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Efficacy of Jojoba oil compared to its Nano particles on biological and physiological aspects of *Agrotis ipsilon* and its histological effect on albino rats

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ABSTRACT

In recent years, different formulations such as Nano emulsions have been widely used for the target delivery, and enhanced biological functions of pesticide combinations. In this work, contact toxicity, biological aspects, haemocyte counts (THCs) and haemolymph volume against 4th instar larvae of the black cutworm, *Agrotis ipsilon* was determined under laboratory conditions. Mortality rates 4th instar larvae of *A. ipsilon* increased with raising concentration to 3% to reach (64.21%) for Jojoba oil and (86.31%) for 0.1% Nano oil emulsion. The $^{LC}50$ of crude oil was (2.853%), while that for Nano emulsion was (0.381%). Also, Jojoba oil (crude and Nano) showed decrease in larval and pupal weight. While, the resultant female's showed, significant reduction in emergence and lower number of deposited eggs /female. Data also, showed significant decrease in haemocyte counts (THCs) and the volume of haemolymph compared to control. The obtained data confirmed that Nano emulsion was more effective than Jojoba oil. Histological examination after feeding rats on seeds wheat treated with LC_{50} of Nano Jojoba oil was carried out. Liver, kidney and stomach of the treated rats were affected by treatment. Hence, the Nano emulsion slow-release-formulation may represent a new category of bio pesticides and this should be considered in the integrated pest management programs.

***Keywords*:** Jojoba oil, *Agrotis ipsilon*, albino rats, Nanotechnology.

Introduction

The black cutworm, *Agrotis ipsilon* (Lepidoptera: Noctuidae) is one of the most destructive insect pests in Egypt. It is polyphahous in feeding behavior and attacks a large number of field and vegetable crops. (Boughton *et al.,* 2001). The full grown larvae of the cutworm can do considerable damage by cutting the stems of young plants and a larva may cut several plants in a single night. (Muhammed *et al,* .2007). The larval of cutworm cause several considerable to plants it varies from 20-37% but in severe cases the damage occur as much as 80% depending on severity of infestation (Atwal, 1976).

The natural plant products showed a strong disruption of inset growth and development against a variety of insect pests. (Tanzubil and Mccaffery, 1990).Essential oils may have attractive or repellent effects and also showed insecticidal action against insects. (Rodriguez and Levin, 1975). The use of Nano oils will complement previous reports on biological and antimicrobial activities of this Nano oils. (Elumalai *et al.*, 2010). So, a diversified use of Nano oils by the development of their use in pest management sector could be of both economic and ecological benefit (Bixby, 2011).

The presented study aimed to find out the effect of essential oil comparing with Nano essential oil against the black cutworm, *Agrotis ipsilon* under laboratory.

Materials and Methods

Tested insect:-

A laboratory strain of *Agrotis ipsilon* was obtained from Plant Protection Research Institute, A.R.C at Giza, Egypt. It was reared on castor bean leaves under constant conditions of 25 ±2 °C and 75 ± 5% RH. The 4th larval instars were used in laboratory experiments.

Tested oil:-

1-Jojoba oil (crude oil).
2-Jojoba oil (Nano oil).

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Nano oil preparation:-

Nano oil was prepared using. Jojoba oil, non-ionic surfactant, Tween 20 (Hydrophile-lipophile Balance. Value – 16.7) and water coarse emulsion was prepared by mixing Tween 20 and water. The emulsion was then subjected to sonication using a sonicator at a high frequency of 20 KHz and power output of 750w. (National Research Center, Dokki Cairo). Energy input was given through sonicator probe with a probe diameter of 13 mm for 45 minutes which generates strong disruptive forces and reduces droplet size of emulsion (Ghotbi, *et al*., 2014).

Tested essential oil (crude and Nano) four concentration were prepared (3, 1.5, 0.5 and 0.2%) for tested crude essential oil and in case of Nano essential oil. The tested concentrations were (0.1, 0.5, 0.05 and 0.005%).

-Larvicidal activity of tested oil (crude and Nano).

Five replicates were used for each tested oil (crude and Nano), each replicate contain twenty 4th instar larvae of *Agrotis ipsilon* which were fed on treated and untreated castor bean leaves till pupation. The percentages of larval mortality were calculated and corrected according to Abbott's formula, while LC_{50} value was calculated through probit analysis. Also, larval weight, and pupal weight were estimated. On the other hand, percentage of adults emergence and number of eggs laid were recorded according to the equation of Lwande *et al*., 1988) as D = (1-T/C) ×100; where T and C represent the mean number of deposited eggs per female of the treated and check, respectively.

