastfeeding and studying its effects on growth and development and rate of infection till the age of one year.

6 - The effect of the lyophilized bee venom and royal jelly and bee honey on rats.

6.1 The increased in body weight of rats.

6.2 Mean values of serum hormones in rats.

6.3 Mean blood hemoglobin concentration and hematoorit.

6.4 Mean values of serum total proteins, albumin, globuline and A/G in rats.

6.5 The increased in weight of testis, epididymis (head, body, tail) prostate and seminal vesicle in rats.

6.6 The increased in sperm count, glutathione, and malodialdehyde levels in rats and testosterone hormone.

6.7 The effect on urea and creatinine in blood and urea of rats.

6.8 The effect on glucose concentration and serum and urine of rats.

7 –Effect of the lyophilized bee venom and royal jelly and bee honey on the following diseases of some patients:

- Diabetes mellitus (DM).
- Anemia (Megaloblastic anemia).
- Rheumatoid arthritis (RA).
- Breast cancer.
- Chronic liver disease (CLD).

REVIEW OF LITERATURE

II. REVIEW OF LITERATURE

2.1 Honey:

Codex Alimentarius Commission (2001) defines honey as "the natural sweet substance produced by honey bees from nectar of blossoms or from secretions of living parts of plants or excretions of plant sucking insects on the living part of plants, which honey bees collect, transform and combine with specific substances of their own, store and leave in the honey comb to ripen and mature".

Honey is the most important product of beekeeping both from a quantitative and an economic point of view. It was also the first bee product used by humankind in ancient times. The history of the use of honey is parallel to the history of man and every evidence can be found of its use as a food source and as a symbol mentioned in religious, magic and therapeutic ceremonies **Crane (1990),** an appreciation and reverence it owes among other reasons to its unique position until very recently, as the only concentrated of sugar available to man in most parts of the world. The same cultural richness has produced and equally colorful variety of uses variety of uses of honey in other products.

Honey is the oldest and only available unique natural sweetener to mankind and is the last of natural unprocessed food to be consumed. The mentioned of honey dates back to as long as 2100-2000 B.C in a Sumerian tablet proclaims the use of honey as a drug and ointment. The Bible also mentions King

Solomon's words"Eat honey my son, because it is good" (Old Testament, proverb 24:13) **Ouchemoukh *et al.* (2010).**

Honey has significant nutritional and prophylactic-medicinal value; honey can be produced from the nectar of flowers or from honeydew and in some cases can even be a combination of both, **(Juszczak and Fortuna, 2006).** When derived from the nectar of flowers honey is known as nectar or blossom honey and can be further categorized as mono/unifloral honey and multifloral honey whereas honey produced from honeydew is known as honeydew honey **(Ouchemoukh *et al.*, 2007).** Nectar honeys are classified as monofloral and multifloral based on the pollen content analyzed by microscopic analysis which is known as mellisopalynological studied. Monofloral honeys are those whose pollen frequency from a single plant species is above 45% unless the pollen grains are "under- or over-represented" in which case around 10-46% is accepted as in Lavender, Citrus and Rosemary honeys (**Felsner *et al.*, 2004).**

Bogdanov *et al.* (2008) conducted that, the annual production of honey globally in the year 2008 was 1.2 million tons which was less than 1% of the sugar production. China is the largest producer of honey through its consumption is not the highest. The European Union countries have the largest per capita consumption of honey with Germany, Austria, Switzerland, and Poland (1 – 1.8kg) leading within the continent where as Italy, France and Great Britain have a moderate (0.3 – 0.4kg) per capita consumption. United State of America, Canada and Australia have a per capita consumption of (0.8 – 0.9 kg). In the year (2009) 144.1 million pounds of honey was produced in

the United States of America with Louisiana producing 3.8 million pounds, an increase of 24% from (2008) according to **National Agricultural Statistics Service (2011).**

2.1.1 Honey Composition:

Carbohydrates:

Main sugars are the monosaccharides fructose and glucose. Beyond the two monosaccharides, about 25 different oligosacharides have been detected, between them nutrition relevant ones such as panose, 1-kestose, 6-kestose, palatinose **(http://www.bee-hexagon.net (2012)).** The principal oligosaccharides in blossom honey are the disaccharides sucrose, maltose, trehalose and turanose. Honeydew honey compared to blossom honey contains higher amounts of oligosaccharides, and also trisaccharides such as melezitose and raffinose. During digestion the principal carbohydrates fructose and glucose are quickly transported into the blood and can be utilized for energy requirements of the human body. A daily dose of 20 g honey will cover about 3% of the required daily energy **(Bogdanov *et al.*, 2008).**

Proteins, enzymes and amino acids:

Honey contains about 0.5% proteins, mainly enzymes and amino acids. Three main honey enzymes are diastase (amylase), decomposing starch or glycogen into smaller sugar units, invertase (sucrase, glucosidase), decomposing sucrose into fructose and glucose, as well as glucose oxidase, producing hydrogen peroxide and gluconic acid from glucose. Since the saliva yields a sufficiently high activity of amylase and glucose oxidase, honey's contribution to sugar digestion is of minor

importance. Honey glucose oxidase producing hydrogen peroxide, might exert an antibacterial effect in the oral cavity **(Bogdanov *et al.*, 2008).**

Vitamins, minerals and trace compounds:

The amount of vitamins and minerals is small. It must be born in mind that different unifloral honeys contain different amounts of minerals. Honey contains a number of other trace elements. From the nutritional point of view the minerals chrome, manganese and selenium are of nutritional importance, especially for children of the age of 1 to 15 years. The elements sulphur, boron, cobalt, fluorine, iodine, molybdenum and silicon can be important in human nutrition too. Honey contains 0.3-25 mg/kg choline and 0.06 to 5 mg/kg acetylcholine. Choline is an essential for cardiovascular and brain function, and for cellular membrane composition and repair, while acetylcholine acts as a neurotransmitter **(Bengsch, 1992).**

Aroma compounds, taste-building compounds and polyphenols:

There is a wide variety of honeys with different tastes and colours, depending on their botanical origin **(Bogdanov *et al.*, 2008).**

The sugars are the main taste-building compounds. Generally, honey with high fructose content (e.g. acacia) are sweet compared to those with high glucose concentration (e.g. rape). Beyond sugars the honey aroma depends on the quantity and quality of honey acids and amino acids. In the past decades some research on honey aroma compounds has been carried out and more than 500 different volatile compounds have been

identified in different types of honey. Indeed, most aroma building compounds vary in the different types of honey depending on its botanical origin 48. Honey flavour is an important quality for its application in food industry and also a selection criterion for consumer's choice (**Bogdanov *et al.*, 2007).**

Polyphenols are another important group of compounds with respect to appearance and functional properties. 56 to 500 mg/kg total polyphenols were found in different honey types, depending on the honey type (**Fukuda *et al.*, 2009).**

Polyphenols in honey are mainly flavonoids (e.g. quercetin, luteolin, kaempferol, apigenin, chrysin and galangin), phenolic acids and phenolic acid derivatives. The flavonoid content can vary between 2 and 46 mg/kg of honey and was higher in samples produced during dry season with high temperatures. The polyphenols are responsible for the antioxidant properties of honey **(Kenjeric *et al.*, 2007).**

2.1.2 Microbiological aspects:

Antimicrobial activity:

The antibacterial effects of honey, mostly against gram-positive bacteria, both bacteriostatic and bactericidal effects have been reported, against many strains, many of which are pathogenic.

Honey glucose oxidase produces the antibacterial agent hydrogen peroxide, while another enzyme, catalase breaks it down. Honey with a high catalase activity has a low antibacterial peroxide activity. Honey has both peroxide and non peroxide antibacterial action, with different non-peroxide antibacterial

substances involved: acidic, basic or neutral **(Bogdanov, 2006).** Antimicrobial effect of honey is thus due to different substances e.g. aromatic acids and compounds with different chemical properties **(Bogdanov, 2006)** and depends on the botanical origin of honey **(Molan, 1997).** The high sugar concentration of honey **(Mundo *et al.*, 2004),** and also the low and also the low honey pH is also responsible for the antibacterial activity. Most experiments report on stop of bacterial growth after a certain time of honey action. (The higher the concentration the longer is the period of growth inhibition). Complete inhibition of growth is important for controlling infections **(Molan, 1992).** Honey has also antiviral activity Rubella **(Zeina *et al.*, 1996),** Herpes virus **(Al-Waili, 2004)**.

Honey has also fungicide activity against different dermatophytes **(Molan, 1997).** Honey has been shown to have a prebiotic effect, i.e. its ingestion stimulates the growth of healthy specific Bifidus and Lactobacillus bacteria in the gut. Sour-wood, alfalfa, sage and clover honeys have been shown to have prebiotic activity **(Shin and Ustunol, 2005)**. The prebiotic activity of chestnut honey is bigger than that of acacia honey **(Lucan *et al.*, 2009).** Oligosaccharides from honeydew honey have prebiotic activity. Theoretically honeydew honeys, containing more oligosaccharides should have a stronger prebiotic activity than blossom honeys.

As antibacterial agent:

The antibacterial properties of honey includes, the release of low lives of hydrogen peroxide, some honey have an additional phytochemical antibacterial compounds. The

antibacterial property of honey is also due to osmotic effect of its high sugar content as it has an osmolarity sufficient to inhibit the microbial growth. Hydrogen peroxide was responsible for the antibacterial activity of honey since both the antibacterial activity of honey and hydrogen peroxide were destroyed by light. Hydrogen peroxidase which is produced by the glucose oxidase of honey could be the inhibitory substance against bacteria. However, it is known that honey as well as bacteria produces a catalase that eliminates hydrogen peroxide. **(Osho and Bello 2010)**

But although catalase is active with high concentration of hydrogen peroxide, it is of low activity with physiological levels. The additional group of light sensitive, heat-stable antibacterial factors in honey which inhibited the growth of *Bacillus subtilis, B. alvei, Escherichia coli, Pseudomona pyocyanes, Salmonella and Staphylococcus aureus*. A comparison was made by Cortopassi-Laurino and Gelli between the physico-chemical properties and antibacterial activity of honey produced by Africanized honey bees *(A. mellifera)* and Melliponinae (stingless bees) in Brasil. For both types of honey at a concentration of 5-25%, *Bacillus stearothermophilus* was found to be the most susceptible and *E. coli* the least susceptible of the seven bacterial isolates tested (the other five being, *B. subtilis, B. subtilis Caron, Staphylococcus, Klebsiella pneumoniae* and *Ps. aeruginosa*). Melipona subnitida honey produced from Mimosa bimucronata and Plebia species honey produced from Borreria/Mimosa exhibited the greatest antibacterial activities **(Singh *et al.*, 2012).** Antibacterial activities of the two honey

samples, produced by the honey bee *(Apis mellifera),* were assayed using standard well diffusion method. Both honey samples were tested at four concentrations (5, 25, 50 and 100% w/v) against *Staphylococcus aureus, Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Bacillus subtilis* and *Escherichia coli* .There are many reports of bactericidal as well as bacteriostatic activity of honey and the antibacterial properties of honey may be particularly useful against bacteria, which have developed resistance to many antibiotics **(Osho and Bello 2010).**

As antifungal agent:

Synergistic action of starch on the antifungal activity of honey, a comparative method of adding honey with and without starch to culture media was used. *Candida albicans* has been used to determine the minimum inhibitory concentration (MIC) of five varieties of honey **(Laid, 2008).** Antifungal action of three single samples of South African honey (wasbessie, bluegum and fynobs) against *Candida albicans* and found honey to inhibit the growth of *C. albicans*, while the control, bluegum and fynobs honey produced only partial inhibition (**Frans, 2001).**

As antiviral agent:

Honey has good anti-Rubella activity. These results may justify the continuing use of honey traditional medicines from different ethnic communities worldwide and in some modem medications such as cough syrups (**Bassam, 2007).**

2.1.3 Medicinal Aspect:

As remedy for diarrhea:

The infectious diarrhea exacerbates nutritional deficiencies in various ways, but as in any infection, the calorific demand is increased. Pure honey has bactericidal activity against many enteropathogenic organisms, including those of the Salmonella and Shigella species, and enteropathogenic *E.coli*. In vitro studies of Helicobacter pylori isolates which causes gastritis have been shown to be inhibited by 20% solution of honey. Even isolates that exhibited a resistance to other antimicrobial agents were susceptible **(Ali. *et al.*, 1991).** In a clinical study, the administration of a bland diet and 30 ml of honey three times a day was found to be an effective remedy in 66% of patients and offered relief to a further 17%, while anemia was corrected in more than 50% of the patients.

As Appetite increaser:

Honey consumption delayed the postprandial ghrelin response (p=0.037), enhanced the total PYY (p=0.007) response, and blunted the glucose response (p=0.039) compared with consumption of the sucrose-containing meal **(Singh *et al.*, 2012).**

As medicine for gastric ulcer:

Gastric ulcer have been successfully treated by the use of honey as a dietary supplement **(Kandil *et al.*, 1987).** Honey administered subcutaneously or orally before oral administration of ethanol affords protection against gastric damage and reverses changes in pH include by ethanol **(Ali, 1991).**

For wound healing:

Singh *et al*. (2012) reported that the treatment of wounds with honey has rendered them bacteriological sterile within 7-10 days of the start of treatment and promoted healthy granulation of tissue.

Farouk *et al*. (1988) reported that honey was also found to be more effective as an antibacterial agent against several pseudomonas and Staphylococcus strains than the antibiotic and gentamicin.

Efem (1988) found that honey has cleaning action of wounds, stimulates tissue regeneration, reduces inflammation and honey impregnated pads act as non adhesive tissue dressing.

Rapid healing agent:

Wounds becoming closed in a spectacular fashion in 90% cases, sometimes in few days **(Descottes, 1990).** Rapid healing changes when honey is applied to Fournier's gangrene **(Hejase *et al*., 1996).** Clinical observations are made that open wounds heal faster and ready faster to closure by stitching when dressed with honey (than when dressed conventionally) **(Hamdy *et al*., 1989).**

Stimulation of healing process:

Somerfield (1991) conducted that some wounds, termed chronic wounds, may go for long periods, sometimes for years, without the healing process taking place. Leg ulcers and diabetic ulcers are common examples of this type of wound. Honey has been found to be effective in starting the healing process in non-healing ulcers.

Tovey (1991) reported that honey has also used successfully on chronic foot ulcers in lepers and diabetic foot.

Clearance of infection:

Farouk *et al.* (1988) conducted that honey is effective clearing infection in wounds where other treatments have failed. One report gave the results of treating with honey dressing 47 patents with wounds and ulcers which had been treated for 1 month to 2 years with conventional therapy including antibiotics with no sighs of healing, or the wounds were increasing in size.

Molan (2001) reported that, the range of honey's activities as in table below:

Table (I) Honeys activities:

Bioactivity of honey	**Suggested Rationale**
Prevention of cross-contamination.	Viscosity of honey provides a protective barrier.
Provides a moist wound healing environment.	Osmolarity draws fluid from underlying tissue.
Dressings do not adhere to wound surface. Tissue dose not grow into dressings.	The viscous nature of honey provides an interface between wound bed and dressing.
Promotes drainage from wound.	Osmotic outflow sluices the wound bed.

Table (I) continuous

Removes malodor.	Bacterial preference for sugar instead of protein (amino acids) means lactic acid is produced in place of malodorous compounds.
Promotes autolytic debridement.	Bacterial preference for sugar instead of protein (amino acids) means lactic acid is produced in place of malodorous compounds.
Stimulates healing.	Bio-active effect of honey.
Anti-inflammatory.	Number of inflammatory cells reduced in honey-treated wounds.
Managing infection.	Antiseptic properties found to be effective against a range of microbes including multi-resistant strains.

As medicine for canine recurrent dermatitis:

Antibacterial activity of honeydew honey and propolis was evaluated in vitro against *Staphylococcus aurous* strains isolated from canine patients with dermatitis and found that the honey showed bactericidal effects against the bacterial tested *S. aureus* strains, but was less efficient then the propolis at certain concentrations **(Iulia *et al.*, 2007).**

As skin disinfectant:

Singh *et al.* (2012) reported that typical honeys are about eight times more potent against coagulase-negative Staphylococci than if bacterial inhibition were due to their osmolarity alone. Therefore, honey applied to skin at the insertion points of medical devices may have a role in the treatment or prevention of infection by coagulase-negative staphylococci.

Reduction of inflammation:

Singh *et al*. (2012) conducted that the inflammation of surrounding tissue that results from infection of a wound or directly from the damage to tissue caused by burns, is the major cause of the pain and discomfort associated with wounds. The process of inflammation involves blood capillaries opening up and allowing plasma from the blood to flow out into the surrounding tissue. This causes swelling of the tissue (oedema), the pressure giving rise to damage and discomfort in the healing area. It also causes plasma to exude from open wounds, sometimes in large quantities.

As immune modulation:

Manuka honey increased IL- 1β, IL-6, and TNF-α production from Mono Mac6 cells or human monocytes (**Cooper *et al.*, 2007).** The active component was 5.8 kDa, which increased production of these cytokines via TLR4 **(Tonks *et al.*, 2003).** Oral intake of honey augmented antibody productions in primary and secondary immune responses against thymus-dependent and thymus-independent antigens **Al-Waili and Haq (2004).**

As antioxidant:

Gheldof and Engeseth (2002) reported that honey contains a variety of phytochemicals (as well as other substances such as organic acids, vitamins, and enzymes) that may serve as sources of dietary antioxidants, and compounds depends largely upon the floral source/ variety of the honey, and darker honeys have been shown to be higher in antioxidant content than lighter honeys.

As anti diabetic agent:

Al-Waili (2004) conducted that some complex carbohydrates raise blood glucose levels more significantly than certain simple sugars. Both honey and sucrose have been shown to produce a lower glucose response than starchy foods such as white bread. Moreover, it has been shown that, the total amount of carbohydrate consumed is probably more important than the type of carbohydrate when it comes to blood sugar levels. Thus, experts agree that diabetics may include moderate amounts of "simple sugar" in a balanced diet. Honey compared with dextrose caused a significantly lower rise in plasma glucose levels in diabetic subject. It also caused reduction of blood lipids, homocystenine levels and CRP (C reactive protein) levels in normal and hyperlipidemic subject.

Antimutagenic and antitumor activity:

Wang *et al.* (2002) reported that, the antimutagenic activity of honeys from seven different floral sources (acacia, buckwheat, fireweed, soybean, tupelo and Christmas berry) against Trp-p-1 was tested by the ames assay and compared to a sugar analogue as well as individually tested simple sugar. A

statically significant anti-metastatic effect was achieved by oral application of honey. These findings indicate that honey activities the immune system and honey ingestion may be advantageous with respect to cancer and metastasis prevention. In addition, it is postulated that honey given orally before tumor cell inoculation may have a decreased effect of tumor spreading. In another study of the same group the effect of honey on tumor growth, metastasizing activity and induction of apoptosis and necrosis in murine tumor models (mammary and colon carcinoma) was investigated **(Orsolic *et al.*, 2003).**

Honey against eye disease:

Molan (2001) reported that honey is successful in various ailments of the cornea. The use of honey in Russia has been reviewed: undiluted or 20-50% water solution being applied to the eye under the lower eye lid against chemical and thermal burns of the eye, conjunctivitis and infection of the cornea.The healing effect of honey explained by its anti-inflammatory, antibacterial and antifungal actions of honey.

Emarah (1982) reported that, the successful treatment by honey of keratitis, conjunctivitis and blepharitis in Egypt, the positive effect in keratitis to reducing the levels of angiogenic factors (VEGF and TGF-beta), inflammatory cytokines (IL-12) and chemokines (CC chemokine receptor 5(CCR-5).

Oral health:

Manuka honey is a very potent antimicrobial honey, has positive effect against dental plaque development and gingivitis and thus can be used in the place of refined sugar in the manufacture of candy **(Molan, 2001).**

2.1.4 Raw processed by honey:

Abhishek *et al.* (2010) conducted that the antibacterial activity of raw and processed honey was carried out on the extracts of honey using solvent such as methanol, ethanol and ethyl acetate and compared it with the popular antibiotics. The inhibitory action of extracts of honey were evaluated against six bacterial strains, Gram-posative bacteria viz., *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus* and Gram-negative bacteria, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi* by agar well diffusion method and it was found that raw honey has more antimicrobial activity than processed one.

2.1.5 Reasons for variance:

Water activity of honey varies relatively little, and is not of much importance in the antibacterial effect of the dilute solutions of honey used to study the antibacterial activity of honey. Although the acidity of honey varies considerably, this too is likely to be of little consequence when the honey is in dilute solution in nutrient both for testing its effect on bacterial cultures, as the both buffer the acidity. The major variations seen in overall antibacterial activity are due to variation in the level of hydrogen peroxide achieved, and in some cases to the level of non-peroxide factors. The latter was found to be responsible for much of the activity in honeys with high levels of antibacterial activity in a study of 64 samples (**Molan and Russell, 1988).** The content of non-peroxide factors is obviously related to the floral source, and sometimes it can account for the major part of the antibacterial activity in a honey, as is level of hydrogen

peroxide achieved can also be related to the floral source, as components from some floral sources can affect both production and the destruction of hydrogen peroxide. There is a dynamic equilibrium: the level of hydrogen peroxide depends upon the balance between the rate of its production and the rate of its destruction. Hydrogen peroxide obviously must be degraded, or else full-strength honey would contain substantial amounts of it, and any dilution of honey would eventually achieve inhibitory levels.

From the first work demonstrating that hydrogen peroxide is responsible for antibacterial activity in honey, it was realized that hydrogen peroxide is destroyed by component of honey. When testing *Staphylococcus aureus* for its susceptibility to added hydrogen peroxide it was found that higher levels had to be added to achieve an inhibitory effect unless honey was present'. Hydrogen peroxide was found to rapidly disappear when added to dilute honey, and, except in samples accumulating very high levels, the level of hydrogen peroxide accumulated from enzymatic action was seen to decline with time **(Allen *et al.*, 1991).**The agar diffusion assay demonstrated that Ulmo 90 honey had greater than antibacterial activity against all MRSA isolates tested than manuka honey and similar activity against *E.coli* and *P. aeruginosa* , and unlike manuka honey, Ulmo 90 honey activity is largely due to hydrogen peroxide production **(Louise and Malone, 1998).** Honey from different phytogeographic regions exhibited differential antibacterial activity and susceptibility of yeasts to honey of

either species was greater than that of bacteria **(Singh *et al.*, 2012).**

2.1.6 Infant Nutrition:

The positive effects of honey in infant diet are attributed to effects on the digestion process. One possible cause is the well established effect of *oligosaccharides* on *B. bifidus* **(Rivero and Santamaria, 2011).** When fed on a mixture of honey and milk infants showed a regularly steady weight gain and had an acidophilic microorganism flora rich in *B. bifidus*. In another experiment with honey and milk it was shown that the infants were suffering less frequently from diarrhoea, and their blood contained more haemoglobin compared to a diet based on sucrose sweetened milk. Feeding honey to infants improved calcium uptake into the blood, resulting in lighter and thinner faeces (**ASP and Bryngelsson, 2008)**. There is a health concern for infants regarding the presence of *Clostridium botulinum* in honey. Since the presence of this bacterium in natural foods is ubiquitous and honey is a none sterilized packaged food from natural origin the risk of a low contamination level cannot be excluded. Spores of this bacterium can survive in honey, but they cannot build toxin. But in the stomach of infants younger than one year the bacteria spores from honey can survive and theoretically build the toxin, while humans older than 12 months can ingest honey without any risk. In some cases, infant botulism has been explained by ingestion of honey **(Cox and Hinkle, 2002).** In Germany one case of infant botulism per year is reported (**Müller *et al.*, 2000)**. As a result of the reported infant botulism cases some honey packers (e.g. the British Honey

Importers and Packers Association) place a warning on the honey label that “honey should not be given to infants under 12 months of age”. Recently, a scientific committee of the EU has examined the hazard of *Cl. botulinum* in honey. It has concluded, that no microbiological examinations of honey are necessary, as the incidence of *Cl. botulinum* is relatively low and that tests will not prevent infant botulism. In the EU countries the health authorities have not issued a warning label on honey pots. Also the counter-indication of honey in nourishing of infants in developing countries has been questioned .For safety reasons honey should be given only to infants older than 1 year. **(European Commission, 2002).**

2.2 Royal Jelly:

Royal jelly is a secretion product of the cephalic glands of nurse bees and serves as the most important part of honeybee larvae diet, playing a major role in caste differentiation **(http://www.bee-hexagon.net. (2012))**. For the first days, royal jelly is the only food given to all young larvae in their maturation process; while for the queen, it is the specific food for her whole life period. This is the reason for the longer life of queen bee over other bee. Royal jelly, one of the most effectual and beneficial remedy for human beings, is widely used both in folk and in official medicine and it is a controversial dietary supplement. Due to its complex composition (water, proteins, lipids, carbohydrates, amino acids, minerals, vitamins , enzymes, hormones, oligo-elements and nature antibiotics), royal jelly has a multiplie of pharmacological activities: antioxidant, neurotrophic, hypoglycemic , hypolipidemic and.

hepatoprotective, hypotensive and blood pressure regulatory, antitumor, antibiotics, anti-inflammatory, immunomodulatory and anti-allergic, general tonics, anti aging **(Mărghitaş, 2008).**

2.2.1 Composition:

Royal jelly is a viscous jelly substance. It is partially soluble in water with a density of 1.1 g/ml. Its color is whitish to yellow, the yellow color increasing upon storage. Royal jelly odour is sour and pungent; the taste is sour and sweet. The sensory characteristics are important quality criteria. Old royal jelly, which has not been properly stored, tends to be darker and can develop a rancid taste. For optimum quality it should be stored in frozen state. The viscosity varies according to water content and age- it slowly becomes more viscous when stored at room temperature or in a refrigerator at 5°C. The increased viscosity appears to be related to an increase in water insoluble nitrogenous compounds, together with a reduction in soluble nitrogen and amino acids **(Sabatinti *et al.*, 2009).**These changes apparently due to contained enzymatic activities and interaction between the lipid and protein fractions **(Bogdanov, 2011a).**

Humidity:

The water content with 60-70% is the main component of royal jelly. The dry substance is composed of carbohydrates, proteins, amino acids and fats. Smaller quantities of minerals and vitamins are also present **(Sabatini *et al.*, 2009).**

Proteins and peptides:

Lercker *et al.*(1992) conducted that fresh royal jelly content of proteins 17% and 45% in lyophlized royal jelly is considard the main substance class of royal jelly.

The main nitrogenous substances accounting for about 97- 98% of them. About 60% of them are water-soluble **(Lee *et al.*, 1999).** Free amino acids represent only 0.6-1.5%, the majority of which belong to the L series. The most representative are proline and lysine. Upon storage at 4°C for 10 months no significant changes of amino acids were encountered, while after room temperature storage proline and lysine content increased. This is due probably to proteolytic enzyme activity.

