Comparative evaluation of different herbal formula and L-Carnitine on reproductive performance of male Moshtohor rabbits

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Abstract

The present study was conducted to compare the effects of L–Carnitine LC, *Nigella sativa NS* and *Panax Ginseng* PG on reproductive functions of male Moshtohor rabbits (MR). Bucks were divided into four groups: (1st group a vehicle-treated C, 2nd group treated with L-Carnitine at a dose of 140 mg/kg BW, 3rd group treated with *NS* oil at a dose of 1.3 ml/kg body weight *NS*, and 4th group treated with P.G. at a dose of 260 mg/kg body weight) for a period of 60 days. LC and PG induced a significant ($P<0.05$) increase in serum Testosterone, Follicle-stimulating hormone (FSH), and Luteinizing hormone (LH) as compared to C and *NS* Groups. Libido (reaction time) and semen characteristics (ejaculate volume /ml, sperm concentration / ml X 10^6, live sperm % and sperm abnormalities %). Treatments enhanced physiological responses reflected by a decrease in serum and seminal plasma of Malondialdehyde MDA and increased the reduced glutathione GSH ($P<0.05$). It could be concluded that LC, *NS* and P.G. Treatments could increase physiological and reproductive performance of male rabbits but LC is more potent of them.

Key words: *L-Carnitine, Nigella sativa, Ginseng, Fertility, and male Moshtohor Rabbits.*

Introduction

Plants and derivatives of plant played a key role in world health and have long been known to possess biological activity. Thirty percent of all modern drugs are derived from plants (Burns, 2000). Plants have a long folklore of use in aiding fertility, including fertility-enhancing properties and aphrodisiacal qualities (D'Cruz, *et al.,* 2010). For several hundred years, people around the world have used locally grown plants as supplements to energize, vitalize, and eventually to improve male sexual functions.

Panax Ginseng (PG) is a widely used as dietary supplement and medicinal herb, in many forms of *Panax Ginseng* PG (Chang *et al.,* 2006). Modern chemical and pharmacological studies indicate that multiple components such as flavonoid, saponins and polyacetylenes are the bioactive compounds in PG and their pharmacological activities include antioxidant, hypotensive, neuroprotective، antibacterial, antitumor, cognitive, sedative, analgesic and anti-stress effects (Attele *et al.,*1999).

Nigella sativa NS belongs to the botanical family of *Ranunculaceae* (Mozaffarian, 1998). However, the effects of N. sativa seeds on fertility parameters are not enough. Samir, (2007) showed that administration of N. sativa oil to hyperlipidemic rats improved their reproductive efficiency and produced additional protection against hyperlipidemia. Mukhallad *et al.,* (2009) concluded that the aqueous extracts of NS increased spermatogenesis of male albino rats. From another point of view carnitine is a quaternary amine (β-hydroxy-γ-N-trimethyammonium butyric acid-M.W. 161.2), and has been known as a vitamin like and amino acidlike substances (Cerretelli and Marconi, 1990). Synthetic carnitine occurs as both D & L isomers; however, only L-carnitine (LC) is physiologically active. The main function of carnitine in the body is facilitation lipid oxidation by transporting long-chain fatty acids into the inner mitochondria region where they undergo β-oxidation (Bieber, 1998) and improve sperm number, quality, and motility in patients with oligospermia (Vitali *et al.,* 1995). Therefore, without carnitine, most of the dietary lipids cannot be used as energy sources and body would accumulate fatty-acids resulting in obesity (Hamilton *et al.,* 1983).

The aim of the present study was to investigate the comparative effects of NS, PG and LC on sexual hormonal profile, semen oxidative stress parameters and semen characteristics of male Moshtohor rabbits.

Materials and Methods

Drug

1. Control group that received normal saline as a vehicle oral administration 0.5 mL/kg body weight.
2. L-Carnitine drops. Dose (140 mg / kg BW) oral administration. According to Khademi *et al.,* (2005). Active ingredient L-Carnitine (3-hydroxy-4-(trimethylazaniumyl) butanoate).
3. Baraka capsules. Dose (1.3 mL/kg BW) oral administration. According to Sherif *et al.,* (2013), the active ingredients Nigella sativa L. oil (NS).
4. Ginseng capsules Hydroalcoholic extract 1: 1 of ginseng root (Panax ginseng) containing 4% ginsenozide - 100 mg. Dose (260mg/kg BW) oral administrations. According to Akram *et al.,* (2012). Active ingredient (6, 20-Bis (β-D-glucopyranosyl)-(3β, 6α, 12β, 20S)-3, 6, 12, 20-tetrahydroxydammar-24-ene).