Haemolymph studies:-

The 2nd and 4th instar larvae of *Agrotis ipsilon* were fed for 48 hours on semi artificial diet treated with LC_{50} of Jojoba oil. (Crude and Nano) as previously computed from the susceptibility tests. Larvae were then transferred to untreated food to determine total haemolymph counts and haemolymph volume.

1-Total haemocyte counts (THCs):

Ten replicates were used in this experiment. haemolymph was allowed to flow on a clean glass slide. A portion of the haemolymph was quickly drawn to the 0.5mark of Thomas white blood cell diluting pipette. This tip was then diluted to the mark 11 with Tuerk's solution (1-2% glacial acetic acid slightly coloured with gentian violet). The haemocytes were counted in the four corners and multiplied by a factor of 50 to give the number of cells per cubic millimeter (Levinson and Macfate, 1951).

2-Haemolymph volume:-

The blood volume was determined by (Yeager and Munson, 1950). 0.81ul amaranth dye in 0.9% NaCl was injected into the haemocoel of each of 10 larvae by Alga micrometer syringe adapted with a thin glass needle which was kept inserted into the insect body to avoid oozing of the haemolymph. Slight pressure was applied to ensure complete circulation of the dye. The haemolymph samples were withdrawn by capillary tubes from incisions made into the cuticle of insects. Haemolymph samples were diluted by 2.3 ml distilled water and protein was precipitated by adding 0.2 ml acetone and quickly centrifuged at 350 rounds per minute. Optical density supernatant (OD1) was measured at 250 nm by a spectrophoto meter. The standard was prepared by diluting the 5 ul of dye solution by distilled water and the optical density (OD2) was measured at 250 nm. Blank was prepared by uninjected haemolymph prepared as previously described.

Mammalian histological studies.

Thirty male albino rats were obtained from the Faculty of Veterinary Medicine, Benha University. Experimental design and animal handling were carried out by the Research Ethical Committee of the Faculty of Veterinary Medicine. Benha University, Egypt Rats were clinically healthy, 15 days old sexually mature, and 60 – 100 gm. weigh. Those were accommodated to the laboratory conditions for a period of one week before experiment Rats were randomly assigned to two groups; first group fed on grain mixed with LC_{50} of Nano Jojoba oil for 20 days while the second group was fed on untreated grain as control three replicates were tested out for each group. Specimens

from vital organs (liver, kidney and stomach) of treated and untreated rats were collected at 10, 15 and 20 days after treatment.

Specimens from these organs were collected and fixed in 10% neutral buffered formalin (Disbrey and Rack, 1970 paraffin-section of 5 microns thickness were prepared, stained by haematoxyline and easin (Harris 1989) and were examined under the microscope. The histopathological changes were recorded and photographed by Leica microscope.

Statistical analysis

The obtained mortality data were subjected to Probit analysis Finney (1971), using a computer Program of Noack and Reichmuth (1978)

The statistical analysis was carried out using ANOVA with two factors under significance level of 0.05 for the whole results using SPSS (ver.19) and data were treated as complete randomization design according to Steel *et al*. (1997).

Results and Discussion

A- Efficacy of Jojoba oil (crude and Nano) on *Agrotis ipsilon*.

Results in Tables (1 and 2) indicated a positive correlation between Jojoba oil (crude and Nano) concentrations and also the time after exposure in relation to *A. ipsilon* larval mortality percentages. Mortality rate was low during the first three days in both Jojoba oil (crude and Nano) treatments. The highest cumulative larval mortality percentage were observed with maximum concentration level especially in case of Nano Jojoba oil (0.1%) which produced 86.31% compared with 64.21% larval mortaliy after 12 days post exposure for the crude oil (3%), Tables (1 and 2). Nano Jojoba oil was more effective against the 4th larval instar of *A. ipsilon*. According to LC_{50} values, (Table 3), the $^{LC}50$s were (0.381%). While, Jojoba oil exhibited latent effect on larvae of *A. ipsilon* (2.853%). The highest concentration of Nano oil (0.1%) caused higher reduction in both larval and pupal weights, being (0.22 and 0.21g) compared with (0.24 and 0.23g), respectively in case of Jojoba oil (3%).

Table 1: Mortality Percentages and some biological aspects after treatment of *A.ipsilon* 4th instar larvae by Jojoba essential oil.