Lipids:

The lipids with 3 to 19% of the royal jelly weight **(Karadeniz *et al*., 2011).** 80 to 90% of the lipid fraction consists of free fatty acids, the rest being neutral lipids, sterols, hydrocarbons **Kodai *et al.* (2007)** reported that most of the organic acids are free with rather unusual structure rarely encountered in nature, mono-and dihydroxy acids and dicarboxylic acids with 8 and 10 carbon atoms **(Lercker *et al.*, 1993).** The identification of this fraction- in particular as regards the pattern and quantitative analysis of free organic acids- is believed to represent the criteria of choice for defining the genuineness of royal jelly. The main acid 10-hydroxy-2-decenoic (HAD) is an understand acid, which is determined for the evaluation of royal jelly genuinely **(Caboni *al et*., 2004).** 10-hydroxy-2-decenoic (HAD) and also the other fatty acids of royal jelly have antibacterial properties, thus contributing to the relatively low content of bacteria in this product **(Serra and Escola, 1991).**

Carbohydrates:

Royal jelly composed of mainly fructose, glucose and sucrose **(Serra, 1992).** Royal jelly also composed of maltose, trehalose, melibiose, ribose and erlose also being found **(http://www.bee-hexagon.net (2012)).**

Minerals:

Ash content (minerals) represents 0.8 to 3% of royal jelly on fresh matter. The major elements are K, P, S, Na, Ca, Al, Mg, Zn, Fe, Cu and Mn but there are trace amounts (0.01-1mg/100g) of Ni, Cr, Sn, W, Sb, Ti and Bi. The sodium content of royal jelly varies between 11 to 14 mg/100g **(Stocker *et al.*, 2005).**

Vitamins:

The concentrations of vitamins in royal jelly are distributrd over a broad spectrum; vitamins showing fairly uniform values are riboflavin, thiamine, niacin and folic acid. Likewise present but with greater variations are pyridoxine, biotin, pantothenic acid and inositol. Only traces of vitamin C are present, while the fat soluble vitamins like vitamin A, D, E and K are absent **(Schmidt and Buchmann, 1992).**

Other minor components:

Numerous minor compounds, belonging to diverse chemical categories, have been identified in royal jelly. Among these are two heterocyclic substances, biopterine and neopterine at 25 and 5 µg/g of fresh weight, respectively .These compounds are found in the food of worker bee larvae too, but at about one tenth of these concentration. Other substances identified include several nucleotides as free bases (adenosine, uridine, guanosine,

iridin and cytidine) the phosphates AMP, ADP, and ATP **(Negai, 2001)**, acetylcholine (1 mg/g dry weight, **(Matsuka, 1993)** and gluconic acid (1.4 % of fresh weight, **(Negai , 2001).** Benzoic acid (8-15 mg/kg) has also been found. Samall amounts of malic, lactic and citric acid has also been found **(Kim *et al*., 1989).**

2.2.2 Bio-active ingredients:

10-hydroxy-2-decenoic (HAD) and other fatty acids:

Most of the organic acids are free with rather unusual structure rarely encountered in nature, mono-and dihydroxy acids and dicarboxylic acids with 8 and 10 carbon atoms **(Lercker *et al.*, 1993).**

Proteins and peptides:

Schmitzova *et al.* (1998) conducted that 82-90% of the royal jelly proteins belong to the major royal jelly protein type (RRJP) protein.

Major royal jelly protein type (RRJP) protein belongs to the albumin protein class **(Simuth, 2001).** Royal jelly has immuno-modulating activity **(Okamoto *et al.*, 2003).** It has different glycoproteins **(Miyamoto *et al*., 2004).** Royal jelly also has peptides have been characterized: apisimin. One with antihypertensive activity and so called jeleines with antibacterial properties **(Matsui *et al*., 2006)**.

Adenosine monophosphate NI Oxide:

Adenosine monophosphate NI oxide is a compound found only in royal jelly. Its main effects are on the centrally nervous system, it stimulates neural differentiation, promotes generation of all three types of cells composing the central nervous system:

neurons, astrocytes and oligodenrocytes, against neuronal damage **(Hattori *et al.*, 2010).**

Acetylcholine:

The concentrations is 1mg/g dry weight it is a nerve transmitter, having a number of hormone- like effects in the central and vegetative nervous system **(Wei *et al.*, 2009).**

The hormones (testosterone, progesterone, prolactine and estradiol):

The hormones (testosterone, progesterone, prolactine and estradiol) are also found in royal jelly, they increase of male and female fertility, and also power and endurance **(Vittek and Solmiany, 1984).**

Polyphenols:

Polyphenols also found in royal jelly which has been also identified with antioxidant effect **(Liu *et al.*, 2008).**

2.2.3 Royal Jelly biological activities and effects:

Anti oxidative activity:

Royal jelly has been found to protect tissue DNA against the oxidative damage. Studying the effect of the royal jelly diet on mice, it has been showed that after feeding royal jelly to mice for 16 weeks, the levels of 8-hydroxy-2-deoxyguanosine (an oxidative stress marker) were significantly reduced in kidney DNA and serum and the average life span of C3H/HeJ mice life expectancy was increased through the mechanism of reduced oxidative damage. There is no clinical data on anti oxidative activity **(Inoue *et al.*, 2003)**.

Neurotrophic action:

Royal jelly has been traditionally known to improve memory, prevent senility, increase energy, and reduce anxiety and calm hyperactive subjects **(Mateescu, 2005).** Royal jelly also contains large quantities of acetylcholine, a neurotransmitter in both the peripheral and central nervous systems and the only neuromodulator used in the motor division of somatic nervous system, also royal jelly increases the differentiation of all types of brain cells from neural stem cells, while unsaturated fatty acid characteristic of royal jelly, increased the generation of neurons and decreased that of astrocytes from neural stem cells **.** It has played neurotrophic and neuroprotective roles on the hippocampus of the adult mouse brain **(Hashimoto *et al.*, 2005).** Oral administration of royal jelly has been proven to be responsible for the increase of the number of granular cells in the dentate gyrus of the hippocampus and for the simultaneous improvement of the affected cognitive process **(Hattori *et al.*, 2011).** Trans-10-hydroxy-2-decanoic acid stimulated the in vitro differentiation of nervous stem into neurons **(Hattori *et al.*, 2007a,b).**

Insulin-like action:

Royal jelly reduces blood sugar level via insulin-like peptides and other compounds (like chromium, sulphur, vitamins B_3 and H). Royal jelly is also capable to sustain the optimal blood level of sugars by taking part in the oxidation of glucose to obtain energy, through the insulinic effect of insulin-like peptides found in it **(Batchelder, 2002).** Insulin found in royal jelly very closely resembles the insulin found in mammals

(O'Connor, 1985). In insulin-resistant diabetes patients, royal jelly produced an important decrease of sanguine sugar levels to 33%, after 3 hours administration through injection. Royal jelly has reduced alloxan induced diabetes which affects rats. The simultaneous administration of royal jelly and a fructose solution for 8 weeks to insulin-resistant rats significantly reduced plasmatic concentration of insulin and triglycerides and reduced systolic arterial pressure, without affecting the blood levels of glucose or total cholesterol. Royal jelly can be a functional dietary treatment for the prevention of insulin resistance associated to developing hypertension in diabetes patients**.** Hypo-cholesterolemiant and hepato-protective action in royal jelly is efficacy use in lowering and controlling triglycerides and cholesterol in humans **(Pizzorno *et al.*, 2007).** Administration of 50-100 mg RJ/day lowered serum levels of total cholesterol by 14% and of total lipids by 10% in patients with atherosclerosis **(Vittek, 1995).** In elderly people, eating 10g of royal jelly daily for 14 days raised the levels of serum high density lipids (HDL) and improved the levels of low density lipids (LDL), without affecting serum triglycerides **(Münstedt *et al.*, 2009).**

Kamakura *et al.* (2007) reported that a study on mice suggest that the cholesterol levels decrease effect is seemingly due to the adjustment of squalene epoxidase enzyme (SQLE) and of the low density lipoprotein receptors (LDLR) which are involved in cholesterol incorporation in the liver.

Royal jelly stimulates cellular growth, especially the hepatocytes. 57 kDa protein seems to be responsible for this effect **(Kamakura *et al.*, 2001).** In rat royal jelly prevented

nocotine-1induced cholesterol levels rise **(Abou-Hozaifa and Badr El-Din, 1995).** Royal jelly has a dose-dependent protective effect against fumosins (mycotoxins) in rats, including significant histological and histochemical improvements in the liver and in the kidneys and of the serum levels of renal and hepatic parameters **(El-Nekeety *et al.*, 2007).** In experimental animals and/or humans royal jelly sustained rebuilding glycogen and helped ammonia and latic (responsible for fatigue) detoxification **(Kamakura *et al.*, 2001).** Royal jelly also increased the oxygen flow to the liver **(Vittek, 1995).** Royal jelly also promoted liver health and hepatocytes growth.

Hypotensive and blood regulatory actions:

Takaki- Doi *et al.* (2009) conducted that peptides have acted in a inhibitory manner on the angiotensin 1 conversion enzyme (ACE) and the sanguine pressure has been decreased after the repetitive oral administration of royal jelly doses to spontaneous hypertensive mice.

The anti-hypertensive effect went as high as 38% **(Tokunaga *et al.*, 2004).** The trans-2-octenoic acid and hydroxydecanoic acid from royal jelly may be responsible for the anti-hypertensive action, with different royal jelly fractions exercising bigger or smaller effects on the duration of the action. Royal jelly has also been associated with protective and therapeutic actions in cases of adrenalin-induced arrhythmia; still, there haven't been any observations of an effect on the heart rate **(Librowski and Czarnecki, 2000).**

Antitumor action:

The mechanism of antitumor action was attributed to the 10-hydroxy-2deccenoic acid (10HDA) contained in the royal jelly, a substance that demonstrates inhibitory action on the VEGF (vascular endothelial growth factor) induced angiogenesis, thus cancelling both cell proliferation and cell migration, which leads to tumor vascularization inhibition **(Izuta *et al.*, 2009).** Apalbumin-1 and apalbumin-2, two major proteins in royal jelly, stimulate mouse macrophages to release (tumor necrosis factor) TNF-α **(Simuth and Bilikova, 2004).** Another evidence is that royal jelly has an immune stimulating effect by preventing the myelo suppression induced by tumor evolution and by splenic hematopoiesis suppression in mice carries of Ehrlich ascetic tumor (EAT) and prologues the survival period, depending on the length of the treatment and of the royal jelly doses **(Bincoletto *et al.*, 2005).**

Kamakura *et al.* (2007) and Nakaya *et al.* (2007) conducted that an anti-estrogenic activity of royal jelly by the inhibition of the effect of bisphenol A- an estrogen which stimulates the MCF-7 mammary cancer cells proliferation. Treatment of the MCF-7 cells with the lipid estrogenic royal jelly component has increased the proliferation of these cells **(Suzuki *et al.*, 2008).** The RJP30 proteic fraction is another part of royal jelly that has been found to play an antitumoral role. It was cytotoxic for HeLa cells of cervical-uterine carcinoma decreasing 2.5 times the initial cell density after 7 days of treatment **(Salazar-Olivo and Paz-Gonzalez, 2005).**

Antibiotic effect:

It has been proved strong antibacterial in *vitro* action of the royalisin protein from royal jelly against gram-posative bacteria (*Lactobacilus helveticus, Clostridium, Corynebacterium, Leucnostoc, Staphilococcus, Streptococcus),* but not against the gram-negative ones **(Fujiwara *et al.*, 2004).** Hydrosoluble components of royal jelly, like proteins and peptides, present a high capacity of inhibiting gram positive bacteria and fungi **(Sauerwald *et al.*, 1998).** It is assumed that all these antibacterial components take part in the defensive immune system of bees. *In vitro* cumulative or synergic effects of both royal jelly and starch against *Streptococcus aureus* and *E.coli* . And he also reported that royal jelly and honey agaist *Staphylococcus aureus* have been demonstrated **(Boukraâ *et al.*, 2008).**

Abdelatif *et al.* (2008) conducted that a human study on 60 diabetes patients with limb-threatening diabetic foot infection suggests that cutaneous ulcers and deep tissular infections can be successfully treated with a royal jelly and panthenol ointment.

Anti-inflammatory action and wound healing effect:

In vitro evidence revealed that supernatants of royal jelly suspensions added to mice peritoneal macrophage culture treated with lipopolisacharides and IFNγ, efficiently inhibit the production of pro-inflammatory cytokines (TNF-α, IL-6, IL-1), in a dose dependent manner, without cytotoxic effects on the macrophages, which suggests that royal jelly contains factors (MRJP3 among them) which suppress the secretion of pro-inflammatory cytokines **(Kohno *et al.*, 2004).** Royal jelly has an

anti-inflammatory action and increases wound healing capacity by increasing exudation and collagen formation in granulation tissue formation. Royal jelly shorted the healing period of desquamated skin lesion **(Fujii *et al.*, 1990).** Royal jelly has promoted the collagen production of skin fibroblasts in the presence of ascorbic acid-2-O-α-gluciside (AA-2G), the increase of the collagen production being induced by 10-hydroxy-2-decenoic acid (10H2DA) and 10-hydroxydecanoic acid (10HDA) in a dose-dependent manner. 10H2DA induced the production of TGF-β1, a transforming growth factor important to collagen production **(Satomi *et al.*, 2004).**

Suemaru *et al.* (2008) reported that ointments with different royal jelly concentrations have significantly improved the recuperation of 5-fluorouracil induced damage in a dose dependent manner, which suggest that topical application of royal jelly has a healing effect on severe oral mucositis induced by chemotherapy.

Immunomodulatory and antialergic activity:

Royal jelly contains amino and gamma globulin, unsaturated fatty acids, hormons, enzymes, proteins, vitamin E and vitamin A which helps the immune system fight infections. The fatty acids that isolated from royal jelly (10HDA and 3-10-dihydroxydecanoic acid) modulate the immune response in rat dendritic cell and T-cell cocultures, in different ways depending on concentration (they stimulate the proliferation of T-cells, but, in high concentrations, they inhibit it, decrease IL-2 production and increase IL-10) **(Vucevic *et al.*, 2007).** Similar effects of different components isolated from royal jelly have been found

on rat T-cell and suggest that water extract possesses the most potent immunomodulatory activity in *vitro* **(Gasic *et al*., 2007).**

Okamoto *et al*. (2003) conducted that, the 70 kDa glycoprotein modulated in the *vivo* and *in vitro* immune response, being able to suppress IL-4, IL-2 and gamma-IFN production, along with the stimulaneous inhibition of T lymphocytes proliferation. In another experimental model on mice, the intraperitioneal administration of MRJP3 has suppressed (IgE și IgG1) ovalbumin induced spontaneous hypersenisibility.

In chiken, royal jelly administration for 28 days has led to an increase of the number of circulating leucocytes and lymphocytes, with the decrease in serum globulins levels **(Kurkure *et al.*, 2000).** In the case of animal immunization with sheep erythrocytes (SRBC), in chickens there has been noticed a significant increase in antibodies production among those birds who were administered royal jelly **(Al-Mufarrej and El-Sarag, 1997).** In tumor bearing mice RJ administration increased the survival period and proved myeloprotection through positive effects on stem cells of bone marrow and on the hematopoiesis in induced splenic tumors **(Bincoletto *et al*., 2005).** In systemic lupus erythematosus in mice, royal jelly has inhibited auto-immunity, determining a significant drop in the serum levels of IL-10, of anti-DNA antibodies (mono and double catenaries) and of anti-erythrocyte antibodies, and a reduction of the number of splenic autoreactive B lymphocytes. Administrated orally, before the onset of the illness, it has significantly delayed it, it has decreased the proteinuria and prolonged the life span, while

administrated after the onset of the illness, and royal jelly has produced a significant amelioration of the renal symptoms, leading to the prolongation of life (**Mannoor *et al.*, 2009).**

Erem *et al*. (2006) found that, the immunomodulatory role played by royal jelly in Grave's disease (autoimmune hypothyroidism) has been studied in vitro using lymph cells from both healthy volunteers and from Grave's disease patients. Royal jelly has produced the proliferation of healthy lymphocytes and the increased secretion of various cytokines (gamma-IFN), while decreasing the production of others. In lymphocytes from patients treated with royal jelly, the ratio between Th1/Th2 (IFN γ/IL-4) cytokines has changed in favor of Th1 and the levels of the antibodies against the thyroid stimulating hormone receptor (Ac-TSHR) have registered a significant drop. These results suggest that the effect of royal jelly is similar to that of the usual medication used to lower Ac-TSHR.

Royal jelly stops the evolution of cutaneous lesions from the category of atopic dermatitis, lowering the hypertrophy, hyperkeratosis and epidermis and dermis inflammatory cells infiltration levels, possibly through a blend of TNP-specific low adjustment of the IFN-gamma specific production and of high adjustment of iNOS expression **(Taniguchi *et al.*, 2003).** It is known that royal jelly stimulates the collagen production of the cutaneous fibroblasts in the presence of ascorbic-1-O-alfa-glicozid acid. Also, it has been reported that, the effect of purified RJ is similar to that of 10HDA, the former being able to stimulate fibroblasts to produce the growth factor - beta 1

transformation, and this being an important factor in collagen production **(Koya-Miyata *et al*., 2004).**

Effects on the reproductive system and fertility:

Royal jelly allows the complete development of the larvae in their brood cells and maintains its ovulatory capacity over its entire life-span. This unusual property of royal jelly has spurred a possible connection to fertility and we know that royal jelly has always been used as a stimulator of fertility **(Marghitas, 2008).** Royal jelly is effective in perimenopausal symptoms, osteoporosis, improving hormonal equilibrium and fertility in men and women by increasing ovules and sperm quality **(Lewis, 2005)**.

Suzuki *et al.* (2008) reported that royal jelly is an important source of para-aminobenzoic acid which increases fertility in women who regularly consume this product for at least 6 months. Together with the pantothenic acid (vitamin B_5), this acid induces protein usage for healthy hair growth and for its regimentation and that of the skin's. Several fatty acids from RJ have found to mimic human estrogens.

Royal jelly have been found to have a low estrogenic activity mediated through the interaction with estrogen receptors which leads to modified gene expression and cell proliferation **(Mishima *et al.*, 2005).**

Suzuki *et al.* (2008) reported that feeding rats with a royal jelly supplement has led to a slight hypertrophy of the uterine luminal epithelium.

Oral administration of royal jelly can counter "summer infertility" (significant improvement of a series of spermography

parameters) and improve physiological status in male rabbits **(El-Nagar *et al.*, 2010).** Royal jelly had similar effects equine chorionic gonadotropin, by inducing the estrus and improving gestation and nativity rates **(Husein and Haddad, 2006).** Oral administration of royal jelly has not been effective in improving the estrus in sheep during the transition between the inactive and the active reproductive seasons **(Kridli and Al-Khetib, 2006).** The increase in bone calcium levels and the recuperation of bone mass were interpreted as the result of an improved intestinal absorption of calcium, to the disadvantage of the opposite effect of the parathyroid hormone **(Hidaka *et al*., 2006).**

Fortifying and tonic action:

Conducted that royal jelly was likely to increase muscular effort capacity, vital capacity, respiratory function and energy levels. And he conducted that it is also increased the feeling of being energetic in patients with chronic **(Batchlder, 2002).** Royal jelly had very good results in convalescence and postsurgical recuperation periods **(Balch and Balch, 2000).**

Food and Agriculture Organization of United Nations, (FAO), (1996) reported that in humans, the pantothenic acid is converted to coenzyme A, which helps the body to metabolize lipids and to improve its stress response capacity, by supporting the suprarenal glands. The pantothenic acid (B_5) content of RJ is higher than in any other known source.

Kamkura *et al.* (2001) reported that there is one an experiment on mice, in which royal jelly has prevented physical fatigue, decreasing the lactate and serum azote accumulations.

Toxicity, Counter –Indication and Precautions:

Bogdanov (2006) reported that in humans a safe dose with a therapeutic index of 500 corresponds to 2 mg royal jelly per kg, and a therapeutic index for a 75 kg individual this being 150 mg RJ per day. Increasing of this dose to 750 mg per day, RJ will still have an acceptable therapeutical index of 100.

2.3 Bee venom:

The early ancient civilizations knew about the healing found virtues in the painful bee stings. Bee stings are probably one of the first natural cures for arthritis. In the ancient civilization of China, India, Egypt, Babylon and Greece bee venom was used for apitherapy **(Urtubey, 2005).**

(2.3.1) Composition:

Bee venom is a complex mixture of proteins, peptides and low molecular components. Nowadays its components have been characterised. The main components are proteins and peptides. The composition of fresh and dried bee venom differs mainly in regards to the volatile components; the overall biological activity is similar **(Bogdanov, 2011b).**

Proteins (Enzymes):

The enzymes are proteins catalyzing specific reactions. There are 5 enzymes in bee venom **(Bogdanov, 2011b).**

Polypeptides:

Protein and the melittin electrophoretic patterns are typical of the honeybee species (**Krell, 1996).**

Low molecular compounds:

Urtubey (2005) reported the composition of bee venom on dry matter:

Table (II) Composition of bee venom.

Substance group	Component	% (dry weight basis)
Proteins (Enzymes)	Phospholipase A2	10-12
	Phospholipase B	1
	Hyaluronidase	1-2
	Phosphatase	1
	α - Glucosidase	0.6

Table (II) continuous:

Peptides	Melittin	40-50
	Apamine	2-3
	MCD peptide	2-3
	Secapine	0.5-2
	Pamine	1-3
	Minimine	2
	Adolapine	0.5-1
	Procamine A, B	1-2
	Protease inhibitor	0.1-0.8
	Tertiapine, cardiopep, melittin F	1-2
Phosphoilipids		1 -3
Biogenic amins	Histamine	0.1-o.5
	Dopamine	0.2-1
	Noradrenalin	0.5-2
Amino acids	Aminobutyric acid, α-amino acids	1
Sugars	Glucose, fructose	2-4
Volatiles (pheromones)	Complex ethers	2-8
Minerals	P, Ca, Mg	3-4

2.3.2 The Painful healing sting: bee venom in human history:

Around 300 BC Aristoteles, referred to the stinging apparatus of bees and the powerful properties of bee venom (BV) in his book *Historia animalia* **(Urtubey, 2005)**. The ancient Greek doctor Hippocrates used bee venom for therapeutic purposes. He described it as arcanum, a mysterious substance whose creative properties he did not quite understand. In 14 BC Pliny the elder described BV use in his Natural history Galen (130–200 AD) prescribed the use of honey and bee venom as a cure for baldness **(Hellner *et al*., 2006).**

2.3.3 Therapeutic index:

Son *et al.* (2007) conducted that bee venom, too, has various side effects. Often the therapeutic and the toxic effects lie closely together. Individual bee venom components show toxic effects when their concentration is 20-50 times greater than the therapeutic dose, while whole bee venom is toxic when its therapeutic dose is exceeded by 200-500 times.

The same outher reported that, the main biological and therapeutic effects of bee venom and its components:

Fonts in italic toxic effect

Table (III) Main biological and therapeutic effects of bee venom.

Component %	Effects	Tox. Mg/kg
Melittin *Biologically active peptide* **50-55**	Membrane-active, diminishes surface tension of membranes Anti-inflammatory in very small doses; Stimulates smooth muscles; Increases capillary permeability increasing blood circulation and lowering the blood pressure, lowers blood coagulation, immunostimulatory and immunosuppressive,Radiation protective, influences the central nervous system, Anticancer, antibacterial, antifungal, antiviral *Higher doses are inflammatory and haemolyti*	4
Phospholipas A *Enzyme hydrolysing phospholipids* **10-12**	Destroys phospholipids and dissolves the cell membrane of blood bodies; lowers the blood coagulation and blood pressure, prevents neuronal cell death caused by prion peptides. *Induces inflammation, the strongest allergen and thus the most harmful bee venom Component.*	7.5
Phospholipase B *cleavage of the toxic lysolecetin* **1**	Detoxicating activity.	
Hyaluronidase *Catalyses hydrolysis of hyoloronic acid, the tissue cement* **1-2**	Catalyses the hydrolysis of proteins, thus enabling the penetrating of BV into the tissue; dilates blood vessels and increases their permeability, causing an increase of blood circulation; *Allergenic.*	0

Table (III) continuous

Apamine *Biologically active peptide* **2-3**	Anti-inflammatory stimulating the release of cortisone, antiserotonine action Increases the defence capability Immuno-supressor, stimulates the central nervous system in very small doses. *Higher doses are neurotoxic*	4
MCD, mast cell degranulating-peptide 401 **2-3**	Lyses mast cells, releasing histamine, serotonine and heparine . Melittin-like effect increasing capillary permeability increasing Anti-inflammatory simulates the central nervous system.	40
Adolapin *Biologically active peptide* **1**	Inhibits the specific brain enzymes cyclooxigenase and lipooxigenase.Decreases inflammations by, anti-rheumatic, decreases pain, antipyretic Inhibits the aggregation of erythrocytes Has relatively low toxicity.	
Protease-Inhibitors *Biologically active peptides* **3-4**	Inhibits the activity of different proteases like trypsin, chymotprypsin, plasmin, thrombin, thus decreasing inflammation, anti-rheumatic Low toxicity.	
Secapin, tertiapin, cardiopep, minimin, procamine **3-5**	Peptides, with an uncertain role in the physiological action of BV Antiradiation effects, cardiopep has antiarhythmic effects	
Histamine *Neurotransmitter* **0.7-1.5**	Dilates blood vessels, increasing the permeability of blood capillaries and increases blood circulation; Stimulates smooth muscles; *Allergenic*	192-445

Table (III) continuous

Dopamine, **Noradrenaline** *Neurotransmitters* **0.2-1.5**	The low concentrations in BV do not cause physiological effects in mammals, but active when injected in invertebrates.	
Alarm pheromones **4-8**	Complex ethers, causing alarm of the bee colony and its defensive behavior	

* "Tox" is measured in rat experiments.

2.3.4 Biological effect of bee venom components:

Melittin is the main bee venom component with many positive biological effects and a relatively low toxicity. Mast cell degranulating- peptide 401 (MCD) and phospholipase A are the two most toxic components. In order to achieve definite biological effects, individual bee venom components can be used **(Bogdanov, 2011b).**

2.3.5 Apitherapy:

Arthritis

Rheumatoid arthritis:

Lee *et al.* (2005) conducted that A chronic, systemic inflammatory disorder that may affect many tissues and organs, but principally attacks the joints producing an inflammatory synovitis that often progresses in destruction of the articular cartilage and ankylosis of the joints. About 1% of the world's population is afflicted by rheumatoid arthritis, women three times more often than men.The mechanism of action of BV in treating arthritis is clarified:

• Bee venom blocks the building of the pro inflammarory substances cytokinine.

• Bee venom inhibits the proliferation of rheumatoid synovial cells.

Osteoarthritis (OA):

Osteoarthritis disease process by which joints wear out. As the joint surface wears away it sheds wear particles which stimulate the joint lining to produce fluid, causing the knee to swell. When the articular cartilage wears through, the underlying bone becomes exposed. The exposed bone rubs against exposed bone when walking and this causes pain - often described as a toothache type pain. It is a common disease in adults with a prevalence of about 0.5 % **(Bogdavov, 2011b).** Bee venom does not seem to influence rheumatoid deformation, as shown by patients X-rays, but it acts by controlling pain and inflammation **(Krylov *et al.*, 2007).** Bee venom used in muskoskeletal pain. Bee venom was used in the treatment of different pain conditions: Neck pain, low back pain, herniated lumbar pain, disc pain, shoulder pain after stroke, acute ankle sprain, wrist sprain, rheumatoid arthritis and knee osteoarthritis. Bee sting and apitherapy was useful in all these conditions. Apitherapy relieves pain more effectively than acupuncture **(Lee *et al.*, 2005).**

Bee venom better than hormone therapy against rheumatic pain:

Many researchers attribute the success of bee venom therapy to the activation of main hormone systems of the body: thyroid, pituitary gland, the hypothalamus and the adrenal gland.