Experimental groups

The experiment was done on a pure strain of Moshtohor Bucks rabbits (aged 3 months with average weight 1.9 ± 0.1 kg). The animals were

randomly divided into four groups each one comprise 8 animals. The 1st group was kept as a control (C) and treated with vehicle, The 2nd , 3rd and 4th groups were treated with daily oral dose of LC Dose (140 mg / kg BW) , NS oil (1.3 mL / kg BW), and PG (260mg/kg BW), respectively.

Experimental procedure

Blood samples from rabbits were withdrawn from the marginal ear vein using a butterfly catheter (Moore, 2000). Blood was collected at two intervals: after the end of treatment which are persisted two months and after three months of initiated treatment, using nonheparinized glass tubes for complete blood analysis and biochemical assays of serum. Serum was collected in dry clean centrifuge tubes and allowed to clot then centrifuged at 3000 rpm. for 15 min. Semen collection was performed using an artificial vagina. A female rabbit was used as a teaser. Semen was collected at about 9 a.m. Volume of semen ejaculates was recorded and ejaculates were placed in a water bath at 37 ºC according to Breederman *et al.* (1964). Each ejaculate was evaluated manually and examined under the microscope. Semen volume was measured per mL by using a graduated tube. If the ejaculates contain gel excretion, it was measured and then removed (El-Sherbiny, 1987). Sperm concentration was measured by a hemocytometer slide. The percentages of live and abnormal spermatozoa were assessed by the method reported by Blom, (1983). Seminal plasma was separated to determine MDA and GRS from ejaculates by centrifugation at 5000 rpm for 10 min. The recovered seminal plasma fraction was further centrifuged at 10000 rpm for 15 min. at 4 ºC. Fractions of seminal plasma were stored at -20 ºC until analysis.

Blood and semen analysis

Serum and semen Malondialdehyde (MDA) (nmol/L) was determined by HPLC according to Karatepe, (2004). Serum and semen reduced glutathione (GSH) (mg/L), were determined by HPLC according to Jayatilleke and Shaw, (1993). The GSH reference standard was purchased from Sigma Chemical Co., dissolved in 75% methanol in stock 1 mg/mL and diluted before application to HPLC. The samples were analyzed with the Agilent HP 1100 series HPLC apparatus (USA). The analytical column was a µ-Bondapak column (15 cm* 3.9 mm). The mobile phase consisted of 25 mmol sodium dihydrogen phosphate containing 5 mmol tetrabutylammonium phosphate and methanol (87:13% H_3PO_4, pH 3.5), with a flow rate of 1 mL/min and wavelength adjusted at 190 nm.

Hormones

Testosterone, Follicle Stimulating Hormone (FSH), and Lutenizing Hormone (LH) were assayed in blood serum within three months of storage. Determination of testosterone by DRG International, Inc., USA Kit and FSH, LH by ELISA technique (Enzyme Linked Immunosorbant Assay). The kit was obtained from Fortrees Diagnostic Limited, United Kingdom and North Ireland. According to Tietz, (1995).

Statistical analysis

Statistical analysis of the obtained data was performed using the general linear model (GLM) produced by Statistical Analysis Systems Institute (SAS, 1989). Significant differences among means were evaluated using Duncan's Multiple Range Test. The following linear model was applied:

$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \xi_{ijk}$

Y_{ij} = Observation measured

μ = Overall mean

α_i = Effect of treatment .

β_j = Effect of intervals.

$(\alpha\beta)_{ij}$ = Interaction between treatment and intervals.

ξ_{ijk} = Experimental error assumed to be randomly distributed ($\sigma 2 = 0$).

Results and discussion

Data presented in Table1 record the effect of LC, NS and PG supplementation on the sexual hormonal profile throughout the experimental durations. LC and PG induces a significant ($P<0.05$) increase in the serum Testosterone, FSH and LH compared to C and *NS* Groups.

Table 1. Effect of LC, NS, and P.G. on sexual hormonal profile (Testosterone ng/mL, FSH ng/mL, and LH MIU/mL) in Moshtohor rabbits after 2-month treatment and after month of recovery.