Concentrations	Accumulative larval mortality % at indicated Days						6th instar larval weight (g) ±SE	Pupal weight (g) ±SE	Adults emergence%	No. of eggs laid/female
	1	3	5	7	9	12				
3.0	21.00	26.70	31.29	36.00	42.40	64.21	0.24±0.00	0.23±0.02	34.32	130.00
1.5	15.60	21.00	26.76	32.33	40.00	46.60	0.26 ±0.01	0.27± 0.05	42.33	210.00
0.5	12.30	10.00	22.00	2567	35.70	42.31	0.28 ±0.01	0.03 ±0.11	46.63	350.00
0.2	0.0	6.00	14.33	23.00	30.00	34.76	0.30 ±0.11	0.31 ± 0.00	52.00	560.00
Control	0.0	0.0	0.0	0.0	2.00	4.00	0.38 ± 6.03	0.37 ± 0.03	86.71	914.00

Table 2: Mortality percentages and some biological aspects after A. ipsilon 4th instar larvae treatment by Nano Jojoba oil.

Concentrations	Accumulative larval mortality % at indicated days						6th instar larval weight (g) ±SE	Pupal weight (g) ±SE	Adults emergence%	No. of eggs laid/female
	1	3	5	7	9	12				
0.1	17.76	24.33	35.22	50.00	62.30	86.31	0.22 ±02	0.21 ±0.01	15.30	0.0
0.5	13.26	21.00	23.32	45.07	51.33	75.76	0.26 ±0.01	0.25 ±0.13	20.60	45.0
0.05	7.60	17.67	22.00	40.01	47.50	73.15	0.25 ±0.01	0.25 ±0.12	23.00	60.0
0.005	5.19	13.20	15.70	35.80	42.13	53.42	0.28 ±0.00	0.28 ±0.22	39.10	80.0
Control	0.0	0.0	0.0	0.0	2.22	6.60	0.38 ±0.04	0.35 ± 0.02	85.76	97.00

Table 3: LC_{50} values after *A. ipsilon* 4th instar larvae treatment by Jojoba oil and Nano Jojoba oil.

Compound	LC50 (%)	LC95 (%)	Slope ±SE
Jojoba oil	2.853 (1.08 – 280)	22.930 (10.53 – 143.90)	1.172 ± 0.256
Nano Jojoba oil	0.381 (0.21 – 0.0634)	6.873 (2.65 – 91.61)	1.024 ± 0.253

In addition, the adults emergence% and number of eggs laid per female showed high reduction by treatment with the highest concentration of Nano oil (0.1%) caused 15.30% reduction for adults emergence and the emerged adults didn't deposit any egg compared with Jojoba oil (3%), it produced 34.32% and 130.0 eggs/female. These results agree with El Bendary and El-Healy (2013) and Borie *et al*., (2014) who reported that when neonates of *S. littoralis* treatment with hydrophobic Nano-silica gave highest toxic action at all concentrations after 15 days post application and caused also, reduction on the biological parameters such as larval and pupal durations, adult longevity and females fecundity. Also, Derbalah *et al*., (2014) reported that Nano oils showed slight mortality rate against newly hatched larvae of *Pectionophora gossypiella*. Also, Adel *et al*., (2014) indicated that when geranium essential oil loaded Nano particles more effective on both larval and pupal development as well as longevity, female fecundity and the percentage of hatchability.

Haemolymph studies.

1-Determination of total haemocyte count (THCs) of the larvae treated with jojoba oil (crude and Nano).

Total number of haemocytes in insect varies with development and physiological stages (Beetzetal, 2008). As shown in table (4), the total number of haemocytes decreased gradually by increasing the concentrations of jojoba oil (crude and Nano) compared with control (23262.0±17.25 cells), being 13746.0±23.90 and 11473.0± 200.70 cells when larvae were treated in their 2nd instar and 12430.0 ±16.48 and 10256.0 ±98.72 haemocytes by treatment of the 4th instar with $^{LC}50$ of Jojoba oil (crude and Nano), respectively. The obtained results showed that total haemocyte counts of larvae treated in their 4th instar were less than those recorded from larvae treated in their 2nd instar with LC_{50} level of Jojoba oil (crude and Nano) compared with THCs in control larvae. On the other hand, the total haemocyte counts were reduced in cases of nano Jojoba oil for larvae treated with LC_{50} at the 2nd and 4th instars compared with THCs in Jojoba oil than control.

Table 4: Effect of Jojoba oil (crude and Nano) on the total haemocytes counts per mm^3 haemolymph in fourth instar of *A. ipsilon* larvae.