Hormone therapies, for example cortisone, ACTH hormone, are also applied against rheumatic pain. However, hormone therapy causes with time of use unfavorable side effects such as the reduction in the activity of this vital hormone glands, while the bee venom constantly activates the activity of hormonal systems **(Bogdanov, 2011b).**

Bee venom against diseases of the nervous system:

Glutamate is the predominant excitatory neurotransmitter in the central nervous system (CNS). Changes in glutamate release and uptake due to alterations in the activity of glutamate transporters have been reported in many neurodegenerative diseases, including Parkinson's disease, Alzheimer's disease, and amyotrophic lateral sclerosis. To assess if bee venom can prevent glutamate-mediated neurotoxicity, glutamatergic toxicity in neuronal cells and microglial cells and found that BV protected against cell death. Furthermore, bee venom significantly inhibited the cellular toxicity of glutamate, and pretreatment with bee venom altered kinase activation (e.g., JNK, ERK) following exposure to glutamate. These findings suggest that treatment with BV may be helpful in reducing glutamatergic cell toxicity in neurodegenerative diseases **(Lee *et al.*, 2011).**

Multiple sclerosis (MS):

There is molecular basis for the action of bee venom for this action. Evidence of specific biologic effects of the bee venom component apamin in brain that might be linked to MS has been shown **(Steketee and Kalivas, 1990).** A very good or good improvement of 175 out of 210 cases (improvement rate was 83%) **(Ludyanskii, 1994).**

Parkinson:

In Russia bee venom is successfully used in Parkinson's patients. After the Russian apitherapy school bee venom reduces the Parkinson symptoms. This effect is due to the positive biological effect of bee venom on the brain: the blood supply and the supply of dopamine in the brain is improved, it increases the brain blood vessels and reduces blood coagulation **(Lee *et al.*, 2011).**

Alzheimer:

Several behavioral and electrophysiological studies indicate that small conductance calcium-activated potassium channels-blockade by apamin may enhance neuron excitability, synaptic plasticity, and long-term potentiation in the CA1 hippocampal region, and for that reason, apamin has been proposed as a therapeutic agent in Alzheimer's disease treatment **(Hellner *et al.*, 2006).**

Cancer:

Bee venom acts against different types of cancer in cell and animal experiments **(Orsolic, 2005).** Bee venom (BV) (apitoxin) has been widely used in the treatment of some immune-related diseases, as well as in recent times in treatment of tumors. Several cancer cells, including renal, lung, liver, prostate, bladder, and mammary cancer cells as well as leukemia cells, can be targets of bee venom peptides such as melittin and phospholipase A2. The cell cytotoxic effects through the activation of PLA2 by melittin have been suggested to be the critical mechanism for the anti-cancer activity of BV. The induction of apoptotic cell death through several cancer cell

death mechanisms, including the activation of caspase and matrix metalloproteinases, is important for the melittin-induced anti-cancer effects. The conjugation of cell lytic peptide (melittin) with hormone receptors and gene therapy carrying melittin can be useful as a novel targeted therapy for some types of cancer, such as prostate and breast cancer **(Park *et al*., 2011).**

Skin and eye diseases:

Bee venom use against skin diseases has a long tradition and has been used since the beginning of the 20th century. Following skin diseases have been successfully treated eczemas like dermatitis, psoriasis, furunculosis (recurring boil), for the healing of cicatrices and against baldness **(http://www.hexagon.net (2012))**.For skin application bee venom is applied in the form of creams and ointments and also in electrophoresis. Interestingly enough bee venom has been used also in ophthalmology. Especially, it has been used for the treatment of acute and chronic rheumatic iritis and neuritis of the eye nerve. Aqueous bee venom solutions are used as drops and injections **(Bogdanov, 2011b).**

2.4 Application for the approval of manuka honey with added bee venom:

2.4.1 Natural information of manuka honey with added bee venom:

The anticipated food uses of manuka honey with added bee venom are novel only to the extent that the honey contains a very low level (20µg/g) of added honey bee venom. The table below is for average quantities and the serve size is 10 g, equivalent to one heaped teaspoon **(Sulaiman *et al*., 2012).**

Table (IV) Nutritional information for Manuka honey with added bee venom.

Components	Per Serve	Per 100g
Energy (kJ)	136	1360
Protein (g)	<0.1	0.4
Fat (g)	0.0	0.0
Carbohydrate (g)	8.0	79.6
Sodium (mg)	1.2	12

2.4.2 Relationship of daily intake of manuka honey with added bee venom to total energy expenditure:

Two teaspoons of Manuka honey with added bee venom (i.e. 20 g) represents about 2 – 4% of an average adult's energy expenditure per day (approx. 272 kJ), using Australian values for males of 11.5 – 14 MJ per day, and 7 – 9.5 MJ per day for females **(Sulaiman *et al.*, 2012).**

2.4.3 Microbial information of manuka honey with added bee venom:

Vegetative forms of bacterial pathogens (*e.g. Salmonella spp., Listeria monocytogenes*) have not been detected in honey. However, bacterial spores (*e.g. Bacillus spp.,Clostridium spp.)* are likely to occur in honey. Although, these spores will not grow in honey, when it is used as an ingredient in another food the bacterial spores could be introduced into, and grows in that food. As manuka honey with added bee venom is meant to be consumed on its own or with food and not used as an ingredient

the possible presence of bacterial spores is unlikely to cause a problem. However, as a precautionary measure against possible infant botulism, which could arise from the presence of *Clostridium botulinum* spores, a warning is included on the product label stating "honey should not be given to infants under 12 months of age" **(Sulaiman *et al.*, 2012).**

2.4.4 Suggested daily intake of honey bee from manuka honey with added bee venom is low:

Intake of two teaspoons of manuka honey with added bee venom per day is equivalent to about 20 g honey and 400 µg honey bee venom per day. This dosage (5.3µg/kg/day) is close to the dose that would be expected to be effective for a 75 kg adult from reported clinical studies. In these studies subcutaneous or intradermal doses of 5 µg/kg body weight were effective for treatment of arthritis in rodent models **(Kim *et al*., 2004).** Even lower venom doses of 0.8 and 1.6 µg/kg body weights were found to be antiarthritic **(Park *et al.*, 2004).**

2.4.5 Bee venom is found naturally in honey:

Low level of bee venom is found naturally in honey. They used a total cumulative dosage of 525 µg per month in their sublingual immunotherapy studies, a concentration of 1 – 2 µg venom/g honey would be sufficient to confer immunological benefits to a person consuming 10 – 20 g per day of normal commercial Manuka honey **(Passalacqua *et al.*, 2008).**

Table (V) show naturally occurring bee venom concentration in commercial New Zealand honeys.

Table (V) Naturally occurring bee venom concentration in commercial New- Zealand honeys.

Honey variety	Bee venom concentration (µg venom/g honey)
Uncreamed Manuka	1.3
Creamed Manuka	1.7
Active Manuka	1.5
Multifloral	1.1

Severino *et al.* (2008) reported that these data show normal commercial honey contains bee venom is supported by comments made by bee-keepers who have observed bees stinging directly into honey within hives. The reason bees do this is unexplained at this time.

2.4.6 Rodent toxicity manuka honey with added bee venom:

Manuka honey with added bee venom appears to have no acute or sub-acute toxic effects in both liquid and freeze-dried forms. Animals gained weight, were observed to behave normally, and showed no signs of change in internal organ form or function**. (Regulation (EC) No 258/97 of the European Parliament and the Councilof 27th January 1997 concerning novel foods and novel food ingredients).**

Kim *et al.* (2004) investigated the general pharmacological effects of bee venom and venom extracts on a range of physiological parameters of the central nervous system,

digestive, cardiovascular and respiratory systems in rodents. A single clinical dose of bee venom was taken as 5 µg/kg when administered by intradermal or subcutaneous route to human patients. They administered bee venom to mice, rats and rabbits in doses up to 200-fold the effective clinical dose (i.e. 1,000 µg/kg). Using a variety of indicators the results of this study showed that treatment with whole bee venom (at a dose 200 times the recommended clinical dose) did not produce any significant effect on the central nervous system (as determined by general behavior, sleep induction time and duration, spontaneous activity, motor function, body temperature, or drug-induced convulsions). Bee venom was a potent antinociceptive agent without the side effects associated with many narcotic drugs. Bee venom treatment did not affect motor activity, intestinal peristaltic function or gastric function. Additionally, bee venom did not alter blood pressure and heart rate in rats nor respiratory rates in rabbits.

2.4.7 Level of bee venom in manuka honey with added bee venom is very low compared to a lethal dose:

Bee venom from bee stings and this data can be used to compare lethal levels of bee venom to those found in Manuka honey with added bee venom. However, it should be noted that all of this published toxicity data relates to transdermal venom delivery that is, resulting from bee stings (**Regulation (EC) No 258/97 of the European Parliament and the Councilof 27th January 1997 concerning novel foods and novel food ingredients).** It is clear that comparing data from transdermal delivery to sublingual or oral delivery will be highly

conservative and will over estimate the toxicity of bee venom consumed orally. This is due to the much higher doses of bee venom (and other allergens) used in sublingual immuno therapy, and its better safety profile, compared to subcutaneous immunotherapy **(Serverino *et al.*, 2008).** Comparing efficacy and dose levels of allergens used in subcutaneous and sublingual immunotherapies. They found that sublingual doses could be more than 500 times the customary dosage used in subcutaneous therapy. Furthermore, in sublingual studies comparing response to different doses there was greater improvement in symptom-medication scores with higher doses **(Cox *et al.*, 2006).** Honey bee venom subcutaneous immunotherapy 100 or 200 µg doses are considered effective but that for other allergens efficacy of sublingual immune therapy has been shown using doses up to 500 times those used in subcutaneous courses of immuno therapy **(Severino *et al.*, 2008).** Median lethal dose (LD50) for honey bee venom is 2.8 mg venom/kg body weight for intravenous and 3.8 mg venom/kg body weight for intraperitoneal delivery in mice. We will use the lower value of 2.8 mg venom/kg body weight value for comparison. For a 75 kg person, the value of 2.8 mg venom/kg body weight is approximately 530 times higher than the daily consumption of bee venom found in 20 g per day of Manuka honey with added bee venom. To put it another way, for this person to consume 2.8 mg venom/kg body weight in Manuka honey with added bee venom they would need to consume more than 10.5kg of the product. Considering the high intrinsic level of monosaccharide carbohydrates in honey this would unlikely be possible for an average person **(Schmidt, 1995).**

2.4.8 Risk of manuka honey with added bee venom to those who may be allergic to bee venom:

An induction phase (or up-dosing phase) where dosage is started low and increased is recommended. To reduce the risk of side reactions consumers are requested on the label under *"Directions for use"* to start with a small amount first and to build up to one to two teaspoons full per day **(Cox *et al.*, 2006).**

"Start with ¼ teaspoon per day and increase to one to two teaspoons per day as required.

2.5 Lyophilization:

For the first time it was possible to dry a delicate product from the frozen state under moderate vacuum. In that state, it would be stable at room temperature for a long time and the authors described, in a set of successive notes, that this technique could be applied to the preservation of sera and vaccines. Freeze-drying was then, officially born, despite it had been used centuries ago by the in case who dried their frozen meat in the radiant heat of the sun in the rarified atmosphere of Altiplano **(Bordas and Arsonval, 1906).** Lyophilization (this name derived from the term *lyophilize* coming from the Greek λυος and Ψιλειν, which means "like the solvent," describing the great ability of the dry product to rehydrate again). Freeze-drying was then received a new name that has been in current use since then, together with cry desiccation **(Flosdrof and Mudd, 1935).**

Freeze-drying still holds a remarkable place in our multiple panels of advanced technologies and more particularly in pharmaceutical field. It was thus a wise and sound decision of our publisher to propose that a collective book be devoted to that

topic. **(Handuroy, 1960).** An aqueous solution of sodium chloride at -25°C could be either a sponge- like ice network soaked with highly concentrated fluids if the system has been cooled progressively from +20°C to -25°C. Or else a totally frozen solid where all the interstitial fluids have crystallized as eutectics if the system has been cooled, first to -40°C and then rewarmed to -25°C **(Rey , 1960).** In general, the thermo luminescence center is located preferentially in the defects of the crystalline network and that those defects , in turn, are more reflex ion of the structure of the original liquid . We can, thus to know a little more about the starting solutions **(Grossweiner and Matheson, 1954).**

Rapolu (2012) defined the *lyophilization* as a process which extracts water from foods and other products so that the foods or products remain stable and are easier to store at room temperature (ambient air temperature).

Lyophilization is carried out using a simple principle of physics called sublimation. Sublimation is the transition of a substance from solid to vapor state, without first passing through an intermediate liquid phase. To extract water from foods, the process of lyophilization consists of:

1. Freezing food so that the water in the food becomes ice;
2. Under a vacuum, sublimating ice directly into water vapor;
3. Drawing off the water vapor;
4. Once the ice is sublimated, the foods are freeze-dried and can be removed from the machine.

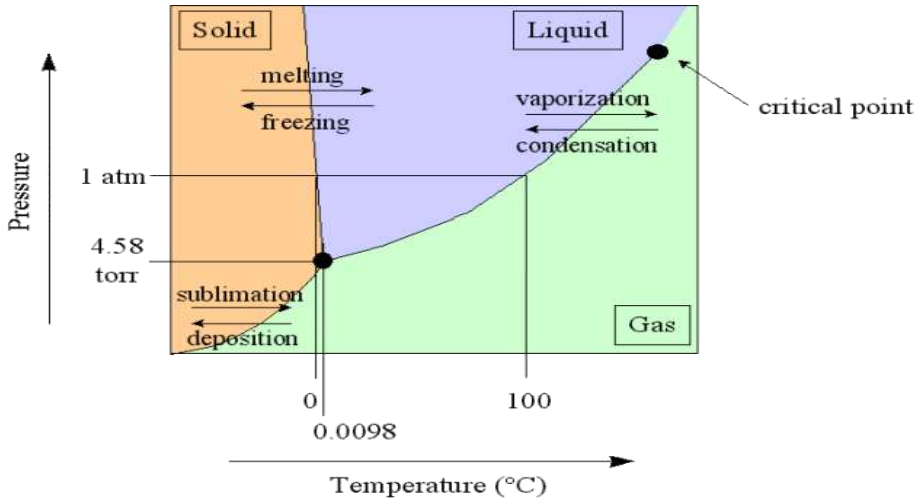

Fig. (I) Diagram of lyophilization.

Snowman and John (1988) found that one milliliter of ice produces more than 1,000,000 ml. of water vapor at typical lyophilization cycle pressures. The more energy –efficient vacuum pumps cannot handle large quantities of water vapor. For this reason, it is usual to fit a refrigerated trap (called ice condenser) between the lyophilization chamber and the vacuum pump.

Wei (1999) mentioned that the developing recombinant protein pharmaceuticals has proved to be very challenging because of both the complexity of protein production and purification , and the limited physical and chemical stability of proteins. To overcome the instability barrier, proteins often have to be made into solid forms to achieve an acceptable shelf life as pharmaceutical products. The most commonly used method for preparing solid protein pharmaceuticals is lyophilization (freeze-drying). The lyophilization process generates both freezing and

drying stresses, which can denature proteins to various degrees. Even after successful lyophilization with a protein stabilizer, proteins in solid state may still have limited long-term storage stability. In the past two decades, numerous studied have been conducted in the area of protein lyophilization technology, and instability/stabilization during lyophilization and long-term storage. Many critical issues have been identified, to have an up-to-date perspective of lyophilization process and more importantly, its application in formulating solid protein pharmaceutical.

2.5.1 Lyophilization and its denaturation stresses:

The amount of non-frozen water for globular proteins is about 0.3 – 0.35 g g^{-1} protein, slightly less than the proteins' hydration shell **(Kuhlman *et al*., 1997).** Some proteins can keep their activity both during freezing and drying processes, such as α1-an-titrypsin in phosphate-citrate buffer **(Vemuri *et al.,* 1994).** Many proteins cannot stand freezing and/or drying stresses, Freeze –thawing caused loss of activity of lactate dehydrogenize (LDH) **(Andersson and Hatti-Kaul, 1999)**.

Interferon (IFN-γ) aggregation in liquid mannitol formulation was more sever at -20°C than at -70°C, 5 and 15°C during storage. To prevent freezing-induced complication in studying cold protein denaturation, cold and heat denaturation of RNase A has been conducted under high pressure (3Kbar) **(Perlman and Ngeryen, 1992).**

The nature of cold denaturation has not been satisfactorily delineated. Since solubility of non-polar groups in water increases with decreasing temperature due to increased hydration

of the non-polar groups, solvophobic interaction in proteins weakens with decreasing temperature **(Graziano *et al.*, 1997).** Decreasing solvophobic interaction in proteins can reach a point where protein stability reaches zero causing cold denaturation **(Jaenicke, 1990).** Normal or thermal denaturation is entropy-driven cold denaturation is enthalpy-driven **(Shortle, 1996).** Oligomeric proteins typically show cold denaturation, i.e. dissociation of subunit oligomers, since association is considered to be a consequence of hydrophobic interaction **(Winsniewski, 1998).**

2.5.2 Concentration effect:

Lowering the temperature reduces the rate of chemical reactions. However, chemical reactions may actually accelerate in a partially frozen aqueous solution due to increased solute concentration (**Pikal, 1999).** Due to solute concentration, the rate of oligomerization of β-glutamic acid at −20°C was much faster than at 0 or 25°C in the presence of a water-soluble carbodiimide , 1-ethyl-3-(3-dimethylaminopropyl) carbodiimid (EDAC) (**Lui and Orgel, 1997).**

2.5.3 Formation of ice-water interface:

Freezing a protein solution generates an ice-water interface. Proteins can be adsorbed to the interface, loosening the native fold of proteins and resulting in surface-induced denaturation **(Starmbini and Gabellieri, 1996).** Rapid (quench) cooling generates a large ice water interface while a smaller interface, is induced by slow cooling **(Chang *et al.*, 1996).**

2.5.4 Changes of pH during freezing:

At extreme pH, increased electrostatic repulsion between like charges in proteins tends to cause protein unfolding or denaturation **(Drill**, **1990).** Freezing a buffered protein solution may selectively crystallize one buffering species, causing pH changes (**Ford and Dawson, 1994).** A significant pH drop during freezing, which then denatures pH-sensitive proteins for example freezing of a LDH solution caused protein denaturation due to a pH drop from 7.5 to 4.5 upon selective crystallization of Na_2HPO_4 **(Anchordoquy and Carpenter, 1996).** The drop of pH during freezing can potentially affect storage stability of lyophilized proteins (**Chang *et al*., 1996).**

2.5.5 Phase separation during freezing:

Freezing polymer solutions may cause phase separation due to polymers' altered solubility at low temperatures. Freezing-induced phase separation can easily occur in a solution containing two incompatible polymers such as dextrin and ficoll **(Izutus *et al.*, 1996).**

Several strategies have been proposed to mitigate or prevent phase separation- induced protein denaturation during freezing. These include use of alternative salts, adjustment of the relative composition of polymers to avoid or rapidly pass over a temperature region where the system may result in liquid-liquid phase separation and chemical modification of the protein such as pegylation **(Heller *et al.*, 1999).**

2.5.6 Dehydration stresses:

Rupley and Careri (1991) reported that proteins in aqueous solution are fully hydrated. A fully hydrated protein has

a monolayer of water covering the protein surface, which is termed the hydration shell. And found that the water content of lyophilized protein product is less than 10%. Therefore, lyophilization removes part of the hydration shell. Removal of the hydration shell may disrupt the native state of a protein and cause denaturation. A hydrated protein, when exposed to a water-poor environment during dehydration, tends to transfer protons to ionized carboxyl groups and thus abolishes as many charges as possible in the protein.

Pikal and Shah (1997) reported that dehydration during lyophilization may cause significant difference in moisture distribution in different locations of a product cake. The uneven moisture distribution may lead to possible localized over drying, which may exacerbate dehydration –induced protein denaturation.

Lyophilization may induce several potential changes in the spectra of proteins. Disruption of hydrogen bonds in proteins during lyophilization generally leads to an increase in frequency and a decrease in intensity of hydroxyl stretching bands **(Carpentar and Crwe, 1989).**

2.5.7 Residual moisture:

The desired residual moisture level in a lyophilized product dictates duration of secondary drying step. An electronic hygrometer or a residual gas analyzer may be used to determine residual moisture level during lyophilization and thus, the end point of secondary drying **(Nail and Johnson, 1992).** Moisture content of lyophilized formulations can be determined by several methods, including less-on-drying Karl Fischer titration, thermal

gravimetric analysis (TGA), gas chromatography (GC), or near IR. The reproducibility of moisture determination by the first three methods was found to be similar for several biological products **(May *et al.,* 1989).** A lower moisture usually content leads to a more stable protein product, although there may not be any significant difference in protein stability between near zero and an intermediate moisture content of about 1% (**Pikal, 1990).** A generally rule, a moisture content in a lyophilized protein formulation should not exceed 2% (**Daukas and Trappler, 1998).**

2.5.8 Protein aggregation:

Costantino *et al.* (1988) defined the aggregation as the one of the major instabilities for lyophilized protein pharmaceutical during storage.

Relative amounts of soluble and insoluble protein aggregates may change with storage conditions such as lysozome aggregation under different relative humidity **(Separovic *et al.*, 1998).** Both physical and chemical aggregation can lead to formation of insoluble aggregates **(Costantino *et al.*, 1994).**

2.5.9 Chemical degradations:

Pikal *et al.* (1992) mentioned that, several chemical degradation have been observed in lyophilized proteins during storage. In some cases, multiple degradation processes proceed simultaneously in a protein.

2.5.10 Oxidation:

Pikal *et al.* (1992) found that, methionine residues in proteins can easily be oxidized by atmospheric oxygen.

2.5.11 Storage temperature:

Many lyophilized proteins show increased loss of activity at high temperatures **(Colaco *et al.*, 1992).** Due to the structural complexities of proteins, the temperature effect on stability of solid proteins cannot be simply described by a single instability mechanism, although in general, the higher temperature, the lower protein stability, both physically and chemically. It should be noted that fluctuating storage temperatures may be more detrimental to a lyophilized protein than a single high storage temperature **(Ford and Dawson, 1994).**

Materials and Methods

III. Materials and Methods

3.1 Materials:

The honey bee, royal jelly and bee venom were collection from honeybee colonies in Borg Alarab in Alexandria, Governorate, the experiment were carried out at Faculty of Agriculture, Moshtohor, Benha University, and Faculty of Medicine, Ismalea ,Chanal Suize University.

3.1.1 Collection the venom:

The extraction device is powered by continuous electric current at 12 volt (using either a battery or a transformer at 220 volts, alternating the current into 12 volt continuous current 3A /hour).

A standard 12 volts ignition coil is used to produce high voltage pulses.

The venom collection was conducted by Moshtohor device. The Moshtohor device was developed by the researcher by developed the plates that used inside the colony from wood material to plastic plates perforated and formatted by Laser and powered by solar cell instead of electric current **(Khattab, 1997).**

Fig. (II) Plastic plates.

Fig. (III) Solar cell power.

Fig. (IV) Collection device.

3.2 Preparing of the sample:

3.2.1 Lyophilized the sample:

Royal jelly and bee venom are lyophilized by a special device which invented by the researcher himself.

3.2.1.1 Lyophilization of bee venom:

1**.** Bee venom was dissolved in few amount of distilled water (1 g bee venom / 1 cm of distilled water).

2. Filtering bee venom by nomination paper (0.02) to get rid of impurities.

3. The samples were frozen at - 50°C in order to separate the freezing bee venom proteins from iced crystal.

4. After that, the sublimating of iced crystal (Sublimation process) by:

- Fixed the volume.
- Reduced the pressure to – 0.8 bar.
- Increased temperature degree to -10°C.

This step lasted 13 hours to get the moisture content less than 5%, so we get a pure lyophlized bee venom with moisture content less than 5% and with active enzymes **(Rapolu, 2012 and Sharoba *et al.*, 2014).**

Fig. (V) Collected venom.

Fig. (VI) Collected venom.

3.2.1.2 Lyophlization of royal jelly:

1**.** Royal jelly was dissolved in few amount of distilled water (1 g royal jelly / 1 cm of distilled water).

2. Filtering royal jelly by nomination paper (0.02) to get rid of impurities.

3. The samples were frozen at - 70°C in order to separate the freezing royal jelly proteins from iced crystal water.

4. After that, the sublimating of iced crystal (Sublimation process) by:

- Fixed the volume.
- Reduced the pressure to – 0.8 bar.
- Increased temperature to -10°C.

This step lasted 48 hours to get the moisture content less than 5%, so we get a pure lyophlized royal jelly with moisture content less than 5% and with active enzymes, according to **Rapolu(2012) and Sharoba *et al.* (2014).**

3.2.2 Specification for honey bee with added lyophilized bee venom and royal jelly:

Honey bee with added lyophilized bee venom and royal jelly are commercial products of honey containing (1mg lyophilized bee venom + 500 mg lyophilized royal jelly+ 25 ml honey).

Regulation (EC) No 258/97 of the European parliament and the council of 27th January 1997 concerning novel foods and novel food ingredients.

3.3 The patients:

Study question:

The addition of a combination of honeybee, royal jelly and bee venom has benefits in different diseases?

This study was carried out:

3.3.1 Patients of both sexes suffering from:

1- Diabetes mellitus (DM).

2- Rheumatoid arthritis (RA).

3- Anemia (megaloblastic anemia).

4- Breast cancer.

5- Chromic liver disease (CLD).

3.3.2 Infants:

For assessment of their growth and length.

3.4 Types of study:

This study is pre and post observational study for patients and comparative analytic study for infants.

* Sample size

The formula is:

n=Z$\times$ $Z[P(1-P)/D \times D]$

Sample size = n/$\left[(1+\left(\frac{n}{population}\right))\right]$

Population = size of population which the sample is represent.

P= true proportion of factor, in the population (guess) according to prevalence.

D= maximum difference between sample mean and population mean.

Z= area under the curve.

3.5 Analytical method:

3.5.1 Moisture, protein, lipid and total carbohydrate:

Moisture content was determined at 60°C, total protein (Nx 6.25) was determined using micro kjeldahl method for total nitrogen, ash was determined at 550°C, the total lipid content of all samples was determined by the standard Soxhlet extraction method using petroleum ether (B.P.40.60°C) for 16 hours and

pH value of device model MA 5736, Metrel, Iskra. Slovenia .According to **A.O.A.C. (2005).** Total carbohydrates were calculated by difference according to **Kerolles (1986).**

3.5.2 Water holding capacity (W.H.C):

Water holding capacity (W.H.C) was estimated according to **Alvarez *el al*., (1992).**

3.5.3 Minerals:

Minerals were determined by atomic absorption spectrophotometer

perkin – elmer (1976) in extracts of dry ashed samples at 550°C, with the phosphorus element was measured on the same dry ash extract by the colorimetric method **(Fiske and Subbarow, 1925).**

3.5.4 Determinations by HPLC:

Fructose, glucose, sucrose, 10-hydroxy-2deconoic acid, meltin, phospholipids A, hyaluronidase, vitamins and hormones were determined according to the method by HPLC analysis.

3.5.5 Determination of physical characteristics:

°Brix, specific gravity, isolectric point, total acid, viscosity, freezing point and color were determined according to the method of **Joslyn (1970).**

3.5.6 Sensory Evaluation:

Honey samples were evaluated by conventional profiling using 14-number sensory panel composed of faculty staff and post- graduate students with previous panel experience from the department of Food Science Fac. of Agric, Benha University. Six

sensory attributes were determined crystallization, flavor, aroma, color, fluidity and acceptability. A description of analysis method using a ten point scale 10=high desirable, 1= extremely poor, according to **Carlos *et al.* (2009).**

3.5.7 Microbiological analysis:

Standard plate count (SPC). One ml was shaken in 10 ml sterilized water duplicate plates for dilutions of 10 to 100 were prepared and pureed with plate count agar (P.C.A) , the technique in compendiums of method for the microbiological examination of foods **(A P H A ,1976)** was used with plates being incubated at 37°C for 48 h.

E.coli was enumerated using (Sorbitol MacConkey Agar). Dilutions of (1ml) were pouring in plate and incubated at 32°C for 24 hrs. according to **Yousef and Carlstrom(2003).**

One ml aliquots from dilutions promptly were poured into the Petri-dish of 10-15 ml of Rose Bengal Chloramphenicol Agar (Biolife, cod. No. 4019912 and chloramphenicol antimicrobic supplement cod. No. 421840003) and tempered to 44-46°C. Immediately mixed aliquots with an agar medium by tilting and rotating the Petri-dishes were incubated at 25°C for 3-5 days. Number of molds and yeast were computed per ml of samples and multiplied by the dilution factor **(American public health association, 1976).**

3.6 Biological evaluation experiments:

3.6.1 Experimental animals:

Fifty weanling albino rats, one hundred ninety six adult male and twenty five adult female albino rats (webester breed)

were obtained from the animal house of the Medical Research Center, Alexandria, was aged 2-3 month and weights ranged from220 - 280g. They were housed in polypropylene cages under controlled condition of temperature (24-26°C) and lighting (12hours light/12hours dark) for one week before the initiation of the experiment. During this period, the animals were fed individually adlib where they were allowed to free access of water and basal diet **(AOAC, 2005)**. The basal diet was comprised of casein (15%), wood cellulose (5%), vitamin mixture (1.0%), salt mixture (4.0%) and corn oil (5%). The basal diet was completed to 100g with corn starch. Food consumption and body weight were monitored daily and individually for each animal.

3.6.2 Salt and vitamin mixtures:

Salt mixtures (normal with iron and iron-free) and vitamin mixtures were prepared according to the formula mentioned in **(AOAC, 2005)**, as shown in the following (Table VI and VII). Ferrous sulphate was ignored from the formula to prepare the iron-free salt mixture.

Table (VI) Composition of normal salt mixture (with iron).

Ingredient	Quantity (gm)
Sodium chloride (Na Cl)	139.30
Potassium iodide (K I)	0.790
Potassiumdihydrogenphosphate (KH_2Po_4)	389
Magnesium sulphate (Mg So_4)	57.30
Calcium carbonate (Ca Co_3)	381.40
Ferrous sulphate (Fe So_4. H_2o)	27
Manganese sulptate (Mn So_4 .H_2o)	4.01
Zinc sulphate (Zn So_4. H_2o)	0.548
Cobalt chloride (Co C_{12}. $6H_2o$)	0.023
Cupper sulphate (Cu So_4 . 6 H_2o)	0.477

Table (VII) Composition of vitamin mixture.