Intervals	Treatment	Testosterone	FSH	LH
150 Days After 2 month treatment	C	2.78 ± 0.08^{c}	10.28 ± 0.53^{d}	5.27 ± 0.21^{cd}
	LC	3.21 ± 0.14^{ab}	12.43 ± 0.62^{c}	6.16 ± 0.27^{ab}
	NS	2.91 ± 0.06^{bc}	11.31 ± 0.52^{cd}	6.28 ± 0.23^{a}
	P.G	3.33 ± 0.10^{a}	11.59 ± 0.72^{cd}	5.77 ± 0.34^{abc}
180 Days After Month recovery	C	2.91 ± 0.08^{bc}	12.71 ± 0.53^{bc}	5.387 ± 0.36^{bcd}
	LC	3.40 ± 0.16^{a}	15.16 ± 0.50^{a}	5.28 ± 0.37^{cd}
	NS	3.16 ± 0.10^{ab}	12.53 ± 0.76^{bc}	4.93 ± 0.29^{d}
	PG	3.16 ± 0.15^{ab}	14.04 ± 0.50^{ab}	5.51 ± 0.25^{abcd}

- *Data represents the means ± SEM.*
- *a, b, c and e means having different superscript letters in the same column differ significantly (P<0.05).*

L-carnitine has an important role in increasing testosterone and other steroidal hormone levels in blood stream. This may be due to transports of fatty acids to the mitochondria, where they undergo β-oxidation leading to generation of metabolic energy in the form of ATP needed by the cells to perform their functions. The cytoplasm of the leydige cells shows abundant smooth endoplasmic reticulum, well-developed Golgi complexes, and numerous mitochondria (Bhat *et al.,* 2010). Most of the enzymes involved in the synthesis of testosterone are located in the smooth endoplasmic reticulum and mitochondria of interstitial cells (Austin and Short, 1972).

Two major functions have been identified for the carnitine system (Peluso *et al.,* 2000): firstly facilitating the transport of long-chain fatty acids into mitochondria for their utilization in energy-generating processes and secondly facilitating the removal of short-chain and medium-chain fatty acids from mitochondria that accumulate as a result of normal and abnormal metabolism.

On the other hand, the significant increase in testosterone after treatment and after one month recovery and FSH after recovery from the PG group may be due to structure of PG which is containing Ginsenosides belong to a family of steroids named steroidal saponins (Banthorpe, 1994 and Kim *et al.,* 1998). Steroids possess numerous physiological activities, partly due to the nature of the steroid skeleton. The trans-ring junctions of the skeleton allow substituent groups, which interact with receptors, to be held in rigid stereo chemically defined orientations (Banthorpe, 1994).

In the present study, the assessments of hormones that exist in volatile oil from *Nigella Sativa* was preformed and show a presence of considerable amount of sex hormones (Estradiol, Progesterone, Prolactin, Testosterone, FSH and LH). Increasing of testosterone was not significant but in general the relevant level can be illustrate by stimulation of NS of17 β-hydroxysteroid dehydrogenase, the most important key enzyme in the testosterone biosynthesis (Gromadzka *et al* ., 2002).

In addition, Thymoquinone is the major active component derived from NS and many of the pharmacodynamic effects reported above for N. sativa are due to Thymoquinone (Al-Ali *et al* ., 2008).

Table 2. Effect of LC, NS, and P.G. on serum MDA, seminal plasma MDA, serum GSH and seminal plasma GSH nmol/ml in Moshtohor rabbits after 2 month treatment and after month of recovery.

Intervals	Treatment	Serum MDA	Seminal Plasma MDA	Serum GSH	Seminal Plasma GSH
150 Days After 2 month Treatment	C	5.54 ± 0.31^{a}	1.41 ± 0.08^{bc}	70.94 ± 3.75^{e}	38.53 ± 1.88^{cd}
	LC	3.72 ± 0.22^{c}	1.19 ± 0.05^{cd}	79.12 ± 5.23^{cde}	44.25 ± 1.87^{bc}
	NS	3.18 ± 0.13^{c}	1.54 ± 0.08^{ab}	73.29 ± 3.77^{de}	48.88 ± 2.23^{ab}
	P.G	3.24 ± 0.20^{c}	1.03 ± 0.06^{d}	85.17 ± 3.02^{abcd}	50.65 ± 2.09^{a}
180 Days After Month Recovery	C	5.56 ± 0.34^{a}	1.69 ± 0.07^{a}	81.79 ± 3.41^{bcde}	43.18 ± 1.73^{bc}
	LC	3.41 ± 0.149^{c}	1.34 ± 0.08^{bc}	92.92 ± 3.90^{ab}	40.85 ± 2.12^{cd}
	NS	4.41 ± 0.21^{b}	1.76 ± 0.12^{a}	86.95 ± 3.97^{abc}	37.00 ± 1.28^{d}
	P.G	4.47 ± 0.29^{b}	1.42 ± 0.08^{bc}	96.81 ± 4.08^{a}	39.42 ± 1.94^{cd}

