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Adverse Effects of Abamectin, Sewage Water and Growth Hormone on Nile Tilapia Fish, *Oreochromis niloticus* L.

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ABSTRACT

The biochemical and histopathological changes of abamectin, sewage and growth hormones to the Nile tilapia fish *O. niloticus* L. 60gm bodyweight were studied. The obtained data show that exposed fish to sublethal concentrations of abamectin, sewage (50% diluted with clean water) and growth hormone (6 mg/kg) for 21 days caused some biochemical and histopathological changes in the Nile tilapia fish. *O. niloticus* L. Sewage caused an increase in the level of all serum blood components that took place during the experimental period. Growth hormone caused an increase in the level of ALT, AST, glucose, total protein, albumin, uric acid, creatinine and cholesterol took place during the experimental period. On the other hand, the histopathology studies show degeneration of hepatocytes, and congestion of blood vessels and revealed degenerative changes and cystic dilatation of some renal tubules together with congestion of renal blood vessels and mild to moderate lymphocytic infiltration were detected compared with the control group.

INTRODUCTION

Fish, generally appreciated as one of the healthiest sources of protein, have a Mino acid composition that is higher in cysteine than most other sources of protein in the 1900s, Tilapia species were introduced into most of the world from their original ranges in Africa and the middle east. They are now grown in commercial farming operations in almost 100 countries. Tilapia is likely to be the most important aquaculture species of the 21st century. FAO (1997) estimated that world aquaculture production of Tilapia had reached 659,000 t in 1995. *O. niloticus* was chosen as a model organism due to its high sensitivity in detecting potential adverse effects of chemicals (Uner *et al.*, 2006).

Chemical pollutants and pesticides are the major potential environmental hazards to humans and animals as these are present and concentrated in the food chain. The long-term ecological hazards associated with the use of organochlorine, organophosphate and carbamate pesticides and other pollutants has led to the introduction of a new generation of pesticides. Hendawy and EL-Deeb (2016).

Toxicity testing of chemicals on animals has been used for a long time to detect the potential hazards posed by chemicals to man. The Bioassay technique has been the cornerstone of programmes on environmental health and chemical safety (Ward and Parrish, 1982) and Refaat *et al.* (2018).

Aquatic bioassays are necessary in water pollution control to determine whether a potential toxicant is dangerous to aquatic life and if so, to find the relationship between the toxicant concentration and its effects on aquatic animals (Olaifa *et al.*, 2003). The application of environmental toxicology studies on non-mammalian vertebrates is rapidly expanding, and for the evaluation of the effects of noxious compounds (Ayoola, 2008).

Histopathological changes of gills such as hyperplasia and hypertrophy, epithelial lifting, aneurysm and increase in mucus secretion have been reported after the exposure of fish to a variety of noxious agents in the water, such as pesticides, phenol and heavy metals (Nowak, 1992). Also, the liver is a very important organ that breaks down chemicals and as a result, liver cells are often among those that are damaged by toxic chemicals.

MATERIALS AND METHODS

1-Tested Animal:

Nile tilapia fish, *O. niloticus* L. of average body weight (60 ± 2 g) and total body length (13.5 ± 0.5 cm) from Abbasa, Sharkia governorate. Fish were transferred alive to the Laboratory of pesticide toxicology, Faculty of Agriculture Zagazig University within 2h in plastic bags supplied with sufficient oxygen. Test glass aquaria (60 liter capacity) were used for holding fish (10 fish per aquarium) throughout the experimental period. Fish were left in the test aquaria for one week for acclimatization before starting the experimental study. All the aquaria were kept under the same conditions of temperature (27 ± 2), pH (7.2 ± 0.1), and photoperiod (12 hours light/12 hours' dark) and dissolved oxygen. Fish were fed with a standard diet ad libitum every day a week once a day.

2-Tested Chemicals:

2.1. Abamectin Nasractive (1.8% EC).

2.2. Growth hormone (Boldenone undecylenate), is an androgen and anabolic steroid (AAS) medication which is used in veterinary medicine.

2.3–Sewage water.