Ingredient	Mg/100g
Vitamin A	2000 (IU)
Vitamin D	200 (IU)
Vitamin E	10.0 (IU)
Menadione	0.50
Chorine	200
P. Amino benzoic acid	10.0
Inositol	10.0
Niacin	4.00
Ca D- pantothenate	4.00
Riboflavin	0.80
Thiamin. HCl	0. 50
Pyridoxine. HCl	0.50
Folic acid	0.20
Biotin	0.04
Vitamin B12	0.003
Glucose. to make	1000

3.6.3 Method of procedure for biological experiment:

After a period of adaptation (one week) on basal diet, rats were divided into ten groups where every animal was placed in single cage and fed adlib**.**

3.6.4 Biological evaluation parameters:

Group 1: Determined LD_{50} according to **Son *et al.* (2007).**

Group 2: Determination of body weight gain (BWG) according to **Chapman, *et al.* (1959).**

BWG= (Final weight – Initial weight)/ Initial weight.

3.6.5 Biochemical blood analysis:

The adult male (171 rats) were randomly divided into eight groups. The third group received tap water serve as control. The fourth group received 0.5% hydrogen peroxide (H_2O_2) daily in drinking water for one month to be compared with the sample (**Abdul-Rahman, 1995)**. The fifth group received 0.5% (H_2O_2) daily in drinking water for one month concomitant with (1mg of lyophilized bee venom+500mg of lyophilized royal jelly +25mg honey) at 1g/kg body weight dissolved in distilled water and given daily for one month orally by gavages needle (**Mishima *et al* ., 2005)**. The sixth, seventh and eighth groups received (1mg of lyophilized bee venom +500mg of lyophilized royal jelly +25mg honey) at a dose 1g/kg body weight orally daily. At the end of experiment blood samples were collected into clean dry centrifuge tubes allowed to clot, serum separated after centrifugation at 1500 rpm for 15 minute for testosterone hormone assay, using Enzyme Linked Immune sorbent Assay (ELISA) (BioCheck Company, USA). Rats were sacrificed by ether administration. The abdominal cavity was then opened; the weight of testis, epididymal, seminal vesicles and prostate were

recorded. The test is placed in ice normal saline for glutathione estimation using Moron method as described by malondialdehyde (MDA) estimation using Gilbert method. The epididymis was dissected out, sectioned and immediately the content of the tail of each epididymis was squeezed gently in clean watch glass diluted 10 times with isotonic solution of sodium citrate 2.9% at 37° C, take one drop from isotonic solution on slide and added one drop of eosin - nigrosin stain and made smear, this technique was used for the percentage of live/dead and for morphological abnormal sperms to be counted (**Al-Sadi, 2001).** The content of the head of epididymis was squeezed immediately in clean watch glass contained 9.8 ml buffer formalin with 0.1 ml eosin 5%, this was used for counting the sperm concentration using hemocytometric technique (**Bearden *et al.*, 2004),** and comparing with the control group, (1mg of lyophilized bee venom +500mg of lyophilized royal jelly +25mg honey) in dose of 50 and 100 mg/kg body weight daily which obtained on it the level of creatinine in serum and urine, finally we obtained glucose level in serum and urine to rats which administrated by 50 and 100 mg/kg body weight of (1mg of lyophilized bee venom +500mg of lyophilized royal jelly +25mg honey).

3.6.6 Hematological studies:

Hematological examination included the determination of hemoglobin (Hb), hematocrit (Hct), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH) and mean corpuscular volume (MCV). Determinations of Hematological tests were performed at the

Biochemical Laboratory of Faculty of Veterinary Medicine, Cairo Universality according to the methods of **Jain, (2000)**.

Reagents:

Haymes and Turkeys solution was prepared according to **Jain, (2000)** for counting red blood cells. Drebkins solution according to **(Jain, 2000)** was used for colorimetric estimation of hemoglobin concentration using Diamond diagnostic kit supplied by Modern Laboratory, Egypt.

3.6.7 Determination of serum albumin:

Albumin was determined according to the method of **Doumas and Biggs (1971)** which have the principal of formation of an Albumin /bromcresol – green complex at pH 4.2 and photometric measurement of the absorbance at 630 nm.

Reagents:

1) Albumin Standard (4 g/dl); and **2)** Color reagent made of citrate buffer pH 4.2, (50mmol/L); bromcresol green, (0.12 mmol/L), a detergent and a preservative.

Procedure:

Three tubes were prepared for the determination of albumin as follows: **A)** Blank tube is containing 2.0 of working reagent. B) Standard tube containing 0.01 of standard solution (sol.) and 2.0 of working reagent; and **C)** Sample tube containing 0.01 of serum sample and 2.0 of working reagent. All tubes were mixed well, and then developed color was measured after 5 min. The absorbance of the sample (A^{sample}) and the standard ($A^{standard}$) against blank at 630nm (620- 640 nm). If albumin

concentration exceeded 6 g / dl the sample was diluted and the assay was repeated, then the dilution factor to be multiplied.

Calculation:

Albumin concentration (g / dl**) =** $(A^{sample} / A^{standard}) \times 4$

Where a = the absorbance at 630.nm.

3.6.8. Kidney Function Tests:

3.6.8.1. Determination of serum creatinine:

Creatinine was determined according to the procedure of **Bartles, *et al.* (1972).** The principle of the method is that creatinine in alkaline solution reacts with picric acid to form a colored complex. The amount of the complex formed is directly proportional to the creatinine concentration.

Reagents:

Four reagents:1) Standard reagent:,2mg/dL (177 µmol/L).

2) Picric acid, (25mmol / L); **3)** Sodium hydroxide reagent, (0.4mmol /L) and **4)** working reagent: mix equal volumes of reagents 2 and 3 before the assay. The reagents are stable up to the expiry date specified when stored at 5 – 25 ^{o}C.

Procedure:

Two test tubes: **a)** Standard tube containing 1.00 ml from working reagent and 0.1 ml standard and **b)** Sample tube containing 1.00 ml from reagent4 and 0.1 ml sample. Tubes were mixed and after 20 seconds at 20 – 25 ^{o}C until development of color. The absorbance A1 of the standard (A^{sample}) and sample was measured at 495 (490 – 510 nm) against distilled water, using cuvette of 1 cm light path. After exactly 2 min., the

absorbance A2 of standard (A ^{standard}) and sample (A ^{sample}) was measured. Linearity up to 10 mg / dl in serum or plasma and 500 mg / dl in diluted urine was undertaken.

Calculation:

$$A2 - A1 = \Delta A^{sample} \text{ or } \Delta A^{standard}$$

Creatinine in serum. (mg / dl) = $(\Delta A^{sample} / \Delta A^{standard})$ x 2

3.6.8.2. Determination of serum urea:

Urea was determined according to the procedure of **Fawcett and Soctt (1960)** which depends on the following reaction:

$$Urea + H_2O \xrightarrow{Uriease} 2NH_3 + CO_2$$

Reagents:

Four reagents: **1)** Urea standard, 50 mg /dL (8.3 mmol/L); **2)** Buffer – enzyme containing phosphate buffer and, (50 mmol/L) and urease (> 10000 u/L); **3)** Color reagent containing phenol (100 mmol / L) and sodium nitroprussid (0.2 mmol /L) and **4)** Aalkaline reagent containing sodium hydroxide, (150 mmol/L) and sodium hypochlorite (15 mmol /L). All the reagents are stable up to the expiry date specified on package when stored at 4-8 ^{o}C

Procedure:

Three test tubes: **a)** Blank tube containing 0.2 ml reagent 2; **b)** Standard tube containing 0.01 ml standard and 0.2 ml reagent 2 and **c)** Sample tube containing 0.01 samples and 0.2 reagents 2. Tubes were mixed and incubated for 5 min. at 37 ^{o}C·

Finally to all tubes, 1.00 ml was added of reagent 3 and 1.00 ml of reagent 4, mixed well, incubated for 10 min. 37 ^{o}C until color development. The absorbance of the sample (A^{sample}) and the standard ($A^{standard}$) against blank was measured at 550 nm. (530-570 nm) where color is stable for 5 hours.

Calculation:

Urea concentration = $(A^{sample} / A^{standard})$ x Standard con.

3.6.8.3. Determination of serum uric acid:

Uric acid was determined according to the procedure of **Barham and Trinder (1972)** which depend on the following reactions:

$$\text{Uric acid} + 2H_2O + O_2 \xrightarrow{Uriease} \text{Allantion} + CO_2 + H_2O_2$$

$$2H_2O_2 + \text{3,5,dichloro-2-hydroxybenzensulphonate} +$$

$$\text{4-Amino anatipyrine} \xrightarrow{\text{Peroxidase}} H_2O + HCL + \text{Colored quinoneimine.}$$

Reagents:

Four reagents: **1)** Uric acid standard reagent, 10mg/dL, (595µmol/L); **2)** Chromogen –buffer reagent containing tris buffer (50mmol/L), 3, 5, dichloro-2-hydroxybenzen sulphonate, (5.0mmol/L) and surface- active agent; **3)** Enzymes solution reagent: containing uricase, (>500lu /L); peroxidase, (>2000u /L), aminoantipyrine, (0.20 mmol/L). And stabilizer and a preservative. and **4)** Working reagent prepared by mixing equal volumes of reagent 2 and 3 immediately before the assay. Reagents are stable up to the expiry date specified on package when stored at 4 – 8 ^{o}C.

Procedure:

Three test tubes: **a)** Blank tube containing 1.00 ml of reagent 4; **b)** Standard tube containing 0.05 ml standard and 1.00 ml reagent 4 and **c)** Sample tube containing 0.05 ml sample and 1.00 ml of working reagent. Tubes were mixed well, incubated for 10 min. at 37 ^{o}C until color development. The absorbance of the sample (A^{sample}) and the standard ($A^{standard}$) against blank was measured spectrophotometeric-ally at 510 nm. (490-550 nm).

Calculation:

Uric acid = (A^{sample} / $A^{standard}$) x Standard concentration.

3.6.9. Pancreatic Functions:

3.6.9.1. Determination of serum glucose:

Blood glucose was determined according to the procedure of **Trinder (1969)** with the principle which depends on the following reaction:

$$\text{Glucose} \xrightarrow{\text{Glucose oxidase}} \text{Gluconic acid} + H_2O_2$$

$$2H_2O_2 + \text{Phenol} + \text{4-amino-antipyrine} \xrightarrow{\text{peroxidase}} \text{Quinoneimine} + 4H_2O$$

The produced quinoneimine was colorimetrically determined at a wavelength of 505nm. (490-530 nm.).

Reagents:

Three reagents: 1) standard reagent, (100 mg/dL 5.55 mmol/L); **2)** Buffer- Enzyme reagent containing phosphate buffer, (70 mmol/L); glucose oxidase,(>5000U/L); peroxidase, (>500U/L)

and 4-Amino anatipyrine, (0.5mmol/L) and **3)** Chromogen reagent containing phenol, (15 mmol/L).

The reagents are stable up to the expiry date specified when stored at 4-8 ^{o}C.

Procedure:

Three tests tubes **a)** Blank tube containing 1.00 ml enzyme reagent; **b)** Standard tube content 0.01ml standard glucose and 1.00 ml enzyme reagent and **c)** Sample tube containing 1.00 ml buffer enzyme reagent. Tubes were mixed well, incubated for 10 min at 37 ^{o}C and the absorbance of the developed color (A^{sample}) and ($A^{standard}$) was measured at 510nm. (490-530 nm.) Color intensity is stable for 30 min.

Calculation:

glucose concentration = (A^{sample} / $A^{standard}$) x Standard con.

3.7 Patient:

3.7.1Diabetes mellitus (DM):

According to the sample size and pervalence 60 patients will be included in the study and approved consent will be taken.

- All patients will be asked to take (two teaspoons equivalent to 20 g of honeybee with added bee venom and royal jelly per day) on an empty stomach for 3 months.

- Glaciated hemoglobin (HbA1c) and general condition will be compared pre and post study.

- Glaciated Hb% is accurate in determining blood glucose level in last 3 months.

3.7.2 Anemia (Megaloblastic anemia):

According to sample size and prevalence 30 patients will be included in the study

- All patients will be asked to take (two teaspoons equivalent to 20 g of honeybee with added bee venom and royal jelly per day) on an empty stomach for 3 months.

- The change of Hb% pre and post study will be represented as a percentage.

3.7.3 Rheumatoid arthritis (R.A):

According to sample size and prevalence 30 patients will be included in the study.

- All the patients will be asked to take (two teaspoons equivalent to 20 g of honey bee with added bee venom and royal jelly per day) on an empty stomach for 3 months.

Follow up will be done by:

a) Clinical assessment pre and post study:

1. Assessment of joints swelling and mobility.
2. Morning stillness.
3. Change in the doses of analysis.
4. Satisfaction of the patients.

b) Blood analysis pre and post study:

1. ESR.
2. Rheumatoid factor.

3.7.4 Breast cancer:

According to the sample size and prevalence 40 patients of cancer will be included:

- All patients will be asked to take (two teaspoons equivalent to 20 g of honeybee with added bee venom and royal jelly per day) on an empty stomach for 3 months.

- Assessment:

1. General condition pre and post study.

2. Tumor size pre and post study.

3. Hb% pre and post study.

4. Tumor markers pre and post study.

3.7.5 Chronic liver disease (CLD):

According to the sample size and prevalence 40 patients will be included in the study.

- All patients will be asked to take (two teaspoons equivalent to 20 g of honeybee with added bee venom and royal jelly per day) on an empty stomach for 3 months.

- Assessment pre and post study by:

a) Lower limb edema and ascots.

b) Jaundice.

c) Bleeding profile PT INR.

d) C.B.C.

e) Albumin liver enzymes.

f) PCR for Hev.

3.8 Infants (New born to first year):

A comparative analytic study will be done for 2 groups of infants (20 infants for each).

Group I:

Non breast feeding (formula feeding) artificial milk +300 mg lyophilized royal jelly

Group II:

Control, Breast feeding only.

3.9 Statistical analysis:

Data of thesis, except minerals and lipids analysis were analyzed by one and two-way of analysis of variance using Statistical Analysis System **"SAS" (1991).** Sensory evaluation by significant at 5% (Turkey test).

RESULTS AND DISCUSSION

IV. RESULTS AND DISCUSSION

4.1 Physicochemical characteristics, microbiological and sensory evaluation of bee honey sample:

"Honey is the natural sweet substance produced by honey bees from the nectar of blossoms or from the secretion of living parts of plants or excretions of plant sucking insects on the living parts of plants, which honey bees collect, transform and combine with specific substances of their own, store and leave in the honey comb to ripen and mature. This is the general definition of honey in **the Codex Alimentarius Comission (2001)**, in which all commercially required characteristics of the product are described.

4.1.1 Physical characteristics of honey bee samples:

Data in Table (1) demonstrated that, pH values, spesific gravity, °brix, isolectric point and total acids (as gluconic acid) were ranged from 3.89 – 4.23, 1.42 – 1.59, 78.99 – 80.17%, 264.20 – 445.93 and 4.20 – 4.40%, respectively. The obtanied data are in an agreement with those obtained by **Krell (1996)**, **Codex Alimentarius Comission (2001) and (Felsner *et al.*, 2004).**

Table (1) Physical characteristics of honey bee.

Parameters	Honey samples			
	Citrus	Clover	Cotton	Buckthorn
pH	3.89 ± 0.06	3.99 ± 0.06	3.93 ± 0.06	4.23 ± 0.06
Specific gravity	1.42 ± 0.06	1.59 ± 0.06	1.43 ± 0.06	1.42 ± 0.02
°Brix	80.17 ± 0.06	79.90 ± 0.06	79.93 ± 0.06	78.99 ± 0.06
Isolectric point (ms/cm)	272.10± 0.06	264.20± 0.06	289.90± 0.06	445.93± 0.04
Total acid (as gluconic acid)	4.20 ± 0.06	4.30 ± 0.06	4.30 ± 0.06	4.40 ± 0.06

Values is mean ± SE

4.1.2 Viscosity of honey samples:

Data in Table (2) indicated that, the viscosity at study temperature. Cotton honey exhibited the highest viscosity and Buckthorn honey, Clover honey was second in respect , both of them having lowest water content (Table 2), this result confirms earlier data concerning dependence of honey viscosity on water content **(Al-Malah *et al*., 2001)** and **Sopade *et al*. (2002).**

Table (2) Viscosity of honey bee (p_a.s).

Temperature (°C)	Honey samples			
	Citrus	Clover	Cotton	Buckthorn
20	7.79 ± 0.06	8.79 ± 0.06	9.15 ± 0.07	8.99 ± 0.06
30	2.42 ± 0.06	2.79 ± 0.06	2.93 ± 0.06	2.85 ± 0.06
40	0.92 ± 0.06	1.03 ± 0.01	1.07 ± 0.01	1.05 ± 0.01
50	0.39 ± 0.06	0.42 ± 0.06	0.46 ± 0.06	0.44 ± 0.06
60	0.18 ± 0.01	0.22 ± 0.06	0.25 ± 0.01	0.23 ± 0.01

Values is mean ± SE

4.1.3 Freezing point depression of bee honey samples:

Data in Table (3) indicated that, the freezing point depression of honey bee samples for honey solution (15%) was ranged between -1.53 to -1.22 ºC for Buckthorn honey and Cotton honey, respectively. The same trend was observed for honey solution (25%) was ranged between -2.77 to -2.27 ºC for Buckthorn honey and Cotton honey, respectively. Data in the same table observed that, the freezing point depression of honey bee samples for honey solution (40%) was ranged between -4.70 to -4.42 ºC for Buckthorn honey and Cotton honey, respectively. While the freezing point depression of honey bee samples for honey solution (68%) was ranged between -5.82 to -5.43 ºC for Buckthorn honey and Clover honey respectively. The obtanied data are in an agreement with those obtained by **Krell (1996), Codex Alimentarius Comission (2001) and National Agricultural Statistics Service (2011).**

Table (3) Freezing point depression of honey bee in different solution concitrates.

Honey concentration	Honey samples			
	Citrus	Clover	Cotton	Buckthorn
15%	-1.42 °C	-1.31 °C	-1.22 °C	-1.53 °C
25%	-2.73 °C	-2.61 °C	-2.27 °C	-2.77 °C
40%	-4.65 °C	-4.52 °C	-4.42 °C	-4.70 °C
68%	-5.78 °C	-5.43 °C	-5.61 °C	-5.82 °C

Values is mean ± SE

4.1.4 Color, Pfund and density of honey bee samples:

Data in Table (4) indicated that, the color of honey samples was white for Citrus honey, extra light amber for Clover honey, light amber for Cotton honey and amber for Buckthorn honey. Pfund was ranged between 25- 96 for Buckthorn and Citrus honey, respectively. Density ranged between 0.38 – 3.01 for Citrus honey and Buckthorn honey respectively. The obtanied data are in an agreement with those obtained by **Krell (1996)**, **Codex Alimentarius Comission (2001)**, **(Bogdanov *et al.*, 2008) and National Agricultural Statistics Service (2011).**

Table (4) Color, Pfund and density of honey samples.

Parameters	**Honey samples**			
	Citrus	**Clover**	**Cotton**	**Buckthorn**
Color	white	Extra light amber	Light amber	amber
Pfund	25.0 ± 0.58	41.0 ± 0.58	76.0 ± 0.58	96.0 ± 0.58
Density	0.38 ± 0.0	0.60 ± 0.0	1.39 ± 0.01	3.01 ± 0.0

Pfund.mn by USA department of agriculture (FAO).

Values is mean ± SE

4.1.5 The moisture percentage in honey samples compared with air ratio humidity percentage:

Table (5) Moisture content in honey samples at different relative humidity percentage.

Air (RH%)	Honey samples			
	Citrus	**Clover**	**Cotton**	**Buckthorn**
50	16.30 ± 0.06	16.10 ± 0.06	15.90 ± 0.06	15.80 ± 0.06
55	17.40 ± 0.06	17.20 ± 0.06	16.80 ± 0.06	16.70 ± 0.06
60	18.50 ± 0.06	18.70 ± 0.06	18.30 ± 0.06	18.10 ± 0.06
65	19.90 ± 0.06	20.0 ± 0.06	23.90 ± 3.05	20.70 ± 0.06
70	23.10 ± 0.06	23.22 ± 0.01	24.20 ± 0.06	24.10 ± 0.06
75	26.80 ± 0.06	27.11 ± 0.01	28.30 ± 0.06	28.20 ± 0.06
80	31.90 ± 0.06	32.20 ± 0.06	33.10 ± 0.06	32.0 ± 1.05

RH%: relative humidity. *Values is mean ± SE*

Data in Table (5) observed that, the moisture content in honey samples at ralative humidity (50%) ranged from 15.80 - 16.30 for Buckthorn and Citrus honey, respectively; for (55%) was ranged from 16.70 - 17.40 for Buckthorn and Citrus honey, respectively; for (60%) was ranged from 18.10 - 18.70 for Buckthorn and Clover honey, respectively; for (65%) was ranged from 19.90 - 23.90 for Citrus and Cotton honey, respectively; for (70%) was ranged from 23.10 - 24.20 for Citrus and Cotton honey, respectively; for (75%) was ranged from 26.80 - 28.30 for Citrus and Cotton honey, respectively and for (80%) was ranged from 31.90 - 33.10 for Citrus and Cotton honey

respectively, according to **Krell (1996)** and **Codex Alimentarius Comission (2001)**.

4.1.6 Moisture content, water activity and water holding capacity of honey bee samples:

Data in Table (6) indicated that, moisture content of honey samples was ranged between 17.12-19.16% for Cotton and Citrus honey, respectively.The same trend was observed for water activity it samples was ranged between 0.54- 0.61% for Buckthorn and Citrus honey, respectively. On the other hand, the water holding capacity was ranged between 16.56 - 18.55% for Cotton and Citrus honey, respectively. The obtained data are in an agreement with those obtained by **Krell (1996) and Bogdanov *et al.* (2008).**

Table (6) Moisture content, water activity and water holding capacity of honey samples.

Parameters	Honey samples			
	Citrus	**Clover**	**Cotton**	**Buckthorn**
Moisture %	19.16 ± 0.0	18.24 ± 0.0	17.12 ± 0.0	17.32 ± 0.0
Water activity %	0.61 ± 0.01	0.56 ± 0.01	0.56 ± 0.01	0.54 ± 0.01
Water holding capacity %	18.55 ± 0.01	17.68 ± 0.0	16.56 ± 0.0	16.78 ± 0.0

Values is mean ± SE

4.1.7 Chemical characteristic of honey samples:

Data in Table (7) indicated that, the glucose content of honey samples was ranged between 29.67-31.99% for Clover and Cotton honey, respectively. The same trend was observed for fructose content of honey bee samples which was ranged

between 34.12-35.12% for Clover and Cotton honey, respectively. On the other hand, the sucrose content of honey samples was ranged between 7.81-9.81% for Buckthorn and Citrus honey, respectively. Total sugars content of honey bee samples was ranged between 78.39 – 80.72% for Citrus and Clover honey, respectively. Also, ash content of honey samples was ranged between 0.19- 0.22% for Cotton and Clover honey, respectively. Protein content of honey bee samples was ranged between 0.23-0.27% for Citrus and Clover honey, respectively. The obtained data are in an agreement with those obtained by **Krell (1996), Bogdanov *et al.* (2008) and (http://www.bee-hexagon.net (2012)).**

Table (7) Chemical characteristics of honey samples (g/100g).

Component	Honey samples			
	Citrus	Clover	Cotton	Buckthorn
Glucose (%)	31.06 ± 0.01	29.67 ± 0.01	31.99 ± 0.01	29.82 ± 0.01
Fructose (%)	34.48 ± 0.01	34.12 ± 0.01	35.12 ± 0.01	34.92 ± 0.01
Sucrose (%)	9.81 ± 0.01	8.72 ± 0.01	8.34 ± 0.01	7.81 ± 0.01
Total sugars (%)	75.35 ± 0.01	72.51 ± 0.01	75.45 ± 0.01	72.55 ± 0.01
Ash (%)	0.21 ± 0.01	0.22 ± 0.01	0.19 ± 0.01	0.20 ± 0.01
Protein (%)	0.23 ± 0.0	0.27 ± 0.0	0.24 ± 0.0	0.24 ± 0.0

Values is mean ± SE

4.1.8 Minerals content in honey samples:

Data in Table (8) indicated that, Calcium content of honey samples was ranged between 16.99 - 17.50 (mg/100g) for Clover and Citrus honey, respectively. The same trend was observed with Chlorine content of honey samples was ranged between

11.50 -12.10(mg/100g) for Cotton and Clover honey, respectively. On the other hand Copper content of honey samples was ranged between 0.06 - 0.07 (mg/100g) for Clover honey and Citrus, Cotton and Buckthorn honey, respectively. Iron content of honey samples was ranged from 1.98- 2.20(mg/100g) for Clover and Cotton honey, respectively. Magnesium content of honey samples was ranged between 0.69 - 0.74 (mg/100g) for Clover and Cotton honey, respectively. Phosphorous was ranged between 3.90- 4.50(mg/100g) for Clover and Cotton honey, respectively. Potassium was ranged between 12.10 – 12.50 (mg/100g) for Citrus and Clover honey, respectively. Sodium was ranged between 7.90 – 8.40 (mg/100g) for Clover and Buckthorn honey, respectively.

Table (8) Minerals content in honey samples (mg/100g).

Mineral	Honey samples			
	Citrus	Clover	Cotton	Buckthorn
Calcium	17.50 ± 0.06	16.99 ± 0.01	17.20 ± 0.06	17.30 ± 0.06
Chlorine	11.70 ± 0.06	12.10 ± 0.06	11.50 ± 0.06	11.60 ± 0.06
Copper	0.07 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.07 ± 0.01
Iron	2.10 ± 0.06	1.98 ± 0.01	2.20 ± 0.06	2.10 ± 0.06
Magnesium	0.71 ± 0.01	0.69 ± 0.01	0.74 ± 0.01	0.72 ± 0.01
Phosphorous	4.30 ± 0.06	3.90 ± 0.06	4.50 ± 0.06	4.20 ± 0.06
Potassium	12.10 ± 0.06	12.50 ± 0.06	12.30 ± 0.06	12.20 ± 0.06
Sodium	8.30 ± 0.06	7.90 ± 0.06	8.10 ± 0.06	8.40 ± 0.06
Zinc	0.20 ± 0.01	0.30 ± 0.01	0.25 ± 0.01	0.23 ± 0.01

Values is mean ± SE

Zinc was ranged between 0.20 – 0.30 (mg/100g) for Citrus and Clover honey, respectively. The obtained data are in an agreement with those obtained by **Bengsch (1992), Krell (1996) and Bogdanov *et al.* (2008).**

4.1.9 Vitamin contents in honey samples:

Data in Table (9) indicated that, (B_1) content of honey samples was ranged between 0.004- 0.006(mg/100g) for Citrus, buckthorn and Cotton honey, respectively. The same trend was observed with (B_2) content of honey bee samples which was ranged between 0.002 - 0.005(mg/100g) for Citrus and Cotton honey, respectively.

Table (9) Vitamin contents in honey samples (mg/100g).

Vitamin	Honey samples			
	Citrus	Clover	Cotton	Buckthorn
Thiamin (B1)	0.004± 0.001	0.005± 0.001	0.006± 0.001	0.004 ± 0.001
Riboflavin (B2)	0.002± 0.001	0.003± 0.001	0.005± 0.001	0.002 ± 0.001
Pyridoxine (B6)	0.009± 0.001	0.021± 0.001	0.011± 0.001	0.008 ± 0.001
Pantothenic acid	0.020± 0.006	0.070± 0.006	0.060± 0.006	0.020 ± 0.006
Scorbic acid (C)	2.200± 0.058	2.300± 0.058	2.40 ± 0.058	2.20 ± 0.058

Values is mean ± SE

Pyridoxine content of honey samples was ranged from 0.008- 0.021(mg/100g) for Buckthorn and Clover honey, respectively. Pantothenic acid was ranged between 0.020 - 0.070(mg/100g) for Buckthorn and Clover honey, respectively. Scorbic acid was ranged between 2.20 - 2.40(mg/100g) for

Citrus, buckthorn honey and Cotton honey respectively. The obtained data are in an agreement with those obtained by **Bengsch (1992), Krell (1996) and Bogdanov *et al.* (2008).**

4.1.10 Microbial of honey samples:

Data in Table (10) showed that, the microbial content of honey samples for (THB) was ranged between $1.5x10^3$ - $1.6x10^6$ for Citrus and Cotton honey, respectively. Total coliform count was ranged between $2.2x10^2$ - $1.1x10^3$ for Cotton and Clover honey, respectively. While, (THF) was ranged between 10.0 – 45.0 for Clover and Buckthorn honey, respectively. These results according to **Alwaili (2004), Mundo *et al.* (2004) and Osho and Bello (2010).**

Table (10) Microbial counts of honey samples.