- *Data represents the means ± SEM.*
- *a, b, c and e means having different superscript letters in the same column differ significantly (P<0.05).*

Data presented in Table 2 record the effect of LC, NS and G supplementation on the oxidation system throughout the experimental durations. LC, NS and P.G. enhanced physiological responses reflected by a decrease of serum and seminal plasma MDA and increase of serum GSH (P<0.05).

It is well known also that LC and its acyl derivatives have anti-oxidant properties to decrease MDA and increase GSH, because of their inhibiting effect on xanthine oxidase activity **(**Giacomo *et al.,* 1993). Scavenging effect on Reactive Oxygen Species ROS and suppression of hydroxyl radical production by the Fenton reaction, probably by chelating the iron required for the generation of hydroxyl radicals (Reznick *et al.,* 1992).

Administration of American P.G. was shown to improve antioxidant enzyme activity in rabbits such as GSH. This antioxidant activity may be due to increasing antioxidant enzyme levels and acting as a free-radical scavenger. Our finding is compatible with the finding of (Rana and Verma, 1996). The P.G. Treatment with cadmium administration maintained erythrocyte GSH content. (Ali *et al.,* 2009).

GSH is a tripeptide (L-c-glutamyl-L-cysteinyl-glycine) which forms the largest pool of non-protein thiols in cells and an important intracellular antioxidant.

Under conditions of oxidative stress, GSH reacts either as an electron donor to neutralize hydrogen peroxides and lipoperoxides or as a direct free radical scavenger (Leichtweis and Gi, 2001). In this process, GSH is oxidized to GSSG, which in turn is reduced back to GSH by GSSG reductase at the expense of NADPH, forming a redox cycle (Winterbourn, 1979). In a situation of excess formation of GSSG, cells actively secrete GSSG, and this efflux or tissue accumulation of GSSG has been used as a sensitive

indicator of intracellular oxidative stress could be. (Sakamoto, *et al.*, 2008). In our results, total saponin and panaxatriol could be attributed to an increase in GSH level and a decrease in lipid peroxidation. This suggests that total saponin and panaxatriol would increase GSH and decrease the level of oxidative stress. These results are similar to those of (Zhu *et al.*, 2009). ginsenoside Rg1, one of panaxatriol saponins, obviously reduced ROS generation through increasing the activity of endogenous antioxidant.

Thymoquinone can reduce reactive oxygen species production indirectly and inhibit lipid peroxidation (Al-Majed *et al.*,2006).This may be due to an active effect of NS on the enzymes of oxidative phosphorylation (Azzarito *et al.*, 1996). These results are compatible with Magda *et al.*, (2010) who concluded that NS improved semen characteristics and reduced free radicals in the seminal plasma.

Table 3. Effect of LC, NS, and P.G. on reaction time and semen characteristics in Moshtohor rabbits after 2 month treatment and after one month of recovery.

Intervals	Treatment	Reaction Time	Ejaculate volume	Sperm concentration	Live sperm%	Sperm Abnormalities%
150 Days After 2 month treatment	C	10.35 ± 0.46^{a}	0.91 ± 0.04^{c}	384.51 ± 12.20b	64.61 ± 1.76^{e}	11.72 ± 0.46^{a}
	LC	7.46 ± 0.40^{b}	1.06 ± 0.05^{b}	415.60 ± 10.10^{ab}	80.10 ± 2.21^{ab}	7.99 ± 0.41^{c}
	NS	8.05 ± 0.50^{b}	0.83 ± 0.06^{c}	403.30 ± 11.00^{ab}	72.46 ± 2.88^{cd}	8.31 ± 0.42^{bc}
	P.G	6.86 ± 0.36^{b}	0.83 ± 0.04^{c}	400.40 ± 12.10^{ab}	74.74 ± 3.06^{bcd}	8.55 ± 0.43^{bc}
180 Days After Month recovery	C	5.62 ± 0.24^{c}	0.85 ± 0.03^{c}	385.11 ± 12.70^{b}	71.09 ± 2.42^{de}	12.45 ± 0.39^{a}
	LC	4.86 ± 0.21^{cd}	1.23 ± 0.04^{a}	430.01 ± 8.40^{a}	85.58 ± 1.25^{a}	7.96 ± 0.34^{c}
	NS	4.02 ± 0.26^{d}	0.93 ± 0.04^{bc}	415.40 ± 8.90^{ab}	78.35 ± 2.24^{bc}	6.71 ± 0.33^{d}
	P.G	4.37 ± 0.28^{d}	1.07 ± 0.04^{b}	412.40 ± 10.61^{ab}	79.26 ± 2.31^{abc}	9.48 ± 0.49^{b}