Treatment concentration (LC50)	Treated larval instar	Mean ± S.E
Jojoba oil (2.853)	2nd	13746.6 ±23.90
	4th	12430.6 ±16.48
Nano jojoba oil (0.381)	2nd	11473.4 ± 200.70
	4th	10256.0 ± 98.72
Control		23262.3 ±17.25

2-Effect of Jojoba oil (crude and Nano) on haemolymph volume:

The volume of healthy 4th instar.*A.ipsilon* larval haemolyph measured 0.269 ±0.0006 ml (Table 5). This volume was found to be decreased, significantly, in the *A. ipsilon* larvae of the same age to reach 0.178 ±0.0006 and 0.12 ±0.0004 ml when larvae were fed in their 2nd instar with $^{LC}_{50}$ of Jojoba oil (crude and Nano), respectively. On the other hand, data showed significant decrease in the volume of 4th instar larval haemolymph compared to control (0.097 ±0.0021 and 0.047 ±0.0012 ml).

Table 5: Effect of Jojoba oil (crude and Nano) on the haemolymph volume of *A. ipsilon* 4th instar larvae.

Treatment concentration (LC50)	Treated of larval instar	Total haemolymph volume / larval (ml)
Jojoba oil (2.853)	2nd	0.178 ± 0.0006
	4th	0.097 ± 0.0021
Nano jojoba (0.381)	2nd	0.121 ± 0.0004
	4th	0.047 ± 0.0012
Control		0.269 ± 0.0006

From these data it could be concluded that the haemolymph of *A.ipsilon* larvae decreased, significantly, in volume with Nano Jojoba oil compared to Jojoba oil (crude) and control. Similar results were reported by Ericsson *et al*., (2009). They reported reduction in haemocyte count after

treatment with Nano oils. Also, the present data agree those obtained by (Abu El Magad and El-kifl, 1993).for *spodoptera littoralis* and *A.ipsilon* larvae infected with *Heterorhabditis* heliothidis nematode.

Sharma *et al*. (2008) found that when *Spodoptera litura* larvae were treated with essential oil of a Corus claims the THC_s decreased only after 48-72 h of treatment. Also, Peter and Ananthakrishnan (1995) noticed that haemocoelic injection of azadirachtin into cyrtacanthacres tatarica last nymphal instar decreased the THCs. Rizk (1991) used C14 dilution techniques to determine the blood volume of *S. littoralis* fifth instar larvae found that blood volume decreased when larvae were treated with insecticides by increasing the concentrations. Also, El-Mandarwy (1992) who found that the blood volume of 4th instar larvae of *Pieris rapae* decreased when larvae were fed on Bactospeine compared to untreated larvae. On the other hand, Reza sadeghi *et al*., (2017) found that when the fourth instar larvae of *Sesamia cretica* treated with essential oil of *Ferula ovina* decreased the number of total and differential haemocyte counts. Also, Ali and Ahmed (2018) showed that when the 4th instar larvae of *Spodoptera littoralis* larvae were treated with essential oils of camphor and castor bean the total haemocyte count (THC) and differential haemocyte count (DHC) were reduced significantly after 48h of treatment compared to controls.