Samples	THB (cfu/ml)	TCC (Cell/100ml)	THF (cfu/ml)
Citrus honey	$1.5x10^3$	$2.4x10^2$	40.0
Clover honey	$5.5x10^5$	$1.1x10^3$	10.0
Cotton honey	$1.6x10^6$	$2.2x10^2$	15.0
Buckthorn honey	$2.0x10^5$	$2.1x10^2$	45.0

THB: Total hetrotrophic bacteria. THF: Total hetrotrophic fungi.

TCC: Total coliform count. Cfu: Colony forming unit.

Values is mean ± SE

4.1.11 Sensory evaluation of honey samples:

Data in Table (11) indicated that, values of sensory evaluation found in honey samples for crystallization was ranged between 0.85 – 1.27 for Citrus and Cotton honey, respectively.

Flavor was ranged between 5.62 – 6.27 for Buckthorn and Citrus honey, respectively. Aroma was ranged between 5.65 – 6.92 for Buckthorn and Clover honey,respectively. Color was ranged between2.35 – 4.00 for Clover and Buckthorn honey, respectively. Fluidity was ranged between 3.19 – 7.46 for Citrus and Clover honey, respectively. Acceptability was ranged between 5.08 – 6.19 for Clover and Citrus honey, respectively. The obtained data are in an agreement with those obtained by **Carlos *et al.* (2009).**

Table (11) Sensory evaluation of honey samples.

Honey samples	Parameters					
	Crystallization	**Flavor**	**Aroma**	**Color**	**Fluidity**	**Acceptability**
Citrus honey	0.85a	6.27a	5.81ab	3.58ab	3.19bc	6.19a
Clover honey	1.23a	6.12a	6.92a	2.35c	7.46a	5.08a
Cotton honey	1.27a	6.08a	6.62ab	3.69ab	7.12a	5.85a
Buckthorn honey	1.08a	5.62a	5.65ab	4.00a	6.85a	5.69a

Means followed by same letter in the same column do not differ significant at 5% significance (Tukey test).

Values is mean ± SE

4.2 Effect of lyophilization on chemical composition of royal jelly:

Royal jelly is a bee product from the hypopharyngeal, mandibular and postcerebral gland of young worker bees. It is produced under partial digestion of essentially pollen and nectar. Royal jelly is a viscous jelly substance. It is partially soluble in water with a density of 1.1 g/ mL. Its color is whitish to yellow, the yellow color increasing upon storage. Its odor is sour and pungent, the taste being sour and sweet. The sensory characteristics are important quality criteria. Old royal jelly, which has not been properly stored tends to be darker and rancid taste can develop. For optimum quality it should be stored in frozen state. The viscosity varies according to water content and age. It is said that the ancient Egyptians knew royal jelly and believed royal jelly will keep the pharao's body young and beautiful even after he passes a way, using it also to prepare the mummy and that Cleopatra has used it for her cosmetics in order to keep herself beautiful.

4.2.1 Effect of lyophlization on royal jelly:

Data in Table (12) indicated that, the reduction in moisture content of royal jelly samples was ranged between 92 – 95% after lyophilized. This was in accordance with previous scientific researches **(Messia *et al*. 2005) and Bogdanov (2011a)**.

The proportion of the proteins in royal jelly was large. The average value of total protein was increased from 8.62 – 9.14 % to 25.90 – 27.44% after lyophlization. The obtanied data

are in an agrement with those obtained by **Simúth (2001) and Kim *et al*. (2010).**

The same trend was observed with royal jelly total lipids. The total lipids were increase from 5.19 – 5.72 % to 8.40 – 9.10 % after lyophlization. This is in accordance with **Kodai *et al.*(2007) and Sabatini *et al*. (2009).**

The concentration of the most abundant sugars fructose was ranged between 7.92 – 8.11% and after lyophlization ranged between 5.11 – 16.39%, glucose was 5.53 – 5.81% after lyophlization ranged from 10.21 – 10.98% and sucrose ranged from 0.52 – 0.61% and after lyophlization ranged between 1.20 – 1.32% . These values are comparable with the sugar levels reported in the literature **(Simúth, 2001) and (Sabatini *et al*., 2009).**

Simúth (2001) found that, the average level of glucose in lyophilized royal jelly (10.21 – 10.98%) was higher than that of fructose (5.11 – 16.39%). **Sabatini *et al.* (2009)** reported that fructose was prevalent to glucose which was also confirmed in our study. Sucrose is always present but in highly variable concentrations. Thus the amount of analyzed royal jelly samples was insufficient to draw conclusions about the prevalence of glucose or fructose.

Royal jelly was highly acidic with pH 3.40 – 3.90 and slightly decreased for 3.29 – 3.81 after lyophlization. The results were in accordance with **Lercker (2003) and Scarselli *et al.* (2005)**.

Most of the organic acids are free with rather unusual structure rarely encountered in nature, mono- and dihydroxy

acids and dicarboxylic acids with 8 and 10 carbon atoms (**Lercker *et al.*, 1993)**, the main acid being 10-hydroxy-2decenoic acid, which was antibacterial and immune activating (**Bachanova *et al.*, 2002)**, immune-modulating, anti-cancer (**Dzopalic *et al.*, 2011)**, anti-diabetes (**Okuda *et al.*,1998)**, collagen promoting and skin protecting , anti-ulcer (**Fang,1994)**, facilitates differentiation of brain cells (**Hattori *et al.*, 2007a)** antidepressant in mice experiments (**Ito *et al.*, 2012)**, promotes endothelial health, antihypertensive, antihyperlipoidemia (**Izuta H *et al.*, 2009)** estrogenic(**Matsui *et al.*, 2006)** anti-rheumatic and activation of TRPA1 and TRPV1 (induces thermogenesis and energy expenditure enhancement) (**Terada *et al.*, 2011).** HAD was ranged between 2.71 – 2.91% and after lyophlization it became 8.13 – 8.70% according to **(Bogdanov (2011a)).**

Table (12) Chemical composition of fresh and lyophilized royal jelly produced at different months (g/100g).

Parameters	Royal jelly produced at different months							
	(February)		(April)		(June)		(September)	
	A	B	A	B	A	B	A	B
Moisture (%)	66.28 ± 0.01	5.27 ± 0.01	64.17 ± 0.01	3.41 ± 1.66	67.21 ± 0.01	5.11 ± 0.01	68.15 ± 0.01	5.62 ± 0.01
pH	3.87 ± 0.01	3.62 ± 0.01	3.90 ± 0.06	3.81 ± 0.01	3.60 ± 0.06	3.50 ± 0.06	3.40 ± 0.06	3.29 ± 0.01
Protein (%)	9.14 ± 0.03	27.44 ± 0.01	8.92 ± 0.01	26.41 ± 0.01	8.62 ± 0.01	25.90 ± 0.06	8.91 ± 0.01	26.91 ± 0.01
Fructose (%)	8.11 ± 0.01	16.39 ± 0.01	7.92 ± 0.01	15.11 ± 0.01	7.95 ± 0.01	16.12 ± 0.01	7.99 ± 0.01	16.11 ± 0.01
Glucose (%)	5.53 ± 0.01	10.21 ± 0.01	5.71 ± 0.01	10.61 ± 0.01	5.62 ± 0.01	10.41 ± 0.01	5.81 ± 0.01	10.98 ± 0.01
Sucrose (%)	0.55 ± 0.01	1.21 ± 0.01	0.52 ± 0.01	1.20 ± 0.01	0.61 ± 0.01	1.32 ± 0.01	0.58 ± 0.01	1.28 ± 0.01
Total sugar (%)	14.19±0.01	27.81±0.01	14.15±0.01	26.92±0.01	14.18±0.01	27.85±0.01	14.38±0.01	28.37±0.01
Ash (%)	0.71± 0.01	2.51± 0.01	0.62± 0.01	2.12± 0.01	0.69± 0.01	2.41± 0.01	0.65± 0.01	2.45± 0.01
10-hydroxy-2deconoic acid (%)	2.71± 0.01	8.13± 0.01	2.86± 0.06	8.38± 0.01	2.91± 0.06	8.70± 0.06	2.82± 0.06	8.56± 0.01
Lipids (%)	**5.72 ± 0.01**	9.10 ± 0.06	5.59 ± 0.04	8.92 ± 0.01	5.32 ± 0.01	8.40 ± 0.06	5.19 ± 0.01	8.60 ± 0.06

Values is mean ± SE

A= fresh sample B= Lyophilized sample

4.2.2 Minerals content of royal jelly:

These data are in Table (13) showed that, Potassium(K) content of royal jelly samples was ranged between 600 – 670 (mg/100g) in February and June, respectively. Calcium (Ca) was ranged between 57-60 (mg/100g) in June and February, respectively. Magnesium (Mg) was ranged between 54-59 (mg/100g) in June and February, respectively. Zink (Zn) was ranged from 0.70- 0.90 (mg/100g) in June and February, respectively. Iron (Fe) was ranged between 5.82- 6.0 (mg/100g) in September and February, respectively. Copper (Cu) was ranged between 0.25-0.3 (mg/100g) in September and February, respectively. These results are in agreement with those obtained by **Schmidt *et al*. (1992), Stocker *et al*. (2005) and Bogdanov (2011a).**

Table (13) Minerals content of royal jelly produced at different months (mg/100g).

Mineral	Royal jelly produced at different months			
	(February)	(April)	(June)	(September)
Potassium (K)	600.0 ± 5.77	650.0 ± 5.77	670.0 ± 5.77	630.0 ± 5.77
Calcium (Ca)	60.0 ± 5.77	58.0 ± 3.51	57.0 ± 5.77	58.0 ± 5.77
Magnesium (Mg)	59.0 ± 5.77	57.0 ± 5.77	54.0 ± 5.77	56.0 ± 5.77
Zinc (Zn)	0.90 ± 0.06	0.80 ± 0.06	0.70 ± 0.06	0.80 ± 0.06
Iron (Fe)	6.0 ± 0.06	5.91 ± 0.01	5.95 ± 0.01	5.82 ± 0.01
Copper (Cu)	0.30 ± 0.06	0.29 ± 0.01	0.25 ± 0.01	0.28 ± 0.01

Values is mean ± SE

4.2.3 Vitamin content of royal jelly:

Data in Table (14) showed that, niacin (B_3) content of royal jelly samples was ranged between 10.90-12.20(mg/100g) in June and February, respectively. Pyridoxine (B_6) was ranged between 2.90-3.30 (mg/100g) in June and April, respectively. Thiamin (B_1) was ranged from 1.20 - 01.50(mg/100g) in September and June, respectively. Riboflavin (B_2) was ranged between 1.50- 1.80 (mg/100g) in February and June, respectively. Pantothenic acid was ranged from 3.10- 3.20 (mg/100g) in September and February, June respectively. These results are in agreement with those obtained by **Schmidt *et al*. (1992), Stocker *et al*. (2005) and Bogdanov (2011a).**

Table (14) Vitamin content of royal jelly produced at different months (mg/100g).

Vitamins	Royal jelly produced at different months			
	(February)	(April)	(June)	(September)
Niacin (B3)	12.20 ± 0.06	11.50 ± 0.06	10.90 ± 0.06	11.20 ± 0.06
Pyridoxine (B6)	3.10 ± 0.06	3.30 ± 0.06	2.90 ± 0.06	3.20 ± 0.06
Thiamin (B1)	1.30 ± 0.06	1.40 ± 0.06	1.50 ± 0.06	1.20 ± 0.06
Riboflavin (B2)	1.50 ± 0.06	1.60 ± 0.06	1.80 ± 0.06	1.70 ± 0.06
Pantothenic acid	3.20 ± 0.06	3.10 ± 0.06	3.20 ± 0.06	3.10 ± 0.06

Values is mean ± SE

4.2.4 Hormones content of royal jelly:

Table (15) Hormones content in fresh royal jelly produced at different months (moles/100g).

Hormones	Royal jelly produced at different months			
	(February)	(April)	(June)	(September)
Testosterone	0.20 ± 0.01	0.18 ± 0.01	0.19 ± 0.01	0.20 ± 0.01
Progesterone	4.61 ± 0.01	4.42 ± 0.01	4.52 ± 0.01	4.49 ± 0.01
Prolactine	70.80 ± 0.06	70.10 ± 0.06	69.91 ±0.01	69.96 ± 0.01
Estradiol	52.03 ± 0.04	51.56 ± 0.01	51.72 ± 0.01	51.92 ± 0.01

Values is mean ± SE

Data in Table (15) showed that, testosterone hormone content of royal jelly samples was ranged between 0.18 - 0.20(moles/100g) in April and both February and September respectively. Progesterone hormone was ranged between 4.42-4.61 (moles/100g) in April and February, respectively. Prolactine hormone was ranged from 69.91-70.80 (moles/100g) in June and February, respectively. Estradiol hormone was ranged between 51.56- 52.03(moles/100g) in April and February, respectively. These obtained data are in agreement with this obtained by**Vittek and Slomiany (1984), Vittek (1995) and Bogdanov (2011a).**

4.3 Chemical composition, physiological composition, lethal dose (LD_{50}) and lyophilized bee venom:

Bee venom is a complex mixture of proteins, peptides and low molecular components. Nowdays its components have been characterized. The main components are proteins and peptides.

The composition of fresh and lyophlized BV differs mainly in regards to the volatile components.

4.3.1 Production of bee venom during one year from one colony and its moisture content of fresh and lyophilized samples:

Data in Table (16) indicated that, the production of bee venom for one year from one colony was ranged between 210 mg/day in January and 330 mg/day in April, May, June, July and August, respectively. Moisture content in fresh bee venom sample was ranged between 90% in January and February and 84% in August and September, respectively. While it's content of moisture as a percent after lyophilization was ranged between 8.0% I n January and February and 5.0% in August and September.These results according to **Bogdanov (2011b).**

Table (16) Production of bee venom for one year from one colony and its moisture content of fresh and after lyophilized samples (mg)/ day.

Months	Weight(mg)/ day	Moisture%	
		A	B
January	210.0 ± 5.77	90.0 ± 0.58	8.0 ± 0.06
February	260.0 ± 5.77	90.0 ± 0.58	8.0 ± 0.06
March	300.0 ± 5.77	88.0 ± 0.58	7.0 ± 0.06
April	330.0 ± 5.77	86.0 ± 0.58	6.0 ± 0.06
May	330.0 ± 5.77	86.0 ± 0.58	6.0 ± 0.06
June	330.0 ± 5.77	86.0 ± 0.58	6.0 ± 0.06
July	330.0 ± 5.77	86.0 ± 0.58	6.0 ± 0.06
August	330.0 ± 5.77	84.0 ± 0.58	5.0 ± 0.06
September	310.0 ± 5.77	84.0 ± 0.58	5.0 ± 0.06

Table (16) continuous

October	300.0 ± 5.77	86.0 ± 0.58	5.50 ± 0.06
November	270.0 ± 5.77	87.0 ± 0.58	6.20 ± 0.06
December	240.0 ± 5.77	87.0 ± 0.58	6.20 ± 0.06

A: Fresh bee venom. **B:** Lyophlized bee venom. *Values is mean ± SE*

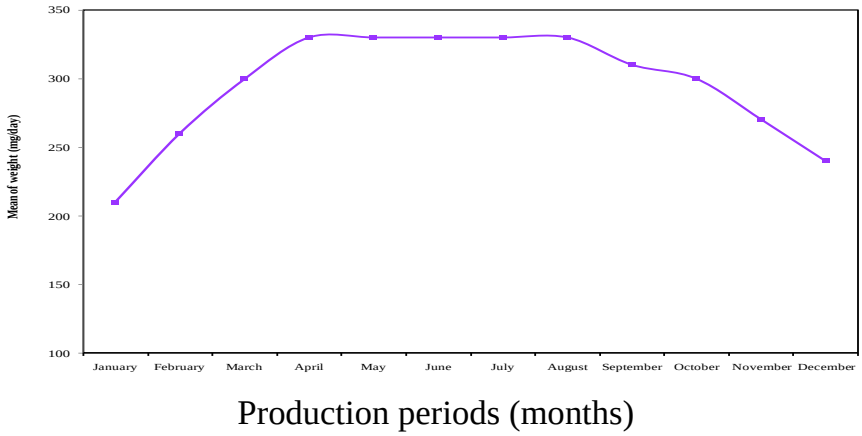

Fig. (1): Mean of production of bee venom from one colony for one year mg/ day.

4.3.2 Chemical composition of fresh and lyophlized bee venom:

Bee venom is a transparent liquid dries up easily even at room temperature, odorless, ornamental pungent smell, a bitter taste, hydrolytic blend of proteins with basic pH (4.5 – 5.5) that is used by bees for defense (**Schmidt and Buchmann, 1999).**. Bee venom is soluble in water and insoluble in alcohol and

ammonium sulfate. When it comes in contact with air it forms grayish-white crystals. Lyophlized venom takes on a light yellow color and some commercial preparations are brown, thought to be due to oxidation of some of the venom proteins. Bee venom contains a number of very volatile compounds which are easily lost during collection, it is considered a rich source of enzymes, peptides and biogenic amines, and it is specific weight (1.1331).The venoms of most stinging insects including honey bees consisted of enzymes, protein, peptides, and a verity of smaller molecules.The pharmacological and biochemical activities of the various stinging insect venoms remarkably convergent. Most venom induces immediate pain. Data in Table (17), show the phospholipids was ranged from 1.20 – 1.50% and 9.60 – 11.20% for fresh and lyophilized samples while, hyaluronidase was ranged from 0.28 – 0.32% and 2.0 – 2.20% for fresh and lyophilized samples and is capable of destroying red blood cells. Most hymenopterous venoms also contain low molecular weight peptides (**Norman *et al.*, (2011)).** On the other hand, lyophilized bee venom content of melitine was ranged between 52.0 – 56.0%, these obtained data are in agreement with those obtained by **Bogdanov (2011b).**

Table (17) Chemical composition of fresh and lyophlized bee venom (g/100g).

Parameters	Bee venom produced at different months							
	(January)		(April)		(October)		(December)	
	A	B	A	B	A	B	A	B
Moisture%	90.0 ± 0.58	8.0 ± 0.06	86.0 ± 0.58	6.0 ± 0.06	86.0 ± 0.58	6.0 ± 0.06	87.0 ± 0.58	6.20 ± 0.06
Melitine %	6.20 ± 0.06	52.0 ± 0.58	7.50 ± 0.06	55.0 ± 0.58	7.50 ± 0.06	55.0 ± 0.58	7.60 ± 0.06	56.0 ± 0.58
Phospholipids A %	1.20 ± 0.06	9.60 ± 0.06	1.50 ± 0.06	10.98 ± 0.01	1.50 ± 0.06	11.20 ± 0.06	1.50 ± 0.06	11.10 ± 0.06
Hyaluronidase %	0.28 ± 0.01	2.0 ± 0.06	0.31 ± 0.01	2.10 ± 0.06	0.32 ± 0.01	2.20 ± 0.06	0.30 ± 0.01	2.10 ± 0.06
Ash %	0.06 ± 0.01	0.46 ± 0.01	0.07 ± 0.01	0.50 ± 0.01	0.07 ± 0.01	0.51 ± 0.01	0.07 ± 0.01	0.51 ± 0.01
Total sugar %	0.31 ± 0.01	1.90 ± 0.06	0.36 ± 0.01	2.10 ± 0.06	0.34 ± 0.01	2.0 ± 0.06	0.34 ± 0.01	2.0 ± 0.06

A: Fresh bee venom. **B:** Lyophlized bee venom.

Values is mean ± SE

4.3.3 The physiological component of lyophlized bee venom:

Data in Table (18) showed that, the physiological component of lyophilized bee venom samples for histamine was ranged between 0.75 – 0.80% in April and January, respectively. Dopamine was ranged between 0.20 – 0.25% in January and December, respectively. Noradrenalin was ranged between 0.08 – 0.10 % in April, and January, respectively. These obtained data are in agreement with those obtained by **Urtubey (2005), Bogdanov (2011b) and** (**Norman *et al.*, (2011)).**

Table (18) Physiological component of lyophlized bee venom.

Parameters	Lyophlized bee venom at different months			
	(January)	(April)	(October)	(December)
Histamine%	0.80 ± 0.01	0.75 ± 0.01	0.77 ± 0.01	0.78 ± 0.01
Dopamine%	0.20 ± 0.01	0.22 ± 0.01	0.24 ± 0.01	0.25 ± 0.01
Noradrenalin	0.10 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.09 ± 0.01

Values is mean ± SE

4.3.4 Lethal dose (LD_{50})of the bee venom after subcutaneous injection in adult and weanling male and female rats:

Data in Table (19) showed that, LD_{50} for male and female adult rats was 2.8 and 1.5, respectively. While for male and female rats weanling rats was 0.9 and 0.7, respectively. This obtained data are in agreement with those obtained by **Hellner *et al.* (2006) and Son *et al.* (2007).**

Table (19) Lethal dose (LD_{50}) of the bee venom after subcutaneous injection in adult and weanling male and female rats.

parameters	Adult rats		Weanling rats	
	Male	Female	Male	Female
Body weight ±SE (g)	386.0±20.7	243.0±19.7	44.0±4.3	40.5±4.1
Dose interval (mg/kg)	1 – 6	1 – 6	1 – 6	1 – 6
LD_{50} (mg/kg)	2.8±0.6	1.5±0.6	0.9±0.4	0.7±0.3

Values is mean ± SE

4.4 Effect of feeding with lyophilized royal jelly on infants:

The positive effects of royal jelly in infant diet are attributed to effects on the digestion process. When fed on a mixture of (royal jelly and milk) infants showed a regularly steady weight and length gain**.**

4.4.1 Effect of non breast feeding on infants (formula feeding) with artificial milk +300 mg lyophilized royal jelly on lengths and weight:

Data in Table (20 and 21) showed that, the weight of infants who had formula feeding was increased from 2642.50 g at the new born weight to 8503.50 g at 12 months and their length was increased from 52.75cm at the new born to 79.0cm at the 12 months beside that, these infants were preterm and was born at 7 months compared with control group infants who had breast feeding only and born at 9 months , their weight was 2982.50g at new born which was increased to 9087.50g at the 12 months and their length was 53.25 cm at new born which increased to 79.84 cm at 12 months. From Table (20and21)we can recognized that the weights and lengths at the end of one year (12 months) of infants who had formula feeding compared with control group infants who had breast feeding only was very close beside that the infants who had formula feeding did not have any antibiotics all the 12 months. The details as follows: at 2months the weight was 4432.50g and length was 60.45cm compared with control group which the weight is 4602.50g and length was 60.70 m, at 4 months the weight was 5572.00 g and the length was65.40cm compared with control group which was 5602.50g and 64.50cm, at 6 months the weight was 6406.0g and

length was 70.15 cm compared with control group which was 6633.50g and 68.55 cm, at 8 months the weight was 6968.50g and the length was 73.10 cm compared with control group which was 7193.00g and 73.45cm, at 10 months the weight was 7778.00 and the length was 76.25cm compared with control group which was 8001.50g and 77.10cm, for the 12 months the weight was 8503.50g and the length was 79.0 compared with control group which was 9087.50g and 79.84cm. **El-Ansari (2008) and Rivero-Urgell *et al.* (2011).**

Table (20) Effect of feeding non breast feeding on infants with artificial milk +300 mg lyophilized royal jelly on lengths and weight.

Infa	W1	L1	W2	L2	W4	L4	W6	L6	W8	L8	W10	L10	W12	L12
1	2200	45	4000	53	5200	57	6000	62	6600	65	7400	68	8100	70
2	3100	55	4950	63	5790	67	6750	71	7300	75	8150	79	8800	82
3	2400	50	4100	58	5400	61	6100	64	6620	67	7360	70	8120	72
4	2650	54	4400	62	5600	65	6450	68	7450	72	8250	75	8900	77
5	2350	47	4100	55	5100	60	6050	64	6700	67	7550	70	8200	72
6	2750	56	4700	64	5800	69	6250	73	6700	77	8500	71	9100	74
7	2600	55	4600	62	5500	68	6400	72	7000	75	7650	79	8400	81
8	2900	54	4750	62	6000	70	6750	75	7250	79	8000	82	8750	85
9	2000	42	3500	50	5000	55	5600	60	6000	63	6600	66	7400	68
10	2800	56	4700	64	5850	69	6700	74	7250	77	8000	80	8800	83
11	2550	54	4400	62	5400	67	6500	72	6900	75	7700	79	8500	84
12	3500	57	5300	64	6200	69	7000	74	7750	78	8400	82	9300	85
13	3100	55	4750	62	5850	68	6970	73	7450	75	8250	79	8900	81
14	2900	56	4700	63	5800	70	6750	75	7250	77	8000	81	8750	84
15	2550	54	4500	62	5500	69	6400	74	7000	76	7650	80	8400	83
16	2700	49	4600	57	5700	60	6600	65	7150	68	7850	71	8500	73
17	3250	50	5000	58	6050	61	7000	66	7600	69	8400	72	9000	74

Table (20) continuous

18	2000	55	3600	62	5000	67	5750	73	6200	75	7100	80	8000	83
19	2250	56	4000	63	5150	68	6000	74	6550	76	7250	81	8000	84
20	2300	55	4000	63	5550	68	6100	74	6650	76	7500	80	8150	85
Mean ± SE	**2642.50 ±91.07**	**52.75 ± 0.93**	**4432.50 ± 104.71**	**60.45 ± 0.89**	**5572.00 ± 79.35**	**65.40 ± 1.03**	**6406.0 ± 92.65**	**70.15 ± 1.08**	**6968.50 ± 103.45**	**73.10 ± 1.07**	**7778.00 ± 110.55**	**76.25 ± 1.17**	**8503.50 ± 104.15**	**79.0 ± 1.30**

W1: The new born weight. L1: The new born length.

W2: The weight at 2 months. L2: The length at 2 months.

W4: The weight at 4 months. L4: The length at 4 months.

W6: The weight at 6 months. L6: The length at 6 months.

W8: The weight at 8 months. L8: The length at 8 months.

W10: The weight at 10 months. L10: The length at 10 months.

W12: The weight at 12 months. L12: The length at 12 months

4.4.2 Effect of (breast feeding only) on infants on lengths and weight:

Table (21) Effect of (breast feeding only) on infants on lengths and weight

Group (2) control group.

Infa	W1	L1	W2	L2	W4	L4	W6	L6	W8	L8	W10	L10	W12	L12
1	3100	55	4700	62	5600	66	6770	70	7300	74	8100	77	9650	80
2	3500	56	5000	64	6000	68	7100	72	7700	77	8600	80	10000	83
3	2800	52	4400	60	5600	63	6450	67	7450	70	8250	73	8900	76
4	2500	50	4250	56	5200	61	6300	65	6750	72	7600	77	8250	79
5	3000	54	4500	61	5550	65	6600	70	7100	75	8000	78	9450	81
6	3400	53	5100	61	6000	66	7000	70	7660	76	8600	79	9750	82
7	2600	57	4400	63	5300	67	6250	71	6850	76	7490	79	8250	83
8	3200	56	4800	63	5600	67	6700	71	7250	75	8100	78	9600	82
9	3500	48	5200	54	6000	58	6900	64	7500	70	8200	78	9450	77
10	2300	51	3700	67	5300	62	5900	66	6400	72	7250	77	7900	78
11	3000	50	4550	56	5600	61	6700	65	7150	71	8000	76	9500	78
12	2700	49	4400	56	5500	60	6800	65	7000	71	7650	75	8300	78
13	3400	56	5000	63	5900	67	7050	72	7600	75	8500	78	9800	81
14	3500	55	5100	63	6000	69	6900	70	7500	74	8200	77	9500	80
15	2500	56	4400	62	5300	67	6300	71	6900	76	7490	78	8300	83
16	3100	54	4500	61	5500	66	6800	70	7100	75	8000	78	9450	81
17	2700	53	4500	61	5600	64	6450	67	7400	70	8250	73	8900	77

Table (21) continuous

18	2600	50	4250	56	5200	61	6300	65	6750	72	7600	77	8250	79
19	3000	54	4500	62	5600	65	6600	69	7200	73	8000	76	9400	79
20	3250	56	4800	63	5700	67	6800	71	7300	75	8150	78	9150	83
Mean ± SE	**2982.50 ± 84.55**	**53.25 ± 0.62**	**4602.50 ± 81.55**	**60.70 ± 0.75**	**5602.50 ± 59.66**	**64.50 ± 0.69**	**6633.50 ± 70.08**	**68.55 ± 0.61**	**7193.00 ± 76.87**	**73.45 ± 0.51**	**8001.50 ± 84.96**	**77.10 ± 0.40**	**9087.50 ± 145.27**	**79.84 ± 0.50**

W1: The new born weight. L1: The new born length.