- *Data represents the means ± SEM.*
- *a, b, c and e means having different superscript letters in the same column differ significantly (P<0.05).*

Data presented in Table 3 record the effect of LC, NS and G supplementation on the reaction time, semen characteristics throughout the experimental durations. LC, NS and PG enhanced reaction time, sperm concentration, live sperm, and decrease sperm abnormalities %, (P<0.05).

These studies demonstrate that LC and PG increases sperm parameters mainly through its antioxidant effect, which is reflected in the increased levels of antioxidant enzymes (i.e., catalase, superoxide dismutase and reduced glutathione) and the Total Antioxidant Capacity TAC, as well as through its androgenic activity, which is indicated by an increased testosterone level that promotes spermatogenic activity.

Increased volume of the ejaculates and higher sperm counts in the ejaculate of rabbits receiving LC were also reported by Jacyno *et al.*, (2007). Increased ejaculate volume and more sperm cells have important practical consequences for artificial insemination. An increased sperm count in the ejaculates is probably not due to LC boosting the spermatogenesis, but it results from the fact that the supplement contributes to increased survival of spermatozoa in the epididymis. LC takes part in mitochondrial acetyl- CoA conversion to acetylcarnitine, which prevents accumulation of acetyl groups that inhibit the activity of pyruvate dehydrogenase responsible for mitochondrial energy metabolism (Rebouche and Seim, 1998). This function of LC enhances sperm survivability and, as a consequence, increases the total number of sperm cells in the ejaculate (Jeulin and Lewin, 1996).

LC has been shown to protect cells against mitochondrial and free radical related nuclear DNA damage and to improve mitochondrial functions by reducing stress-mediated DNA damage through reducing the production of oxidants and enhancing antioxidant status (Arockia and Panneerselvam, 2001). Cetinkaya *et al.*, (2006) found that, LC is involved in energy metabolism which promotes sperm motility, maturation and the spermatogenic process by supplying readily available energy to spermatozoa. LC contributes to cellular energy production by accelerating the lipid metabolism, and is especially critical in mitochondrial β-oxidation of long-chain fatty acids.

Acetylcarnitine is a source of energy needed by sperm cells for their progressive movement (Jeulin and Lewin, 1996). A positive effect of LC on sperm motility was found in men and rats (Palmero *et al.*, 1990).

Daily oral administration of NSO and PE for 6 weeks could produce a significant increase in epididymal sperm concentration and sperm motility accompanied with decreased abnormal sperm concentration that could be related to decreased lipid peroxidation in wistar male rats.

The NS (Nigella sativa) group was significantly higher than the (C) control group from sperm concentration. The NS may have decreased abnormal sperm rate in comparison with the control. In this study, improvements observed in sperm quality may be attributed to prevention of excessive generation of free radicals, produced by antioxidant property of P.G. and NS. It was reported by Gökçe *et al.,* (2011) that consumption of thymoquinone, which is the major active constituent of *NS* oil could decrease total antioxidant capacity and prevent the increase in the myeloperoxidase activity.

A clinical study has confirmed the positive effects of PG on sexual impotence; (Choi *et al*., 1995). Our results indicated that PG increased sperm count. In this regards Hwang *et al*., (2004) and (Yamamoto *et al*., 1977) reported that PG increased the survival rate and sperm quality in guinea pigs exposed to 2, 3, 7, 8-Tetrachlorodibenzo-p-dioxin (TCDD) and stimulate the spermatogenesis

Conclusion

L-carnitine (LC), Nigella Sativa *(NS)* and Pamax Ginseng (PG) have increased physiological and reproductive performance of male rabbits and LC is more potent of them.

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