Histopathological results:-

Liver

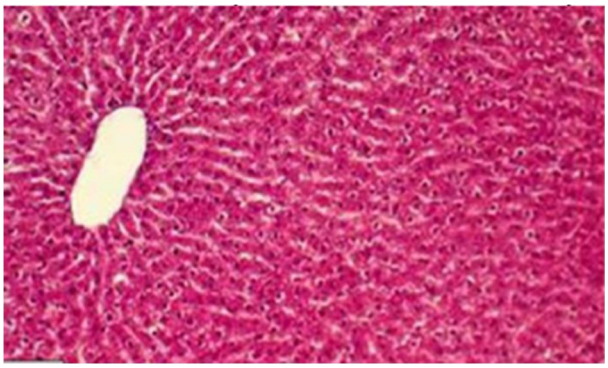

Fig. 1: Control

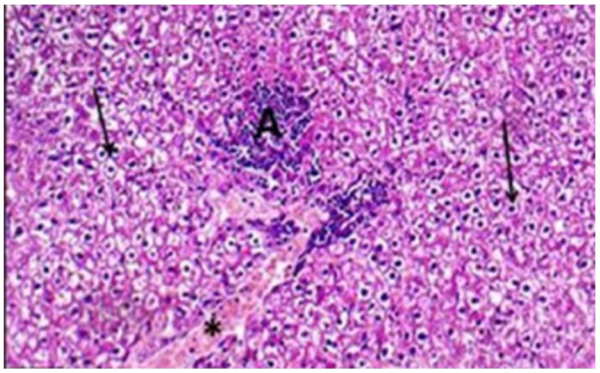

Fig. 2: After 10 days from treatment

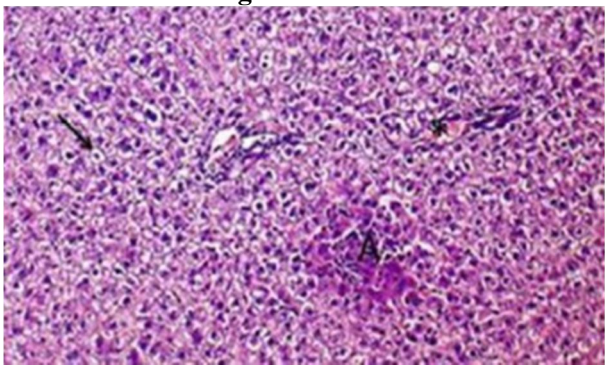

Fig. 3: After 15 days from treatment)

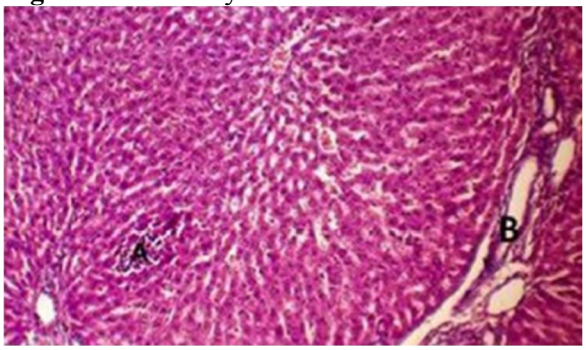

Fig. 4: After 20 days from treatment

Livers of control rats showed normal feature of hepatocytes, central veins and blood sinusoids of the liver. H & E Stain. (Fig-1) At 10 days after treatment, showed wide spread of degenated hepatocytes with severe cytoplasmic vacuolation and midzonal necrosis associated with severe Vascular Congestion (Fig 2). At 15 days after treatment, showed moderate helptoeytic necrosis (A) and mild congestion. (Fig 3). After 20 days of treatment, mild hepatocytic necrosis and mild inflammatory cell in filterates (A) occurred with biliary hyperplasia (B) (Fig 4).

Showing (Fig 5 and 6) control and 10 days after treatment, respectively, having normal structure of renal glomeruli and tubules. At 15 days after treatment, showed mild dilation of (Fig 7) renal tubules (while arrow), while after 20 days of treatment showing glomerular atrophy (star),

sloughing of epithelial cells of renal tubules, dilation and intratubular cellular casts (white arrow) and wide capsular space black arrow) (Fig 8).

Fig (9) shows normal structure of stomach of control group. At 10 days after treatment, Fig (10) showed nearly normal stomach structure and no histopathological changes were detected. Intestine of rat treated with Nano Jojoba oil after 15 and 20 days from treatment showed activation of mucous secreting epithelium with hemorrhagic area Also monoclcar cells ifiltration and large degenerative area.

These results are similar to those obtained by Ragavan *et al*., (2017) who found that the use of garlic oil Nano emulsion/kg for rats treatments, the LD_{50} (0.4 ml/kg) did not exhibit any toxicity and show fewer changes in hemalogical and histological parameters Also, Mivon and setorki (2014) indicated that all concentration of copper nanoparticles induced toxicity and changed of histopathological changes in liver and lung tissues of rats.

On the other hand, Deapsari *et al*., (2017) found that olive oil Nano emulsion caused many changes in liver and kidney of rats but did not cause any changes in stomach. Amal and Elsaid (2012) found that when rats were fed on a diet containing a mixture of olive and fried oil showed many alteration in liver and kidney tissues also, Mossad *et al* (2016) indicated that when rats were fed on low dose of Jojoba seed extract,those showed severe biochemical and histological changes in livers