W2: The weight at 2 months. L2: The length at 2 months.

W4: The weight at 4 months. L4: The length at 4 months.

W6: The weight at 6 months. L6: The length at 6 months.

W8: The weight at 8 months. L8: The length at 8 months.

W10: The weight at 10 months. L10: The length at 10 months.

W12: The weight at 12 months L12: The length at 12 months.

Values is mean ± SE

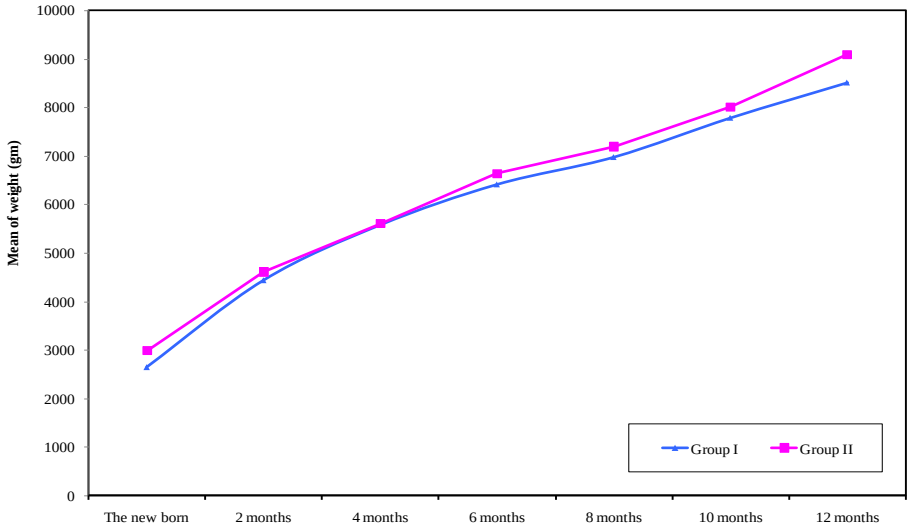

Figure (2) Comparison between the weights (gm) of the two studied groups.

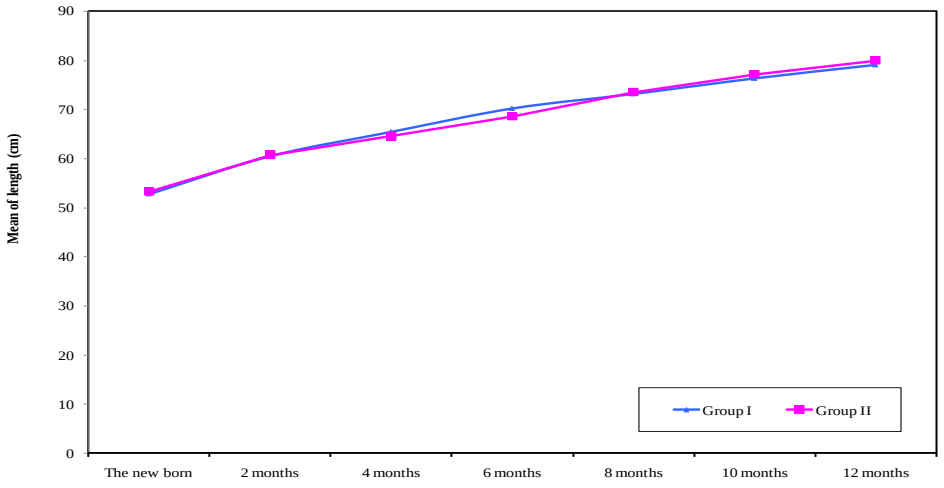

Figure (3) Comparison between the lengths (cm) of two studied groups.

4.5 Biological experimental:

This part designed to investigate the effect administration of 1g (1mg lyophilized bee venom + 500 mg lyophilized royal jelly + 25 ml honey)/ Kg of body weight orally or for one month to the male adult rats induced oxidative stress by hydrogen peroxide on sexual efficacy, glutathione and malondialdehyde.

4.5.1 Effect of feeding with lyophilized bee venom and royal jelly on body weight of rats:

Data in Tables (22 and 23) indicated that, the total and weekly increases in body weight of rats treated with 1g (1mg lyophilized bee venom + 500 mg lyophilized royal jelly + 25 ml honey)/ Kg of body weight orally compared with control group which treated orally with saline for the same period. There was an increase in body weight of rats that treated with 1g (1mg lyophilized bee venom + 500 mg lyophilized royal jelly + 25 ml honey)/ Kg of body weight orally compared with control group. The total increase in body weight of rats that treated with 1g (1mg lyophilized bee venom + 500 mg lyophilized royal jelly + 25 ml honey)/ Kg of body weight orally is 604g (46.32%), while the weekly increased was 296g (23.33%),177g (13.08%) and 131g (9.92%) for 1,2 and 3 weeks respectively compared with control group which the total increase was 309g (44.9%) while the weekly increase was. 145g (20.5%), 84g (12.61%) and 80g (11.81%) for 1,2 and 3 weeks respectively. These results are in an agreement with those obtained by **Antinelli *et al.* (2003) and Mannoor *et al.* (2009).**

Table (22): Total and weekly increases in body weight of rats treated with 1g (1mg lyophilized bee venom + 500 mg lyophilized royal jelly + 25 ml honey)/ Kg of body weight orally.

No. of rats	Initial weight (g)	Weight before sacrifice (g)	Total increase		Weekly increase					
			g	%	1st week		2nd week		3rd week	
					g	%	g	%	g	%
1	140	214	74	52.9	35	25	25	17.86	14	10
2	140	191	51	36.4	30	21.4	13	9.29	8	5.72
3	90	169	79	87.8	45	50	21	23.33	13	14.44
4	100	141	41	41	20	20	5	5	16	16
5	130	180	50	38.5	30	23.1	15	11.54	5	3.84
6	140	185	45	32	10	7.1	12	8.57	23	16.43
7	90	138	48	53.3	35	38.9	5	5.55	8	8.89
8	100	149	49	49	20	20	10	20	9	9
9	145	188	43	29.7	20	13.8	20	13.79	3	2.07
10	160	229	69	43.1	25	15.6	25	15.63	19	11.87
11	120	175	55	45.8	26	21.66	15	13.33	13	10.83
Total	1355	1959	604	509.5	296	256.63	177	143.89	131	109.08
Mean	**123.18**	**178.09**	**54.9**	**46.32**	**27**	**23.33**	**16.09**	**13.08**	**11.91**	**9.92**
S.E	**7.39**	**8.59**	**3.9**	**4.76**	**2.88**	**3.56**	**2.1**	**1.76**	**1.81**	**1.42**

Values is mean ± SE

Table (23): Total and weekly increases in body weight of rats treated orally with saline (control).

No. of rats	Initial weight (g)	Weight before sacrifice (g)	Total increase		Weekly increase					
			g	%	1st week		2nd week		3rd week	
					g	%	g	%	g	%
12	95	140	45	47.37	15	15.79	15	15.79	15	15.79
13	120	191	71	59.17	25	20.83	17	14.17	29	24.66
14	100	140	40	40	10	10	20	20	10	10
15	100	140	40	40	30	30	5	5	5	5
16	110	161	51	46.36	25	22.73	15	13.63	11	10
17	170	232	62	36.47	40	23.53	12	7.06	10	5.88
Total	695	1004	309	269.37	145	122.88	84	75.65	80	70.84
Mean	115.8	167.3	51.5	44.9	24.17	20.5	14	12.61	13.3	11.81
S.E	9.9	13.25	4.5	3.32	4.36	2.81	2.09	2.29	3.39	2.92

Values is mean ± SE

4.5.2 Effect of treatment by injected subcutaneous of lyophilized bee venom and royal jelly on body weight of rats:

Data in Tables (24 and 25) indicated that, the total and weekly increases in body weight of rats treated with 0.1ml (1mg lyophilized bee venom + 500 mg lyophilized royal jelly + 25 ml honey)/ Kg of body weight injected subcutaneous compared with control group (2) which treated injected subcutaneous with saline for the same period. . The total increase in body weight of rats that treated with 0.1ml (1mg lyophilized bee venom + 500 mg lyophilized royal jelly + 25 ml honey)/ Kg of body weight injected subcutaneous is 517g (42.2%), weekly increase was 235g (19%), 198g (16.2%) and 84g (7%) for 1,2,and 3 week respectively compared with control group(2) which the total increase was 446g (47.3%), weekly increase was 260g (27.81%), 66g (6.81%) and 120g (12.69%) for 1,2 and 3 weeks respectively. These results are in an agreement with those obtained by **Abreu *et al.* (2000), Antinelli *et al.* (2003) and Mannoor *et al.* (2009).**

Table (24): Total and weekly increases in body weight of rats injected subcutaneous with 0.1ml (1mg lyophilized bee venom + 500 mg lyophilized royal jelly + 25 ml honey)/ Kg of body weight.

No. of rats	Initial weight (g)	Weight before sacrifice (g)	Total increase		Weekly increase					
			g	%	1st week		2nd week		3rd week	
					g	%	g	%	g	%
26	140	204	64	45.72	30	21.43	32	22.86	2	1.43
27	100	140	60	40	20	20	22	22	2-	-2
28	90	130	40	44.44	10	11.11	20	22.22	10	11.11
29	90	128	38	42.22	10	11.11	17	18.89	11	12.22
30	40	142	52	57.78	30	33.3	5	5.56	17	18.89
31	90	123	33	36.66	20	22.22	10	11.11	3	3.33
32	125	185	60	48	25	20	20	16	15	12
33	90	132	42	46.66	20	22.22	20	22.22	2	2.22
34	165	244	79	47.88	35	21.21	25	15.15	19	11.52
35	115	158	43	37.39	15	13.04	20	17.39	8	6.96
36	145	171	26	17.93	20	13.79	7	4.83	-1.0	-0.69
Total	12.40	1757	517	464.4	235	209.46	198	178.23	84	77
Mean	112.7	159.7	47	42.2	21.4	19	18	16.2	7.6	7
S.E	8.2	11.54	4.63	3	2.44	1.97	2.4	1.97	2.13	1.9

Values is mean ± SE

Table (25): Total and weekly increases in body weight of rats injected with saline (control 2).

No. of rats	Initial weight (g)	Weight before sacrifice (g)	Total increase		Weekly increase					
			g	%	1st week		2nd week		3rd week	
					g	%	g	%	g	%
18	100	165	56	56	30	30	10	10	16	16
19	90	144	54	60	25	27.7	5	5.56	24	26.67
20	90	140	50	55.56	40	44.4	5	5.56	5	5.56
21	125	192	67	53.6	50	40	10	8	7	5.6
22	140	193	53	37.86	30	21.4	13	9.29	10	7.14
23	165	241	76	46.06	40	42.2	15	9.09	21	12.73
24	145	194	49	33.8	25	17.24	-	-	24	16.55
25	115	156	41	35.65	20	17.39	8	6.96	13	11.3
Total	970	1416	446	378.5	260	222.51	66	54.46	120	101.55
Mean	121.3	177	55.75	47.3	32.5	27.81	8.25	6.81	15	12.69
S.E	9.72	12.11	3.83	3.67	3.54	3.54	1,7	1.14	2.65	2.52

Values is mean ± S

4.5.3 Serum hormones in rats treated with (1mg lyophilized bee venom + 500 mg lyophilized royal jelly + 25 ml honey) or saline (1g/kg) orally or (0.1ml/kg) injected:

Data in Table (26) indicated that, the thyroxine (T4) hormone was 39.623 when treated with (BV+RJ+H)1 decreased to 33.965 when treated with saline orally, 29.296 when treated with (BV+RJ+H)2, increased to 34.127 when treated with saline injected subcutaneous. Triiodothyroine (T3) hormone was 1.078 when treated with (BV+RJ+H)1, increased to 1.328 when treated with saline orally, 0.78 when treated with (BV+RJ+H)2 and increased to 0.86 when treated with saline injected subcutaneous. Cortisol hormone was 23.336 when treated with (BV+RJ+H)1, decreased to 18.183 when treated with saline orally, 17.575 when treated with (BV+RJ+H)2 and increased to 18.7 when treated with saline injected subcutaneous. Lutenizing (LH) hormone was 7.35 when treated with (BV+RJ+H)1, decreased to 6.1 when treated with saline orally, 6.764 when treated with (BV+RJ+H)2 and decreased to 5.271 when treated with saline injected subcutaneous. Progestrone hormone was 8.74 when treated with (BV+RJ+H)1, decreased to 8.225 when treated with saline orally, 10.6911 when treated with (BV+RJ+H)2 and decreased to 7.25 when treated with saline injected subcutaneous. Testosterone hormone was 4.5 when treated with (BV+RJ+H)1, decreased to3.3166 when treated with saline orally, 4.957 when treated with (BV+RJ+H)2 and decreased by small amount to 4.82 when treated with saline injected subcutaneous. These results are in an agreement with those

obtained by **Abreu *et al.* (2000), Strum *et al.* (2002), Antinelli *et al.* (2003) and Mannoor *et al.* (2009).**

Table (26) Mean values of serum hormones in rats treated with (1mg lyophilized bee venom + 500 mg lyophilized royal jelly + 25 ml honey) or saline (1g/kg) orally and (0.1ml/kg) injected.

Parameters	Oral treatment		Subcutaneous	
	(BV+RJ+H)1	Saline control (1)	(BV+RJ+H)2	Saline control (2)
Thyroxine(T4) (nmo1/L)	39.623 ±5.899	33.965 ±4.458	29.296 ±2.94	34.127 ±5.716
Triiodothyroine(T3) (nmo1/L)	1.078 ±0.125	1.328 ±0.166	0.78 ±0.096	0.86 ±0.2
Cortisol (nmo1/L)	23.336 ±5.01	18.183 ±3.66	17.575 ±2.01	18.7 ±0.2
Lutenizing hor. (LH) (nmo1/L)	7.35 ±0.65	6.1 ±1.24	6.764 ±0.82	5.271 ±1.03
Progestrone (nmo1/L)	8.74 ±1.78	8.225 ±3.1	10.6911 ±2.42	7.25 ±1.19
Testosterone (nmo1/L)	4.5 ±1.4	3.3166 ±0.92	4.957 ±2.0	4.82 ±1.77

Values is mean ± SE

(BV+RJ+H)1=1g (1mg lyophilized bee venom + 500 mg lyophilized royal jelly + 25 ml honey)/ Kg of body weight orally.

(BV+RJ+H)2=0.1ml (1mg lyophilized bee venom + 500 mg lyophilized royal jelly + 25 ml honey)/ Kg of body weight injected or subcutaneous.

4.5.4 Hemoglobin and hematoorit values in rats treated with 1mg lyophilized bee venom + 500 mg lyophilized royal jelly + 25 ml honey)or saline (1g/kg) orally or (0.1ml/kg) injected:

Data in Table (27) indicated that, the increase in hemoglobin values from 16.267 by (BV+RJ+H)1 treatment to 16.663 by saline orally , there was no change in Hemoglobin 13.551 by (BV+RJ+H)2 and 13.597 by saline injected treatment while hematoorit there was decreasing from 40.5714 by (BV+RJ+H)1 treatment to 38.0 by saline orally. On the other hand an increasing was from 39.44 by (BV+RJ+H)2 treatment to 40.5714 by saline subcutaneous. These results are in an agreement with those obtained by **Abreu *et al.* (2000), Strum *et al.* (2002), Antinelli *et al.* (2003) and Mannoor *et al.* (2009).**

Table (27) Mean blood hemoglobin concentration and hematoorit values in rats treated with 1mg lyophilized bee venom + 500 mg lyophilized royal jelly + 25 ml honey) or saline (1g/kg) orally or (0.1ml/kg) injected.

Parameters	Oral treatment		Subcutaneous	
	(BV+RJ+H)1	Saline control 1	(BV+RJ+H)2	Saline control 2
Hemoglobin (gm. /d1.)	16.2667 ±1.3768	16.663 ±1.44	13.551 ±0.45	13.597 ±1.059
Hematoorit %	40.5714 ±1.85	38.0 ±1.79	39.444 ±1.625	40.5714 ±2.114

(BV+RJ+H)1=1g (1mg lyophilized bee venom + 500 mg lyophilized royal jelly + 25 ml honey)/ Kg of body weight orally.

(BV+RJ+H)2=0.1ml (1mg lyophilized bee venom + 500 mg lyophilized royal jelly + 25 ml honey)/ Kg of body weight injected or subcutaneous.

Values is mean ± SE

4.5.5 Mean values of serum total proteins, albumin, globuline and A/G in rats treated with (25ml honey + 500mg royal jelly + 1mg bee venom) or saline (1g/kg) orally or (0.1ml/kg) injected:

Data in Table (28) indicated that, the increase in total protein from 6.294 by (V+R+H)1 treatment to 6.88 by saline orally , there was decreasing in total protein from 6.364 by (V+R+H)2 to 5.87 by saline injected treatment. Albumine there

was no change 3.695 by (V+R+H)1 treatment, 3.64 by saline orally, on the other hand there was very small amount increasing from 3.37 by (V+R+H)2 treatment to 3.477 by saline subcutaneous. For Globulin there was an increasing from 2.593 by (V+R+H)1 treatment to 3.24 by saline orally, on the other hand there was decreasing from 3..0 by (V+R+H)2 treatment to 2.397 by saline subcutaneous. Finally, A/G ratio there was decreasing from 1.485 by (V+H+R)1 treatment to 1.153 by saline orally, on the other hand there was an increasing from 1.199 by (V+R+H)2 treatment to 1.46 by saline suncutaneous. These results are in an agreement with those obtained by **Abreu *et al.* (2000), Strum *et al.* (2002), Antinelli *et al.* (2003) and Mannoor *et al.* (2009).**

Table (28) Mean values of serum total proteins, albumin, globuline and A/G in rats treated with (1mg of lyophilized bee venom + 500mg royal jelly + 25 ml honey) or saline 1g/kg) orally or (0.1ml/kg) injected.

Parameters	Oral treatment		Subcutaneous	
	(BV+RJ+H)1	Saline control (1)	(BV+RJ+H)2	Saline Control (2)
Total protein (gm. /d1.)	6.294	6.88	6.364	5.87
	±0.19	±0.22	±0.176	±0.174
Albumin (gm. /d1.)	3.695	3.64	3.37	3.477
	±0.1	±0.2	±0.154	±0.136
Globulin (gm. /d1.)	2.593	3.24	3.0	2.397
	±0.139	±0.24	±0.283	±0.1
A/G ratio	1.485	1.153	1.199	1.46
	±0.09	±0.135	±0.136	±0.08

(BV+RJ+H)1=1g (1mg lyophilized bee venom + 500 mg lyophilized royal jelly + 25 ml honey)/ Kg of body weight orally.

(BV+RJ+H)2=0.1ml (1mg lyophilized bee venom + 500 mg lyophilized royal jelly + 25 ml honey)/ Kg of body weight injected or subcutaneous.

Values is mean ± SE

4.5.6 Effect of treatment by royal jelly and bee venom on fertility and efficiency nationality:

Data in Table (29) showed that, the administration by hydrogen peroxide (0.5%) in drinking water for one month did not affect the weight of testis, epididymis (head, body, and tail), prostate and seminal vesicles compared with control group value. While the administration by hydrogen peroxide (0.5%) in drinking water with 1g (1mg of lyophilized bee venom + 500mg of lyophilized royal jelly +25ml honey) /kg of body weight orally for one month were a companied by increasing in the weight of testis and body of epididymis. Whereas no changes in the weight of head and tail of epididymus, prostate and seminal vesicles compared with hydrogen peroxide group. On the other hand, the administration by (1mg of lyophilized bee venom + 500mg of lyophilized royal jelly + 25ml honey) /kg of body weight orally for one month increased the weight of testis and body of epididymis. whereas no changes in the weight of head and tail of epididymus, prostate and seminal vesicles compared with hydrogen peroxide group, and this results were in agreement with those obtained by **Abreu *et al.* (2000), Strum *et al.* (2002), Antinelli *et al.* (2003) and Mannoor *et al.* (2009).**

Table (29) Effect the treatment of 1g (1mg of lyophilized bee venom +500mg of lyophilized royal jelly+25ml honey)/kg of body weight orally on the weight of the testis, epididymis (head, body, tail) prostate and seminal vesicle in rats receiving hydrogen peroxide for one month.

Treated animals	Testis mg/100g B.Wt.	Head of epididymus mg/100g B.Wt	Body of epididymus mg/100g B.Wt	Tail of epididymus mg/100g B.Wt	Prostate mg/100g B.Wt	Seminal vesicle mg/100g B.Wt
Control	473.4±27.7	79.2±4.2	21.1±0.9	93.2±7.4	447.5±44.2	100.9±5.9
1	501.5±10.8	85.0±3.9	20.8±0.6	92.9±5	431.35±35.3	108.9±7.4
2	604.9±212	89.4±5.5	23.7±0.3	93.4±2.9	455.3±33.2	99.9±6
3	636.1±21.7	76.5±4.1	24.1±0.3	90.4±1.5	427.1±26.7	105.7±6.4

1: Hydrogen peroxide (0.5%) in drinking water for one month.

2: Hydrogen peroxide (0.5%) in drinking water for one month +1g (1mg of lyophilized bee venom +500mg of lyophilized royal jelly+25ml honey) /kg of body weight orally for one month.

3: 1g (1mg of lyophilized bee venom +500mg of lyophilized royal jelly+25ml honey)/kg of body weight orally for one month.

Values is mean ± SE

4.5.7 Effect of treatment by royal jelly and bee venom on fertility and efficiency nationality:

Data in Table (30) revealed that, there was decrease in the sperm count in hydrogen peroxide group compared with control group. While,treatment with 1g (1mg of lyophilized bee venom +500mg of lyophilized royal jelly+25ml honey)/kg of body weight orally and hydrogen peroxide caused increase in sperm count compared with hydrogen peroxide group and approximately returned to the normal control value(1.43). Data in the same table showe increase in the sperm count by 1g (1mg of lyophilized bee venom +500mg of lyophilized royal jelly+25ml honey)/kg of body weight orally treated group compared with hydrogen peroxide group, and 1g **(**1mg of lyophilized bee venom +500mg of lyophilized royal jelly+25ml honey)/kg of body weight orally concomitantly with hydrogen peroxide group. Also, data in the same table demonstrated that, there was decrease in glutathione level in hydrogen peroxide group compared with control group. While the administration of 1g (1mg of lyophilized bee venom +500mg of lyophilized royal jelly+25ml honey) /kg of body weight orally with or without hydrogen peroxide caused increase in the glutathione level compared with hydrogen peroxide group. On the other hand, the increase of malondialdehyde level in hydrogen peroxide group compared with control group while treatment with 1g(1mg of lyophilized bee venom +500mg of lyophilized royal jelly+25ml honey) /kg of body weight orally concomitant with hydrogen peroxide was no affect in the malondialdehyde level, but the treatment with 1g (1mg of lyophilized bee venom +500mg of

lyophilized royal jelly+25ml honey)/kg of body weight orally alone caused decrease in malondialdehyde level. These results are in an agreement with those obtained by **Krell (1996) and WHO (2002).**

Table (30) Effect of the treatment of 1g (1mg of lyophilized bee venom +500mg of lyophilized royal jelly+25ml honey)/kg of body weight orally on sperm count, glutathione, and malonaldehyde levels in the rats receiving hydrogen peroxide for one month.

Treated animals	Sperm concentration $x10^6$	Glutathione µm/g	Malonaldehyde nm/g
Control	1.4320±0.02	1.04±0.02	264.82±12.48
1	0.80±0.02	0.59±0.02	311.0±17.6
2	1.3440±0.13	1.23±0.14	233.9±14.3
3	1.6260±0.02	1.26±0.02	219.7±5.7

1: Hydrogen peroxide (0.5%) in drinking water for one month.

2: Hydrogen peroxide (0.5%) in drinking water for one month + (1mg of lyophilized bee venom +500mg of lyophilized royal jelly+25ml honey)(1g/kg orally) one month.

3: (1mg of lyophilized bee venom +500mg of lyophilized royal jelly+25ml honey) (1g/kg orally) one month.

Values is mean ± SE

4.5.8 Effect of treatment by royal jelly and bee venom on fertility and efficiency nationality:

Data in Table (31) demonstrated that, there was decrease in the percentage of the live sperms in hydrogen peroxide group (84) compared with control group (91.6). Treatment with 1g (1mg of lyophilized bee venom +500mg of lyophilized royal jelly+25ml honey)/kg of body weight orally concomitantly with hydrogen peroxide did not affect in the percentage of the live sperms compared with hydrogen peroxide group. Whereas the treatment with 1g (1mg of lyophilized bee venom +500mg of lyophilized royal jelly+25ml honey)/kg of body weight orally alone caused increase in the percentage of the live sperms and returned to normal control value. Data in the same table revealed that increasing in the percentage of sperms deformity in hydrogen peroxide group compared with control group. Also, treatment with 1g (1mg of lyophilized bee venom +500mg of lyophilized royal jelly+25ml honey)/kg of body weight orally with or without hydrogen peroxide caused decrease in the percentage of sperm deformity compared with hydrogen peroxide group**.** Data in the same table showe decreasing in the testosterone hormone level in hydrogen peroxide group compared with control group. While, administration by 1g (1mg of lyophilized bee venom +500mg of lyophilized royal jelly+25ml honey)/kg of body weight orally concomitant with or without hydrogen peroxide caused increase in testosterone hormone compared with hydrogen peroxide group. These results are in an agreement with those obtained by **Abreu *et al.* (2000),**

Strum *et al.* (2002), Antinelli *et al.* (2003) and Mannoor *et al.* (2009).

Table (31) Effect of treatment by 1g (1mg of lyophilized bee venom +500mg of lyophilized royal jelly+25ml honey) /kg of body weight orally on the percentage number of live sperm, sperm deformity, and testosterone hormone concentration in rats receiving hydrogen peroxide for one month.

Treated animals	Live sperm%	Sperm deformity%	Testosterone hormone mg/ml
Control	91.6±1.5	4.2±0.37	2.37±0.16
1	84±1.51	11.2±1.06	1.72±0.30
2	87±0.54	9.0±7.03	2.51±0.13
3	94.6±0.81	4.6±0.4	4.24±0.27

1: Hydrogen peroxide (0.5%) in drinking water for one month.

2: Hydrogen peroxide (0.5%) in drinking water for one month + (1mg of lyophilized bee venom +500mg of lyophilized royal jelly+25ml honey) (1g/kg orally) one month.

3: (1mg of lyophilized bee venom +500mg of lyophilized royal jelly+25ml honey) (1g/kg orally) one month

Values is mean ± SE

4.5.9 Effect of treatment by royal jelly and bee venom on kidney function:

Data in Table (32 and 33) show the oral administration with (1mg of lyophilized bee venom +500mg of lyophilized

royal jelly+25ml honey) orally in doses of 50 mg and 100 mg/kg body weight increased urea concentration in serum and decreased in urine. Urea clearance in urine was also decreased as compared to control group.While, oral administration by (1mg of lyophilized bee venom +500mg of lyophilized royal jelly+25ml honey) orally in a dose of 100 mg/kg body weight decreased serum and urine creatinine level which might be attributed to the increased cortisol level. These results are in an agreement with those obtained by **Abreu *et al.* (2000), Strum *et al.* (2002), Antinelli *et al.* (2003) and Mannoor *et al.* (2009).**

Table (32) Effect of oral administration of 50 and 100mg/kg body weight of (1mg of lyophilized bee venom +500mg of lyophilized royal jelly+25ml honey) for 5 days on urea concentrations (mg/100ml) in serum and urine and urea clearance (ml/min) of rats.

Groups	Group (1) (Control)			Group (2) (50m/g kg b. wt)			Group (3) (100 m/g kg b. wt)		
	Urea concentration (mg %)		Urea clearance (mg %)	Urea concentration (mg %)		Urea clearance (mg %)	Urea concentration (mg %)		Urea clearance (mg %)
	Serum	urine		Serum	Urine		Serum	Urine	
Values	40.19± 0.93	145.00 ± 5.69	0.019 ± 0.0007	78.33 ± 1.60	105.0 ± 5.54	0.009 ± 0.0004	88.28 ± 1.28	46.33 ± 3.13	0.0044 ± 0.0003

Values is mean ± SE

Table (33)Effect of oral administration of 50 and 100mg/kg body weight of (1mg of lyophilized bee venom +500mg of lyophilized royal jelly+25ml honey) for 5 days on creatinine level (mg %) in serum and urine and creatinine clearance (ml/min) of rats.

Groups	Group (1) Control			Group (2) (50m/g kg b. wt)			Group (3) (100 m/g kg b. wt)		
	Creatinine concentration (mg %)		Greatinine clearance (mg %)	Greatinine concentration (mg %)		Greatinie clearance (mg %)	Greatinine concentration (mg %)		Greatinie clearance (mg %)
	Serum	urine		Serum	Urine		Serum	Urine	
Values	0.29 ± 0.015	133.16 ± 4.00	2.57 ± 0.0007	0.30 ± 0.023	120.68 ± 3.98	2.89 ± 0.148	0.22 ± 0.019	88.75 ± 3.86	0.0855 ± 0.050

Values is mean ± SE

4.5.10 Effect of treatment by royal jelly and bee venom in glucose concentrations in serum and urin (Diabetics Rats):

Decreasing in serum glucose level was also observed when the test animals were treated with (1mg of lyophilized bee venom +500mg of lyophilized royal jelly+25ml honey) orally in a dose of 50 and 100 mg/kg body weight (Table 34). Present findings suggest that (1mg of lyophilized bee venom +500mg of lyophilized royal jelly+25ml honey) possessed insulin like effects. These results are in an agreement with those obtained by **Abreu *et al.* (2000), Strum *et al.* (2002), Antinelli *et al.* (2003), Mannoor *et al.* (2009) and Münstedt *et al*. (2009).**

Table (34) Effect of oral administration of 50 and 100mg/kg body weight of(1mg of lyophilized bee venom +500mg of lyophilized royal jelly+25ml honey) for 5 days on glucose concentrations (mg/100 ml) in serum and urine of rats.

Rats Group No	Group (1) Control		Group (2) (50 mg/kg .b. wt)		Group (3) (100 mg/kg .b. wt)	
	Serum	Urine	Serum	Urine	Serum	Urine
Values	96.73 ± 0.584	0.00	88.42 ± 0.766	0.00	84.14 ± 1.21	0.00

Values is mean

4.6 Utilization of (1mg of lyophilized bee venom +500mg of lyophilized royal jelly+25 ml honey) for treated some human disease:

The modern utilization of bee venom in apitherapy was initiated through the effects of Austrian physican Philip Trec in his published results "Report about a peculiar connection between the bee sting and rheumatism" in 1888. After the first word war Bogog Beck brought bee venom apitherapy to the US and published a book on bee venom therapy in 1935, mainly against rheumatoid arthritis. In Europe the first commercial bee venom preparation was released in 1928. Charles Mraz, a student of Beck, popularized bee venom therapy in the USA.

4.6.1 The effect of the oral dose with 1g (1 mg lyophilized bee venom + 500mg of lyophlized royal jelly + 25 ml honey)/kg of body weight orally to diabetes mellitus (DM) patients:

Data in Table (35) demonstrated that, the effect of treat 60 patient with 1g (1 mg lyophilized bee venom + 500mg of lyophlized royal jelly + 25 ml honey)/kg of body weight orally to diabetes mellitus (DM) patients for two months, that there were decrease in HbA1c from 8.29% before the treatment to 6.22% after the treatment *(Glaciated HbA1cis accurate in determining blood glucose in last three months).* These results according to **Batchelder (2002) and Bogdanov *et al*. (2011b).**

Table (35) Effect of the oral dose with 1g(1 mg lyophilized bee venom + 500mgof lyophlized royal jelly + 25 ml honey)/kg of body weight orally to diabetes mellitus (DM) patients.

Patients	HbA1c (before treatment)	HbA1c (after treatment)
P1	9	7
P2	8.7	7
P3	7.8	6
p4	9.1	6.2
P5	8.5	5.9
P6	7.7	5.5
P7	7.9	6
P8	8.2	7
P9	9.1	7.4
P10	7.1	6.2
P11	8.2	7
P12	6.7	5.5
P13	9.5	7.1
P14	7.7	6.2
P15	7.6	5.9
P16	8.4	6.1
P17	8.2	6.1
P18	7.8	6.2
P19	8.1	6.5
P20	7.9	5.5

Table (35) continuous

P21	8.3	6
P22	9.2	6.2
P23	8.5	6
P24	9.4	7.1
P25	8	6.2
P26	8.4	5.9
P27	9.1	6.5
P28	9.3	6.5
P29	10	7.2
P30	9.5	6.5
P31	8.8	6.1
P32	9.2	6.4
P33	7.1	5.5
P34	8.2	6.1
P35	7.2	5.9
P36	6.9	5.5
P37	7.3	5.5
P38	8	6.1
P39	7.5	5.9
P40	8.4	5.5
P41	7.6	5.1
P42	6.8	5
P43	9.2	6.2
P44	9.1	6.5
P45	8.5	6.3
P46	7.8	5.9
P47	8.2	6.1
P48	7.6	5.8
P49	8.4	6.4
P50	7.7	6.9
P51	9.1	6.4
P52	8.8	6.2
P53	7.7	5.7
P54	7.1	5.5
P55	8.4	6.1
P56	8.3	5.9
P57	9.1	6.3
P58	8.9	6.5

Table (35) continuous

P59	7.9	6
P60	8	6.4
Mean ± SE	**8.29 ± 0.13**	**6.22 ± 0.08**

NB: (Glaciated HbAc% is accurate in determining blood glucose in last three months).

Values is mean ± SE

4.6.2 The effect of the oral dose with1g (1mg of lyophlized bee venom + 500mg of lyophlized royal jelly + 25 ml honey)/kg of body weight orallyto anemia patients.

Data in Table (36) demonstrated that, the effect of treat 30 patient with 1g (1 mg lyophilized bee venom + 500mg of lyophlized royal jelly + 25 ml honey)/kg of body weight orally to Anemia patients for two months , that there was increase in Hb%from 8.16% before the treatment to 10.32% after the treatment. These results are in an agreement with those obtained by **Bogdanov *et al*. (2011a) and Bogdanov *et al*. (2011b).**

Table (36) Effect of the oral dose with 1g (1mg of lyophlized bee venom + 500mg of lyophlized royal jelly + 25 ml honey)/kg of body weight orallyto anemia patients.

Patients	Hb% (before treatment)	Hb% (after treatment)
P1	8.2	10.5
P2	7.9	10.2
P3	9.5	11
p4	10	12
P5	8.7	11
P6	7.9	10.5
P7	6.5	9
P8	7.8	10
P9	8	10.5
P10	8.2	10.6
P11	8	10.7
P12	7.6	10
P13	7.8	10.2
P14	8	10
P15	9	11.2

Table (36) continuous

P16	9.2	11
P17	8.1	10.5
P18	7.9	10
P19	6.9	9.5
P20	8	10.5
P21	8.4	10.4
P22	8.5	10.2
P23	9	11.2
P24	7.5	9.8
P25	6.7	8.9
P26	7.6	8.5
P27	8	10
P28	8.5	10.2
P29	8.5	10.4
P30	9	11
Mean ± SE	**8.16 ± 0.14**	**10.32 ± 0.13**

Values is mean ± SE

4.6.3 The effect of giving 1g (1mg of lyophilized bee venom + 500mg of lyophilized royal jelly + 25ml honey)/kg of body weight orally to rheumatoid arthritis (R.A) patients.

A: clinical assessment.

Data in Table (37) showd that, the effect of treat 30 patient with 1g (1 mg lyophilized bee venom + 500mg of lyophlized royal jelly + 25 ml honey)/kg of body weight orally to Rheumatoid arthritis (R.A) patients for two months , that for Clinical assessment , Joint swelling & mobility , Morning st.fitnessthere, Analgesia and Satisfaction the results were 23 patients improved which can translate as percentage (76.7%) and 7 patients have no change which can translate as a percentage (23.3%). These results are in an agreement with those obtained by **Lee *et al.* (2005), Son *et al*. (2007), Bogdanov *et al*. (2011a) and Bogdanov *et al*. (2011b).**

Table (37) Effect of giving 1g(1mg of lyophilized bee venom + 500mg of lyophilized royal jelly + 25ml honey)/kg of body weight orally to rheumatoid arthritis patients.

Patients	Joint swelling & mobility	Morning st.fitness	Analgesia	Satisfaction
P1	1	1	1	1
P2	1	1	1	1
P3	1	1	1	1
P4	0	0	0	0
P5	1	1	1	1
P6	1	1	1	1
P7	1	1	1	1
P8	0	0	0	0
P9	1	1	1	1
P10	1	1	1	1
P11	1	1	1	1
P12	1	1	1	1
P13	0	0	0	0
P14	1	1	1	1
P15	1	1	1	1
P16	1	1	1	1
P17	1	1	1	1
P18	1	1	1	1
P19	0	0	0	0
P20	1	1	1	1
P21	1	1	1	1
P22	1	1	1	1
P23	1	1	1	1
P24	0	0	0	0
P25	1	1	1	1
P26	0	0	0	0
P27	0	0	0	0
P28	1	1	1	1
P29	1	1	1	1

Table (37) continuous

P30	1	1	1	1
No change	**7 (23.3%)**	**7 (23.3%)**	**7 (23.3%)**	**7 (23.3%)**
Improved	**23(76.7%)**	**23 (76.7%)**	**23 (76.7%)**	**23 (76.7%)**

No change: 0 Improved: 1 worsend: 2

Values is mean ± SE

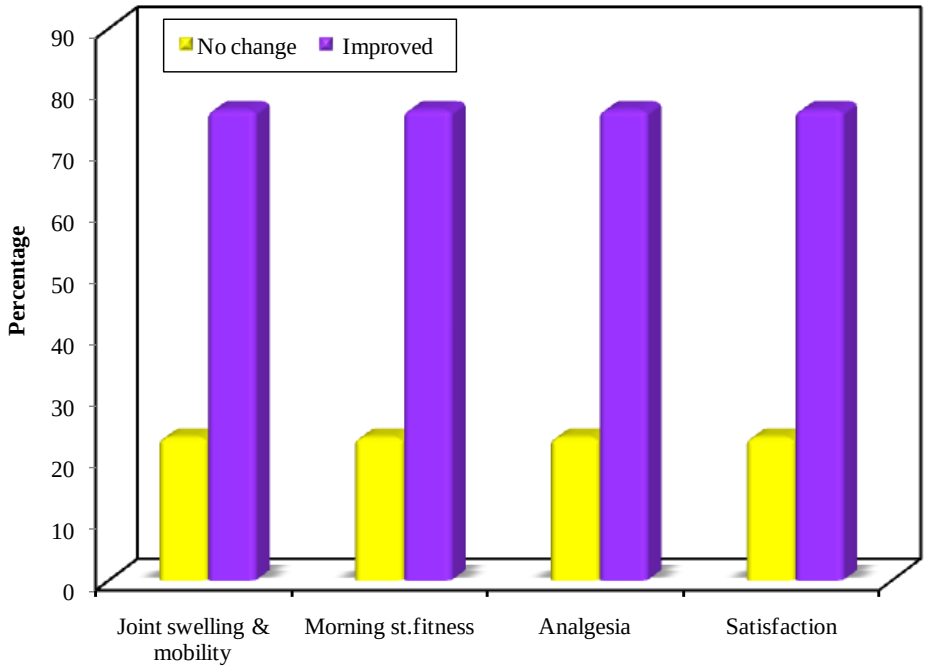

Fig. (4): Effect of the oral dose giving (25ml honey + 500mg royal jelly + 1mg bee venom) to rheumatoid arthritis patients.

4.6.4 Effect of the oral dose with 1g(1mg lyophilized bee venom + 500mg lyophlized royal jelly + 25 ml honey)/kg of body weight orally to rheumatoid arthritis (R.A) patients (blood analysis):

Data in Table (38) showed that, the effect of treat 30 patient with 1g (1 mg lyophilized bee venom + 500mg of lyophilized royal jelly + 25 ml honey)/kg of body weight orally to rheumatoid arthritis patients for two months ,for blood analysis that for ESR was 69.33 before the treatment which decreased to 46.67 after the treatment and rheumatoid factor was 53.00 before the treatment which decreased to 36.40, these results according to **Lee *et al*. (2005), Son *et al*. (2007), Bogdanov *et al*. (2011a) and Bogdanov *et al*. (2011b).**

Table (38) Effect of the oral dose with 1g(1mg of lyophilized bee venom + 500mg of lyophilized royal jelly + 25ml honey)/kg of body weight orally to rheumatoid arthritis patients.

Patients	ESR		Rheumatoid factor	
	Before treatment	After treatment	Before treatment	After treatment
P1	100	70	120	60
P2	77	48	24	12
P3	46	32	62	38
P4	55	38	48	40
P5	65	30	28	12
P6	70	44	38	16
P7	62	40	22	10
P8	78	62	68	60
P9	56	38	47	32
P10	48	25	52	25
P11	62	36	114	72
P12	72	42	92	60
P13	48	36	68	62
P14	55	30	74	70
P15	61	38	82	44
P16	72	42	47	30
P17	58	30	39	16
P18	68	33	52	25
P19	47	40	66	58
P20	53	31	30	30

Table (38) continuous

P21	62	40	37	15
P22	100	70	48	40
P23	92	72	52	50
P24	78	62	15	10
P25	66	55	19	15
P26	80	64	22	20
P27	67	52	72	72
P28	70	50	38	30
P29	100	80	70	40
P30	112	70	44	28
Mean ± SE	**69.33 ± 3.16**	**46.67 ± 2.81**	**53.00 ± 4.79**	**36.40 ± 3.69**

Values is mean ± SE

4.6.5 Effect of the oral dose with 1g (1mg of lyophilized bee venom + 500mg of lyophilized royal jelly + 25ml honey)/kg of body weight orally to cancer patients:

Data in Table (39) showed, that the effect of treat 40 patient with 1g (1 mg lyophilized bee venom + 500mg of lyophilized royal jelly + 25 ml honey)/kg of body weight orally to cancer patients for two months, the result was 14 patients which can translate in percentage (35%) improved and 26 patient have no change which can translate in percentage (65%), the

details were as follows HB% before the treatment was 9.35 which was decreased and become 7.53 after the treatment while the Tumor marker (CEA) was 94.55 before the treatment which decrease a little amount by 0.05 and became 94.50 after the treatment. These results are in an agreement with those obtained by **Orsolic (2005), Park *et al. (*2011), Bogdanov *et al*. (2011a) and Bogdanov *et al*. (2011b).**

Table (39) Effect of the oral dose giving 1g(1 mg of lyophilized bee venom + 500mg of lyophilized royal jelly + 25ml honey)/kg of body weight orally to cancer patients.

Patients	General condition	HB%		Tumor marker (CEA)	
		Before treatment	After treatment	Before treatment	After treatment
P1	1	75	8	112	110
P2	0	8	7.8	130	120

Table (39) continuous

P3	0	7.8	7.4	120	120
P4	1	6.4	7.5	92	96
P5	0	5.5	6.1	160	160
P6	1	6.2	6.5	125	125
P7	0	7.8	7.4	130	130
P8	0	9	8	92	92
P9	0	7.2	7	80	80
P10	1	6.9	6.5	30	30
P11	0	8	8	66	70
P12	0	8.5	8	82	80
P13	1	8.1	8	120	130
P14	1	8.2	7.5	116	115
P15	0	7	7.5	146	140
P16	0	7.1	7.2	92	94
P17	0	7.4	7	90	90
P18	0	7.8	7.4	88	88
P19	1	6.8	6	98	98
P20	0	6.5	6	112	112
P21	0	5.9	6	120	122
P22	0	9.4	9	90	96
P23	0	8.5	8.5	66	70
P24	0	7.5	7.6	36	34
P25	1	7.4	7	48	50
P26	1	6.8	7	62	60
P27	0	6.4	6.5	112	110
P28	0	7.5	7	120	120
P29	0	8.2	8	90	90
P30	0	8.1	8.1	88	90
P31	1	8.4	8	90	88
P32	1	8.3	8	115	112
P33	0	7.5	7.6	96	96
P34	0	7.6	7.7	90	90
P35	0	7.8	7.8	70	70
P36	1	8.2	8.1	60	60
P37	1	8.5	8	58	58
P38	1	9.1	9	46	46
P39	0	8.7	8.5	120	118

Table (39) continuous

P40	0	9.1	9	124	120
Mean ± SE	26 Zero (65%) 14 One (35%)	9.35 ± 1.69	7.53 ±0.13	94.55 ±4.72	94.50 ± 4.63

No change:0 Improved:1 Worsened:2

Values is mean ± SE

4.6.6 Effect of the oral dose with 1g (1mg of lyophilized bee venom + 500mg of lyophilized royal jelly + 25ml honey)/kg of body weight orally to chronic liver disease (CLD) patients:

Data in Table (40) showed that, the effect of treat 40 patient with 1g (1 mg lyophilized bee venom + 500mg of lyophilized royal jelly + 25 ml honey)/kg of body weight orally to chornic liver disease (CLD) patients for two months , the result was 24 (60%) patients improved and 16 (40%) patient have no change. The details were as follows brilirbin before the treatment was 2.97 which was decreased and become 2.04 after the treatment. While the PT was 17.82 before the treatment and decreased to 15.60, also the SGPT was 70.40 before the treatment and decreased to 50.08 after the treatment. Albumin was 2.55 before the treatment and increased by 0.33 and become 2.88 after the treatment. Finally, PCR million was 2.09 before the treatment and decreased to 1.10 after the treatment. These results are in an agreement with those obtained by **Son *et al.* (2007), Bogdanov *et al*. (2011a) and Bogdanov *et al*. (2011b).**

Table (40) Effect of the oral dose with 1g(1mg of lyophilized bee venom+ 500mg of lyophilized royal jelly + 25ml honey)/kg of body weight orally to chronic liver disease (CLD) patients.

Patients	L L edma ascites	Bilirbin		PT		SGPT		Albumin		PCR milion	
		pre	post	pre	post	pre	post	pre	post	Pre	Post
P1	1	2.4	1.8	18	16	113	40	2.6	2.9	1.2	0.8
P2	1	1.5	0.9	16	14	70	45	2.2	2.6	0.7	0.5
P3	0	5.2	3.2	22	18	68	55	2.2	2.4	2.5	0.4
P4	1	3.5	1.8	20	18	112	60	2.2	2.8	3.2	1
P5	1	2.6	1.4	18	10	90	77	3	3.2	1.4	0.6
P6	0	3.1	2.5	17	15	100	48	2.5	2.5	0.4	0.2
P7	0	1.8	0.9	16	14	120	55	2.6	2.7	0.6	0.6
P8	1	1.7	0.8	15	13	92	60	2.4	2.8	1.6	1
P9	1	1.2	0.8	15	14	58	38	2.5	3.2	2.4	0.8
P10	1	3	1.9	18	15	40	40	2.4	3.1	1.8	0.9
P11	0	2.5	1.2	17	14	58	40	2.5	2.6	1.7	1
P12	0	4.6	2.5	20	15	62	55	2.3	2.4	1.5	0.5
P13	0	3.5	2	19	15	39	35	2.2	2.3	2.2	0.4
P14	1	2.6	1.8	10	14	112	60	2	2.5	2.5	1
P15	0	3	2.6	17	13	70	50	2.8	2.9	1.6	0.8
P16	0	3.7	2.4	17	14	70	35	2.2	2.4	1.8	0.2
P17	1	2	0.9	15	14	82	48	2.6	3	1	0.3
P18	0	4.2	3.6	20	17	92	90	2.7	2.7	1.1	0.5
p19	1	2.5	1.4	18	16	116	52	2.9	3.2	0.9	0.4
P20	1	2.6	1.5	18	15	48	48	2.8	3.1	1.5	1
P21	1	2.8	1.6	17	15	56	40	3	3.4	1.4	0.8

Table (40) continuous

P22	1	3.1	2	18	15	40	40	2.5	2.8	2	0.6
P23	1	1.9	1	16	14	78	44	2.6	3.2	3	1.5
P24	1	1.7	0.9	15	13	111	68	2.7	3.1	5	2
P25	0	5.2	9	24	20	92	80	3	3.2	2.5	1.2
P26	1	4	2.5	22	20	88	70	2.8	3.2	4.2	2.5
P27	1	3.4	2.1	20	18	68	50	2.7	3	3.1	3
P28	1	2.6	1.8	18	18	77	52	2.5	2.9	2.9	3
P29	1	2.8	1.9	19	17	48	40	2.6	3	2.7	2.5
P30	0	3.1	2	17	15	55	50	2.2	2.3	5.1	2.5
P31	0	3.6	2.5	18	16	49	40	2.5	2.8	2.5	1.5
P32	1	2.7	1.6	16	14	60	42	2.2	2.6	3.1	2.5
P22	1	2.8	1.7	15	14	72	35	2.4	2.8	1.7	1
P34	0	3.1	2	20	18	78	40	2.3	2.5	2.1	1.5
P35	0	2.4	1.7	19	18	49	42	2.5	3.6	0.9	0.5
P36	0	2.6	1.9	18	17	55	50	2.6	2.7	0.8	0.4
P37	1	4.1	2.5	20	16	78	47	2.7	3.2	1.2	0.8
P38	1	5.1	3.7	22	20	67	52	2.8	3.2	2.1	1.0
P39	1	2.5	1.9	18	18	66	50	2.9	3.2	3	0.9
P40	0	1.9	1.5	15	14	77	40	3	3.2	2.7	1.5
Mean ± SE	16 Zero (40%) 24 One (60%)	**2.97 ± 0.16**	**2.04 ± 0.21**	**17.82 ± 0.40**	**15.60 ± 0.35**	**74.40 ± 3.59**	**50.08 ± 2.0**	**2.55 ± 0.04**	**2.88 ± 0.05**	**2.09 ± 0.17**	**1.10 ± 0.12**

L L ascites : No change :0 Improved:1 Worsend:2

Values is mean ± SE

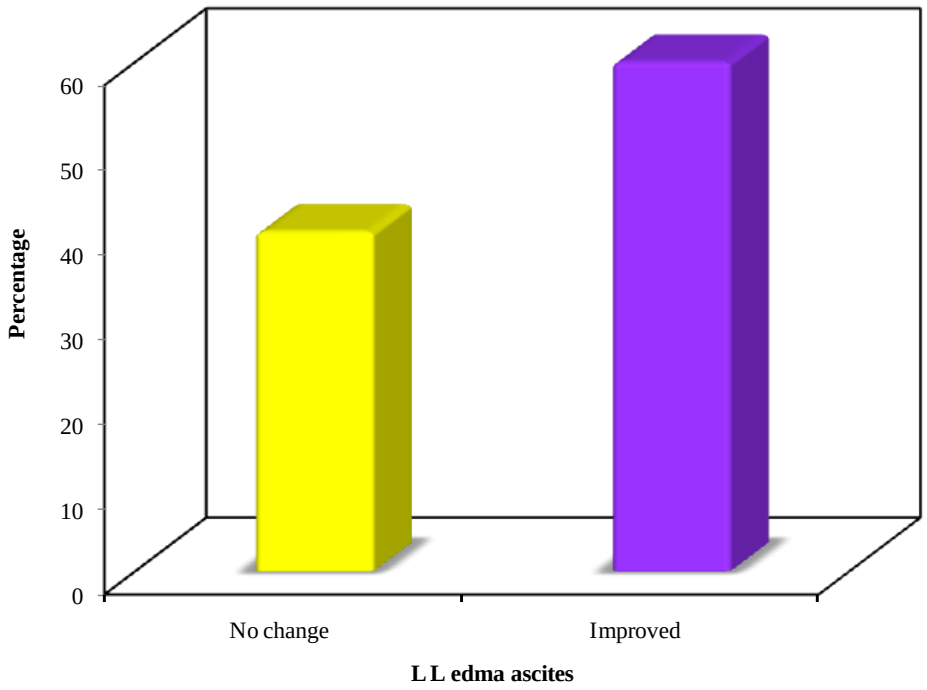

Fig. (5): The Effect of oral dose of with 1g(1mg of lyophilized bee venom+ 500mg of lyophilized royal jelly + 25ml honey)/kg of body weight orally to chronic liver disease (CLD) patients.

SUMMARY

V.SUMMARY

Honey bee produces seven types of productions:

1 – Honey.

2 – Wax.

3 – Royal jelly.

4 – Pollen (bee bread).

5 – Propolis.

6 – Bee larva.

7 – Bee venom.

These products are considered to be as food and medicine. This study was for honey, royal jelly and bee venom, in this study we were saved bee venom and royal jelly by lyophlization then dissolved them in honey to have a dietary supplement (1mg of lyophilized bee venom +500 ml of lyophilized royal jelly +25 ml honey) Therefore, this study was conducted in order to investigate the effect of having the dietary supplement orally and to study its effect on the following diseases:

1 – Diabetes.

2 – Anemia(megaloblastic anemia).

3 – Cancer.

4 – Chronic liver disease (CLD).

5. Rheumatoid arthritis (R.A).

This study aimed at the following points:

1 - Physical, chemical, Microbiological and Sensory evaluation of bee honey samples of Clover, Citrus, Cotton and Buckthorn.

2 - Study the Chemical composition of royal jelly after and before lyophilization.

3 - The study of the chemical, physiological prosperities and calculate the lethal dose LD_{50} of bee venom.

4 - Study the effect of feeding newborns lyophlized royal jelly and the impact on weights and lengths of them.

5 - Biological experiments on rats.

6 - Study of the using of the dietary supplement and its therapeutic effect on some human disease like cancer, chronic liver disease (CLD), anemia, rheumatoid arthritis (R.A) and diabetes.

Part (1):

1. Physicochemical characteristic, microbiological and sensory evaluation of honey bee samples.

1.1 Physical characteristics of honey bee sample:

PH of honey bee samples was ranged between 3.89-4.23 for Citrus honey and Buckthorn honey respectively, the Specific gravity of honey bee samples which was ranged between 1.42 - 1.59 for Citrus, Buckthorn and Clover honey bee samples respectively. Brix of honey bee samples was ranged between 78.99 -80.17 for Buckthorn honey and Citrus honey respectively. The total acids as (gluconic acid) samples was ranged between 4.20 – 4.40 for citrus honey and Buckthorn honey respectively.

1.2 Viscosity of bee honey samples:

Cotton honey exhibited the highest viscosity and Buckthorn honey, Clover honey was second in respectively, the viscosity in honey bee samples inversely proportional with temperature.

1.3 Freezing point depression, moisture percentage of bee honey samples:

Freezing point depression of honey bee samples for honey solution (15%) was ranged between -1.53 Cº to -1.22Cº for Buckthorn honey and Cotton honey respectively, for honey solution (68%) was ranged between -5.82Cº to -5.43Cº for Buckthorn honey and Clover honey respectively. Moisture percentage of bee honey samples was ranged between 17.12-19.16 for Cotton honey and Citrus honey respectively.

1.4 Chemical characteristics of bee honey samples:

Total sugars content of bee honey samples was ranged between 78.39 – 80.72 for Citrus honey and Clover honey respectively. Protein content of honey bee samples was ranged between 0.23-0.27 for Citrus honey and Clover honey respectively.

Part (2):

2. Effect of lyophlization on chemical composition of royal jelly.

2.1 The effect of lyophlization on royal jelly:

This study recognize that the moisture content of royal jelly ranged between 64.17 – 68.15 before lyophlization, after lyophlization moisture was ranged between 3.41 – 5.62. Protein content of royal jelly ranged between 25.90 – 27.44 after

lyophlization, before lyophlization the protein was 8.62- 9.14. (HAD) 10- hydroxyl-2deconoic acid was ranged between 2.71 – 2.91 before lyophlization, after lyophlization it was 8.13 – 8.70.

2.2 Mineral content of royal jelly:

The mineral content of royal jelly of Potassium, Calcium, Magnesium, ZinK, Iron, Copper was (600.0, 60.0, 59.0, 0.90, 6.0, 0.30) mg/100 g.