Kidney

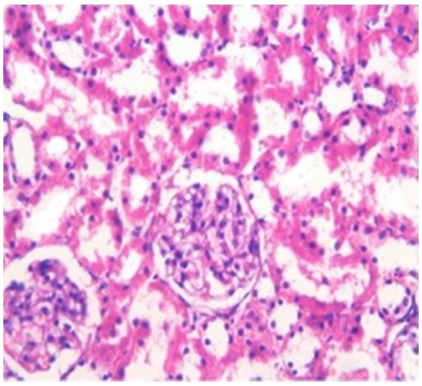

Fig. 5: Control

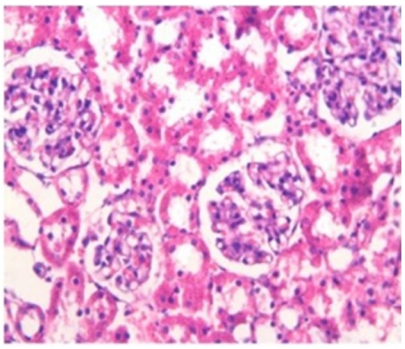

Fig. 6: After 10 days from treatment

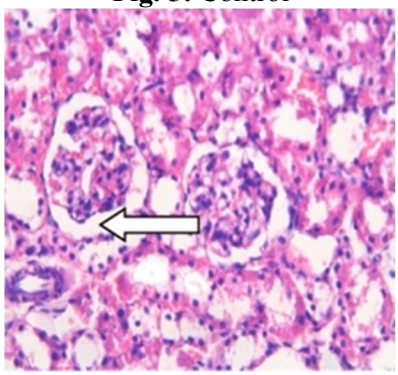

Fig. 7: After 15 days from

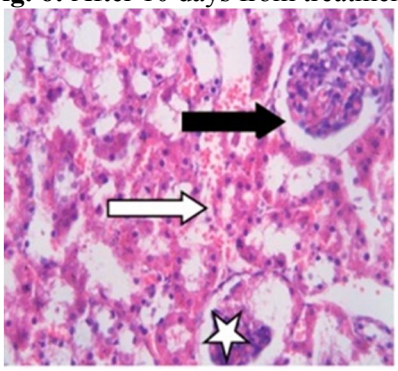

Fig. 8: After 20 days from treatment

Stomach

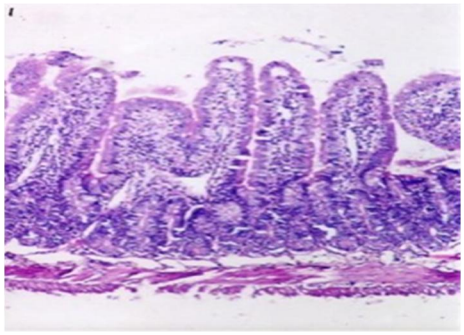

Fig. 9: Control

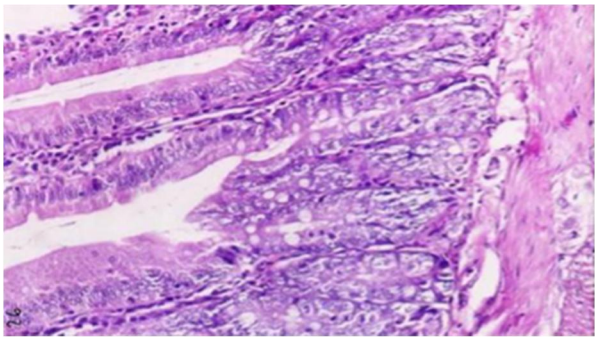

Fig. 10: After 10 days from treatment

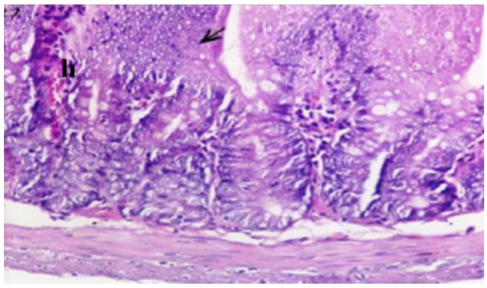

Fig. 11: After 15 days from treatment

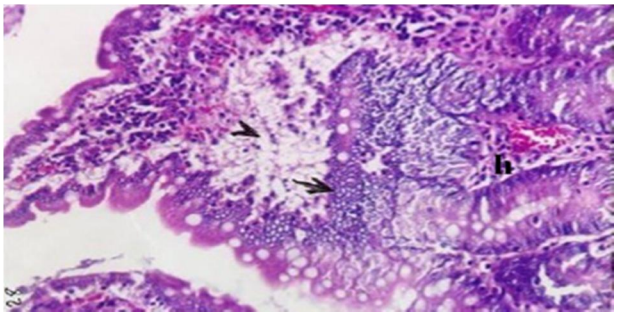

Fig. 12: After 20 days from treatment

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