2.3 Vitamin content of royal jelly:

The vitamins content of royal jelly of Niacin (B3), Pyridoxine (B6), Thiamin (B1), Riboflavin (B2) and Pantothenic acid was (12.20, 3.10, 1.30, 1.50 and 3.20) mg/100g.

2.4 Hormones content of royal jelly:

The hormones content of royal jelly of Testosterone, Progesterone, Prolactine and Estradiol was (0.20, 4.61, 70.80 and 52.03) (moles/100g).

Part (3):-

3. Chemical composition, physiological prosperities and lethal dose LD_{50} of bee venom.

3.1 The production of bee venom for one year from one colony (mg)/ day and its moisture content (fresh and after lyophilization):

The highest production of bee venom from one colony was in September, reaching to 310mg /day, while the lowest production was in January reaching to 210mg / day.

3.2 Chemical composition of fresh and lyophlized bee venom:

The moisture content of bee venom was 84 - 90 % before lyophlization and it was 5 - 8 % after lyophlization, the phospholipids was ranged from 1.20 – 1.50% and became 9.60 – 11.20% after lyophlization, hyaluronidase was ranged from 0.28 – 0.32% after lyophlization it became 2.0 – 2.20% **.** On the other hand lyophilized bee venom content of melitine was ranged between 52.0 – 56.0%. Ash content bee venom was 0.06 % before lyophlization and after lyophlization it was 0.46 while a total sugar was 0.31% before lyophlization and after lyophlization it was 1.90.

3.3 The physiological composition of lyophlized bee venom:

Physiological composition of lyophilized bee venom samples for Histamine % was ranged between 0.75 – 0.80 on April and January respectively. Dopamine% was ranged between 0.20 – 0.25 on January and December respectively. Noradrenalin% was ranged between 0.08 – 0.10 on April, October and January respectively.

3.4 Mean lethal dose L.D50 of the bee venom after subcutaneous injection in adult and weanling male and female:

LD_{50} for adult's male and female rats was (1 – 6) mg/kg of body weight; the same result was for weanling male and female rats.

Part (4):

4. Effect of feeding with lyophlized royal jelly on infants.

4.1 The effect of feeding non breast feeding on infants (formula feeding) using artificial milk +300 mg lyophilized Royal Jelly on (lengths and weight):

Weight of infants who had formula feeding of using artificial milk with 300mg of lyophilized royal jelly was increased from 2642.50 g (the new born weight) to 8503.50 g at 12 months and their length was increased from 52.75cm at the new born to 79.0cm at the 12 months, beside that these infants were preterm and was born at 7 months, so there curves of weights and lengths is similar to infants that born on 9 months and feeding breast feeding only. The most important thing that infants who had formula feeding did not have any antibiotics as a treatment all the period of having that formula.

Part (5):

5. Biological experiments of rats.

5.1 Effect of treatment with 1g(1mg of lyophilized bee venom +500 mg of lyophilized royal jelly + 25 ml honey)/kg of body weight on some diseases.

The use of bee venom and royal jelly improved the therapeutic efficiency of some diseases: improving the sexual efficiency, the functions of the kidney and decrease the rate of glucose by using the doses of 500 mg and 1 gram of (1mg of lyophilized bee venom +500mg of lyophilized royal jelly +25mg honey) / kg of body weight orally daily for adult male rats for a month. This study showed that the treatment of adult male rats

by 1 gram (1mg of lyophilized bee venom +500mg of lyophilized royal jelly +25mg honey) / kg of body weight orally alone resulted in increasing the weights of the testis and the body of the epididymus, sperm count, testosterone hormone, the percentage of live sperm, and glutathione level, accompanied with decrease in malondialdehyde level and the percentage of sperm abnormality. The oral administration of (1mg of lyophilized bee venom +500mg of lyophilized royal jelly +25mg honey) in doses of 500 mg and 1 g/kg body weight today for one month increased urea concentration in serum and decreased in urine, urea clearance in urine was also decreased as compared to control group. (1mg of lyophilized bee venom +500mg of lyophilized royal jelly +25mg honey) in a dose of 1 g/kg body weight daily decreased serum, urine and creatinine levels. Decrease in serum glucose level was also observed when the test animals were treated with (1mg of lyophilized bee venom +500mg of lyophilized royal jelly +25mg honey) in a dose of 500mg and 1g /kg body weight daily. Remarkable for one month was showed increasing in body weight of adult rats that treated with 1g (1mg of lyophilized bee venom +500mg of lyophilized royal jelly +25mg honey) /kg of body weight compared with control group.

Part (6):

6. Utilization of the dietary supplement and its therapeutic effect on some human disease like cancer, chronic liver disease (CLD), anemia, rheumatoid arthritis (R.A) and diabetes.

6.1 Effect of using of the dietary supplement of 1g(1mg of lyophilized bee venom +500 mg of lyophilized royal jelly + 25 ml honey)/kg of body weight and its therapeutic effect on Diabetes mellitus (DM) patients:

Effect of treat patients with 1g (1 mg lyophilized bee venom + 500mg of lyophlized royal jelly + 25 ml honey)/kg of body weight orally to diabetes mellitus (DM) patient was decrease in HbA1c%from 8.29% before the treatment to 6.22% after the treatment.

6.2 Effect of using of the dietary supplement of 1g(1mg of lyophilized bee venom +500 mg of lyophilized royal jelly + 25 ml honey)/kg of body weight and its therapeutic effect on Anemia patients:

Effect of treat patients with 1g (1 mg lyophilized bee venom + 500mg of lyophlized royal jelly + 25 ml honey)/kg of body weight orally to Anemia patients there was increase in Hb%from 8.16% before the treatment to 10.32% after the treatment.

6.3 Effect of using of the dietary supplement of 1g(1mg of lyophilized bee venom +500 mg of lyophilized royal jelly + 25 ml honey)/kg of body weight and its therapeutic effect on Rheumatoid arthritis (R.A) patients:

Effect of treat patients with 1g (1 mg lyophilized bee venom + 500mg of lyophlized royal jelly + 25 ml honey)/kg of body weight orally to Rheumatoid arthritis (R.A) patients that for Clinical assessment , Joint swelling & mobility , Morning st.fitnessthere, Analgesia and Satisfaction the results was (76.7%) improved, ESR was 69.33 before the treatment which

decreased to 46.67 after the treatment and Rheumatoid factor was 53.00 before the treatment which decreased to 36.40.

6.4 Effect of using of the dietary supplement of 1g(1mg of lyophilized bee venom +500 mg of lyophilized royal jelly + 25 ml honey)/kg of body weight and its therapeutic effect on Cancer patients:

Effect of treat patients with 1g (1 mg lyophilized bee venom + 500mg of lyophilized royal jelly + 25 ml honey)/kg of body weight orally to Cancer patients, the result was 35% improved, HB% before the treatment was 9.35 which was decreased and become 7.53 after the treatment while the Tumor marker (CEA) was 94.55 before the treatment which decrease a little amount by 0.05 and became 94.50 after the treatment.

6.5 Effect of using of the dietary supplement of 1g(1mg of lyophilized bee venom +500 mg of lyophilized royal jelly + 25 ml honey)/kg of body weight and its therapeutic effect on Chronic Liver Disease (CLD) patients:

Effect of treat patients with 1g (1 mg lyophilized bee venom + 500mg of lyophilized royal jelly + 25 ml honey)/kg of body weight orally to Chornic liver disease (CLD) patients 60% improved, Brilirbin before the treatment was 2.97 which was decreased and become 2.04 after the treatment while the PT was 17.82 before the treatment which decreased and become 15.60, while the SGPT was 70.40 before the treatment which decreased and become 50.08 after the treatment, Albumin was 2.55 before the treatment which increased by 0.33 and become 2.88 after the treatment, Finally PCR million was 2.09 before the treatment which decreased and become 1.10 after the treatment.

CONCLUSION AND RECOMMENDATION

VI. CONCLUSION AND RECOMMENDATION

1 -Application of lyophilization technique in saved royal jelly.

2 - Application of lyophilization technique in saved bee venom.

3 -Using mixtures of lyophilized bee venom and royal jelly which are dissolved in honey as therapy of sexual efficiency.

4 - Using mixtures of lyophilized bee venom and royal jelly which are dissolved in honey as therapy of kidney disease.

5 - Using mixtures of lyophilized bee venom and royal jelly which are dissolved in honey as therapy of Diabetes mellitus (DM).

6 - Using mixtures of lyophilized bee venom and royal jelly which are dissolved in honey as therapy of Anemia.

7 - Using mixtures of lyophilized bee venom and royal jelly which are dissolved in honey as therapy of Cancer.

8 - Using mixtures of lyophilized bee venom and royal jelly which are dissolved in honey as therapy of Rheumatoid arthritis (R.A).

9 - Using mixtures of lyophilized bee venom and royal jelly which are dissolved in honey as therapy of Chronic liver Disease (CLD).

10 – Using lyophilized royal jelly for preterm infants who feed artificial milk to increase their weights and lengths.

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Arabic Summary

دراسات على عسل النحل المصرى والغذاء الملكى

الملخص العربى

لعب النحل دورا هاما فى تاريخ الإنسان حيث أنه قبل 700 عام لم يكن قصب السكر موجود فى أفريقيا وأوروبا والذى نقل من جنوب الباسفيك عبر الصين إلى منطقة البحر المتوسط أما بنجر السكر فإن تطويره كمصدر هام للسكر كان حديثا للغاية كان المصدر الرئيسى للسكر قبل هذه الفترة يأتى من عسل النحل وبعض ثمار الفاكهة. ذكر العسل فى القرآن الكريم وغيره من الكتب السماوية كما أستخدم فى الطب الشعبى وكذلك فى دهان الجروح وينتج نحل العسل أيضا الشمع والذى استخدم قديما لعمل القماش الغير منفذ للماء حماية الحديد من الصدأ وكثير من الأغراض الأخرى يستخدم البروبوليس الذى يجمعه النحل فى دهان الجروح وفى بعض التركيبات الطبية وذلك لكونه مضاد حيوى وقد حاز نحل العسل الاعجاب بواسطة الانسان فى العديد من الحضارات.

أنواع النحل التي تربى في مصر:

- *Apis mellifera carnica.*
- *Apis mellifera ligustica.*
- *Apis mellifera jmentica.*

ينتج نحل العسل سبعة منتجات من الطائفة:

1 – العسل. 2 – الشمع

3 – الغذاء الملكي. 4 – حبوب اللقاح (خبز النحل).

5 – البروبوليس. 6 – اليرقات.

7 – سم النحل.

تعريف عسل النحل:

هو السائل الذى تجمعه شغالات النحل السارح من رحيق الأزهار والغدد الرحيقية فى النباتات ويتم تصنيعه داخل معدة نحل العسل فى بطن الشغالة ثم يسلم إلى شغالات الخلية لإنضاجه وتخزينه فى الأقراص الشمعية حيث يفرز ويعبأ.

ويمكن لطائفة النحل إنتاج حوالي من 5–15 كجم سنويا من عسل النحل كما يمكنها إنتاج 10–12 جرام من سم النحل سنويا كما أن إنتاج غذاء الملكات من الطائفة الواحدة سنويا يكون حوالي 100 جرام في جمهورية مصر العربية إلا أن بعض البلاد مثل الصين واليابان تمكنوا من استخدام تكنولوجيا إنتاج ملكات النحل بطريقة تجارية حيث وصلوا إلى إنتاج حوالي 8 كجم من غذاء الملكات من الطائفة الواحدة في السنة . كما أن انتاج الطائفة الواحدة من حبوب اللقاح يصل الى 100 جرام يوميا في موسم إنتاج حبوب اللقاح. كما يمكن إنتاج حوالي 8 كجم من اليرقات سنويا من الطائفة الواحدة بجانب إنتاج الخلية للبروبوليس (صمغ النحل).

سم النحل:

هو عبارة عن إفراز سائل حامضى تفرزه الغدد الحامضة Acid gland تسمى غدد السم حيث ان LD_{50} لسم النحل 2,5ملجم/كجم.

ويتم إنتاج السم عن طريق تعريض شغالات النحل لتيار كهربي 12 فولت و 3 أمبير لمدة أربع ساعات حيث تقوم شغالات النحل تحت هذا التأثير بلدغ كيس من البلاستك أسفله لوح زجاجي حيث يوضع سم النحل على اللوح الزجاجي. و بمجرد خروج السم من شغالات النحل يفقد حوالي 50% من رطوبته ويكون به نسبة من شعر النحل وحبوب اللقاح وأحيانا من البروبوليس ولايصلح السم في هذا الوضع في الإستخدامات الطبية والعلاجية أو الغذائية.

تجهيز السم:

1– يتم إذابة سم النحل بكمية قليلة من الماء المقطر (1جم سم نحل / 1سم ماء مقطر).

2- يتم ترشيح السم باستخدام مرشحات 0,02 ملم للتخلص من الشوائب.

تجفيد السم:

1- يتم بتجميد السم على درجة حرارة -50 °م حتى يمكن فصل بروتينات السم المجمدة عن بلورات الماء المجمدة.

2- يتم تبخير بلورات الثلج (التسامي) وتتم هذه الخطوة من خلال الاتي:

أ. تثبيت الحجم.

ب. خفض الضغط إلى -0,8 بار.

ج. رفع درجة الحرارة إلى -10° م.

وتستمر هذه العملية لمدة 13 ساعة حتى نتخلص من الرطوبة وتكون رطوبة السم أقل من 5% وبذلك نحصل على سم النحل نقي خالي من الشوائب وبنسبة رطوبة 5% لاتسمح باتمام التفاعلات الإنزيمية للحفاظ على إنزيماته بصورة نشطة لحين الإستخدام الطبي أو الغذائي.

غذاء الملكات:

هو إفراز غدى لشغالات النحل صغيرة السن بواسطة زوجين من غدد بلعومية ويسمى (لبن النحل).

خطوات إعداد وتجهيز غذاء الملكات:

1- يتم الحصول على غذاء الملكات عن طريق تربية ملكات النحل حيث يفرز غذاء الملكات من غدة تسمى hypo pharyngeal gland .

2- يتم الحصول على غذاء الملكات من كل بيت ملكي بمعدل (كل بيت ملكي 100-250 ملجرام) من البيت الملكي الواحد.

3- يتم إذابة غذاء الملكات بكمية مناسبة من الماء المقطر (1جم غذاء ملكات/ 1سم ماء مقطر) ثم تجمد عند درجة حرارة -70°م لمدة ثلاث ساعات.

4- المرحلة الثانية من التجفيد وتتم عن طريق:

أ. تثبيت الحجم.

ب. خفض الضغط إلى −0,8 بار.

ج. رفع درجة الحرارة إلى −10°م.

وتستمر هذه العملية حوالي 48 ساعة حتى يتم تبخير بلورات الثلج (التسامي) ونحصل على غذاء ملكات في صورة مسحوق نسبة الرطوبة به أقل من 5% وبذلك تصلح للإستخدامات الطبية والعلاجية والغذائية.

يستخدام في اليابان غذاء ملكات النحل كمادة مضادة لنمو الأورام الخبيثة حيث تحطم الأحماض النووية في خلايا الأورام السرطانية كما أثبتت الأبحاث أن غذاء ملكات النحل مادة فعالة في قتل الميكروبات.

وتعتبر هذه المنتجات غذاء ودواء. وتم في هذه الدراسة حفظ كل من سم النحل وغذاء الملكات بواسطة التجفيد وتم اذابتهما في عسل النحل لعمل مكمل غذائي يتكون من(1مجم سم نحل مجفد+ 500 مجم غذاء ملكات مجفد +25 مل عسل نحل) لذلك أجريت هذه الدراسة من أجل معرفة تأثير استخدام المكمل الغذائي السابق عن طريق الفم وتأثيره على الأمراض الآتية:

1 – السكر. 2 – الأنيميا.

3 – السرطان. 4 – أمراض الكبد.

5 – الروماتيد.

استهدفت هذه الدراسة النقاط الآتية:

1 – دراسة الصفات الكيميائية والفيزيائية والميكروبيولوجية والتقييم الحسي لعينات العسل من عسل الموالح ، عسل البرسيم ،عسل القطن وعسل النبق .

2 – دراسة الخواص الكيميائية لغذاء الملكات الطازج.

3 – دراسة بعض الخواص الكيميائية لغذاء الملكات بعد إجراء عملية التجفيد.

4 – دراسة الخواص الكيميائية والمركبات الفسيولوجية وحساب الجرعة النصف مميتة لسم النحل قبل وبعد التجفيد.

5 – دراسة تأثير تغذية الأطفال حديثي الولادة على غذاء الملكات المجفد وتأثير ذلك على أوزان وأطوال الأطفال ومقارنته بالأطفال حديثي الولادة الغير خاضعين في تغذيتهم على غذاء الملكات.

6 – الإختبارات البيولوجية على فئران التجارب .

7 – دراسة استخدام المكمل الغذائي وتأثيره العلاجي على بعض الأمراض في الإنسان مثل السكر ،السرطان ،الأنيميا ،الروماتويد وأمراض الكبد.

تم تقسيم النتائج المتحصل عليها من هذه الدراسة على ستة أجزاء رئيسية وهي كالتالي:

الجزء الأول:

الصفات الكيميائية والفيزيائية والميكروبيولوجية والتقييم الحسي لعينات العسل من عسل الموالح ، عسل البرسيم ،عسل القطن وعسل النبق .

وأظهرت النتائج المتحصل عليها:

– تراوحت قيم ال pH حيث كانت تتروح قيمه من 3,89 لعسل الموالح و 4,23 لعسل النبق . تم دراسة ظاهرة التوتر السطحي في عينات العسل وكانت 1,42 لعسل الموالح والنبق والقطن و 1,59 لعسل البرسيم كما تمت دراسة رقم ال Brix التركيز بالبركس لعينات العسل وكانت تتراوح مابين 78,99 لعسل النبق و80,17 لعسل الموالح وتمت دراسة الحموضة الكلية وكانت 4,20 لعسل الموالح و4,40 لعسل النبق.

– كانت قيم اللزوجة تتناسب تناسبا عكسيا مع درجات الحرارة في عينات العسل المختلفة.

– كانت نقطة التجميد– 1,22 و –1,53 لكل من عسل القطن و النبق على التوالي عند تركيز 15% بينما كانت نقطة التجميد –5,43 و–5,82 لكل من عسل البرسيم و النبق عند تركيز 68%. بينما كانت نسبة الرطوبة 17,12 و19,16% لكل من عسل القطن والموالح على التوالي.

– كانت أيضا نسبة السكريات الكلية والبروتين 78,39 و 80,72 و 0,23 و 0,27% لكل من عسل الموالح والبرسيم على التوالي.

الجزء الثاني:

الخواص الكيميائية لغذاء الملكات قبل وبعد التجفيد:

– تراوحت قيم نسبة الرطوبة قبل التجفيد لغذاء الملكات 64,17 و 68,15 % وتراوحت نسبة الرطوبة بعد التجفيد 3,41 و 5,62% . وتراوحت نسبة البروتين في غذاء الملكات 25,90 و 27,44% بعد التجفيد بينما تراوحت قبل التجفيد 8,62 و 9,14% وتراوحت نسبة الحمض الدهني (HDA) 2,71 و 2,91% قبل التجفيد وأصبحت النسبة بعد التجفيد 8,13 الى 8,70%.

– تراوحت قيم البوتاسيوم ، الكالسيوم، المغنسيوم، الزنك ، الحديد والنحاس (600، 60، 59، 0,90، 6,0، 0,30) مجم/ 100 جم على التوالي.

– تراوحت قيم فيتامين B_3 ، B_6 ، B_1 ، B_2 وPantothenic (12,20، 3,1، 1,30، 1,50، 3,20) مجم/ 100 جم على التوالي.

– تراوحت قيم هرمون التيستسترون ، هرمون البروجسترون ، هرمون البرولاكتين وهرمون الاستيرويل (0,20، 4,61، 70,80، 52,03) مول/ 100 جم على التوالي في غذاء الملكات .

الجزء الثالث:

دراسة الخواص الكيميائية والمركبات الفسيولوجية وحساب الجرعة النصف مميتة لسم النحل قبل وبعد التجفيد:

– كانت أعلى نسبة لإنتاج السم في شهر سبتمبر حيث كان انتاج الطائفة الواحدة 310مجم/ يوم بينما كانت أقل نسبة في شهر يناير حيث وصلت 210مجم/يوم.

–تراوحت نسبة الرطوبة في السم قبل التجفيد بين(84 – 90) % بينما كانت نسبة الرطوبة في السم بعد التجفيد (5 – 8)% بينما كانت نسبة الملتينين والفسفولبيد2أ الهالوروندين ونسبة الرماد والسكريات للسم قبل التجفيد (6,20، 1,20، 0,28، 0,06و 0,31%) بينما كانت نسب هذه المركبات للسم بعد التجفيد (52,0، 9,60، 2,0، 0,46و1,90 %) .

– فكانت نسبة الهستامين ، الدوبامين والنورادرينالين تتراوح بين(0,77 – 0,80 %) ، (0,20 – 0,25 %) و (0,08 – 0,10 %)على التوالي لسم النحل .

– كانت النسبة النصف مميتة لكل من ذكور واناث فئران التجارب البالغة وحديثة الولادة تتراوح بين (1 – 6 مجم/كجم).

الجزء الرابع:

تأثير تغذية الأطفال حديثي الولادة على غذاء الملكات المجفد وتأثير ذلك على أوزان وأطوال الأطفال:

– أظهرت النتائج المتحصل عليها ان استخدام 0,33 جم من غذاء الملكات المجفد يوميا للأطفال حديثي الولادة المولودين قبل موعدهم والخاضعين للرضاعة الصناعية تشابه منحنيات أوزانهم أطوالهم مقارنة بالأطفال غير الخاضعين لهذه المعاملة والذين تغذوا على الرضاعة الطبيعية لمدة عام ألا أنه لوحظ أن الأطفال حديثي الولادة الذين خضعوا لهذه المعاملة لم يحتاجوا إلى أي علاج باستخدام المضادات الحيوية طوال فترة التجربة.

الجزء الخامس:

الإختبارات البيولوجية على فئران التجارب:

– تم استخدام سم النحل والغذاء الملكي المجفدين لتحسين الكفاءة العلاجية لبعض الأمراض وهي : تحسين الكفاءة الجنسية وتحسين وظائف الكلى وانخفاض نسبة الجلوكوز في الدم حيث تم استخدام جرعات 500 ملي جرام و 1 جرام من المخلوط (25 ملي عسل+ 500 ملي جرام غذاء ملكات مجفد + 1ملي جرام سم النحل المجفد) / كيلو جرام من وزن الجسم عن طريق الفم في الذكور البالغة من فئران التجارب يوميا لمدة شهر حيث اظهرت النتائج المتحصل عليها ان معاملة ذكور الفئران البالغين بجرعة 1 جرام من كل المخلوط السابق / كيلو جرام من وزن الجسم عن طريق الفم يوميا لمدة شهر وحدها نتج عنها زيادة في أوزان الخصية وجسم البربخ وعدد الحيوانات المنوية وهرمون التستوستيرون ونسبة عدد الحيوانات المنوية الحية ومستوى الجلوتاثيون وترافق ذلك مع انخفاض لمستوى المالونالدهيد والنسبة المئوية للحيوانات المنوية المشوهة كما اظهرت النتائج ايضا أن معاملة ذكور الفئران بجرعة مقدارها 500 ملي جرام و 1 جرام من المخلوط السابق / كيلو جرام من وزن الجسم عن طريق الفم يوميا لمدة شهر سببت زيادة تركيز اليوريا في مصل الدم وانخفاضه في البول إضافة إلى نقص اليوريا في البول بشكل ملحوظ مقارنة مع مجموعة الكنترول .كما اظهرت النتائج ايضا ان معاملة ذكور الفئران بالمخلوط السابق بواقع اجرام/ كجم من وزن الجسم عن طريق الفم يوميا لمدة شهر أدت إلى انخفاض مستوى الكرياتتين في الدم والبول.كما لوحظ انخفاض نسبة الجلوكوز في الدم في الفئران المعاملة بالمخلوط السابق بجرعة مقدارها 500 ملي جرام و 1 جرام/ كجم من وزن الجسم لمدة شهر يوميا .كما أوضحت النتائج ايضا حدوث زيادة فى أوزان فئران التجارب المعاملة بالمخلوط السابق بواقع اجرام/ كجم من وزن الجسم مقارنة بمجموعة الكنترول.

الجزء السادس:

دراسة استخدام المكمل الغذائي وتأثيره العلاجي على بعض الأمراض في الانسان مثل مرض السكر ،السرطان ،الأنيميا ،الروماتويد وأمراض الكبد.

– أظهرت النتائج المتحصل عليها أن اعطاء المرضى 1جم(1مجم سم نحل مجفد+ 500 مجم غذاء ملكي مجفد + 25 مل عسل)/ كجم من وزن الجسم عن طريق الفم لمرضى السكر أن نسبة ال HbA% قلت من 8,29% قبل العلاج إلى 6,22% بعد العلاج .

– أظهرت النتائج أن اعطاء المرضى 1جم(1مجم سم نحل مجفد+ 500 مجم غذاء ملكي مجفد + 25 مل عسل)/ كجم من وزن الجسم عن طريق الفم لمرضى الأنيميا أن نسبة ال Hb% زادت من 8,16% قبل العلاج إلى 10,32% بعد العلاج.

– أظهرت النتائج أن اعطاء المرضى 1جم(1مجم سم نحل مجفد+ 500 مجم غذاء ملكي مجفد + 25 مل عسل)/ كجم من وزن الجسم عن طريق الفم لمرضى الروماتويد وأن نسبة التحسن لل Joint sweeling & mobility و Morning st.fitness و Analgesia و Satisfaction كانت 76% وأن ال ESR كان 69,33 قبل العلاج وانحفض إلى 46,67 بعد العلاج وأن ال Rheumatoid factor كان 53,00 قبل العلاج و انحفض إلى 36,40 بعد العلاج.

– أظهرت النتائج المتحصل عليها أن اعطاء المرضى 1جم(1مجم سم نحل مجفد+ 500 مجم غذاء ملكي مجفد + 25 مل عسل)/ كجم من وزن الجسم عن طريق الفم لمرضى السرطان حيث كانت نسبة ال Hb% قبل العلاج 9,35 وانخفضت إلى 7,53 بعد العلاج وأن ال Tumor marker (CEA) كانت قبل العلاج 94,55 وانخفضت بنسبة 0,05 لتصبح 94,50 بعد العلاج وكانت نسبة التحسن 35%.

– أظهرت النتائج أيضا أن اعطاء المرضى 1جم(1مجم سم نحل مجفد+ 500 مجم غذاء ملكي مجفد + 25 مل عسل)/ كجم من وزن الجسم عن طريق الفم لمرضى الكبد حيث تحسنت حالتهم بنسبة 60% حيث كان Bilirbin قبل العلاج 2,97 وانخفضت إلى 2,04 و ال PT كانت 17,82 قبل العلاج وانخفضت إلى 15,60

وكان ال SGPT قبل العلاج 74,40 وانخفضت إلى 50,08 بعد العلاج أما ال Albumin فكان 2,55 قبل العلاج وارتفع إلى 2,88 بعد العلاج في حين كان ال PCR 2,09 قبل العلاج وانحفض إلى 1,10 بعد العلاج.

التوصيات

من النتائج المتحصل عليها يوصى بالآتي:

1 – تطبيق تقنية التجفيد في حفظ كل من الغذاء الملكي وسم النحل.

2 – استخدام مخاليط سم النحل والغذاء الملكي المجفدان المذابان في عسل النحل لزيادة الكفاءة الجنسية.

3 – استخدام مخاليط سم النحل والغذاء الملكي المجفدان المذابان في عسل النحل لعلاج أمراض الكلى، السكر، الأنيميا، الروماتويد ،الكبد والسرطان.

4 – استخدام الغذاء الملكي المجفد للأطفال المبسترين الذين يرضعون رضاعة صناعية لزيادة أوزانهم وأطوالهم.

دراسات على عسل النحل المصرى والغذاء الملكى

رسالة مقدمه من

سمير يوسف عبد السيد يوسف

بكالوريوس علوم زراعية كلية الزراعة بمشتهر - جامعة الزقازيق/ فرع بنها (1992)

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للحصول على درجة دكتوراه الفلسفة

في العلوم الزراعية

(صناعات غذائية)

من

قسم علوم الأغذية

كلية الزراعة بمشتهر

جامعة بنها

2015