Samples of sewage water obtained from the Bahr EL-Baker stream and translated to the Laboratory of Pesticide Toxicology, for fish treatment by 50% dilution with clean water.

3-Acute Toxicity:

The toxicity tests of abamectin were performed according to the USEPA procedure (EPA, 1975). Fish individuals were starved for 48 hours before treatment and 96 hours during the experiment. Mortality was less than 1% during the acclimatization period. A preliminary screen test was carried out to determine the appropriate concentration for the test compound. Each test consisted of a control and three concentrations. Three replications per each concentration with ten fish in each replicate were used. 60-liter glass aquaria were used for fish (60 gm) at the beginning of tests and every 24h, the symptoms and holding period (4 days). The results of LC₅₀ were computed using the EPA probity analysis programs.

4-Biochemical Studies:

To detect the role of increasing the exposure period of fish individuals to a sub-lethal concentration of toxicants on disturbance of the detected biochemical aspects, other groups of fish individuals were continuously exposed to a sub-lethal concentration of the insecticide abamectin the most toxic compound tested. In this respect, a preliminary study was conducted to establish the 96-h LC₅₀ of the insecticide abamectin against the 60 ± 2 gm fish which was found to be 3.69 mg/l. The tested fish (60 ± 2 gm) were divided into three groups, 30 fish of each in three replicates, and then each replicate was placed in glass aquaria (60 Liter). The first group was kept in pesticide-free water as a control, whereas the second group was exposed to a pesticide solution with a concentration of 0.37 mg /l. The

concentration used represents the value of 0.1 of 96-h LC₅₀. The third group was treated with sewage water. The fourth group was treated with growth hormone. Fish transferred to clean aquaria containing clean untreated dechlorinated tap water for 14 days (i. e. recovery period). Fish samples were taken from each group after 1, 2 and 3 weeks as well as 14 days after the recovery period. Three individuals were taken from each group then blood samples were taken from the caudal vein of treated and untreated fish using a clean syringe, collected in centrifuge tubes and left at room temperature until it has clotted. After that, the blood samples were centrifuged at 3000 rpm for 15 minutes and the serum was separated for estimation as some blood serum contents as follows:

- a- Transaminases (AST and ALT)
- b- Total protein
- c- Glucose
- d- Uric acid
- e- Creatinine
- f- Albumine
- g- Sugar
- h- Lipids
 - 1- Triglyceride
 - 2- Cholesterol

5- Histopathological Studies:

This study was conducted to investigate the effects of a sublethal concentration of abamectin, sewage water and growth hormone on Nile Tilapia fish tissue. Some organs (gill, liver, ovary, brain, kidney, testes, intestine and muscle) were removed from samples of treated and untreated fish after 21 days of treatment, as well as the end of the recovery period, and kept in plastic cassettes and preserved in 10% formalin. Fish tissues were processed in an automated tissue processor. The processing consisted of an initial 2-step fixation and dehydration. Fixation comprising tissue immersion in 10% buffered formalin for 48 hours, followed by removal of fixative in distilled water for 30 minutes. Dehydration was then carried out by running the tissues through a graded series of alcohol (70%, 90, % and 100%). The tissue was initially exposed to 70% alcohol for 120 minutes followed by 90% alcohol for 90 minutes and then two cycles of absolute alcohol, each for one hour. Dehydration was then followed by clearing the samples in several changes of xylene. It consisted of tissue immersion for an hour in a mixture comprising 50% alcohol and 50% xylene, followed by pure xylene for one and a half hours. Samples were then impregnated with molten paraffin wax, then embedded and blocked out. Paraffin sections (4–5 μ m–thick sections) were stained with hematoxylin and eosin, the conventional staining technique. Stained sections were examined for necrosis, apoptosis, inflammation, and vascular changes along with the presence of granulomas, and degenerative and or fatty change in different tissues of different groups (Suvarna *et al.*, 2013).

Statistical Analysis:

All data were expressed as mean \pm standard deviation for 30 fish in each group. All the grouped data were statistically evaluated with Co Stat 6.303 software (2004). hypothesis testing methods included one-way analysis of variance (ANOVA) P. p-value of less than 0.05 were considered to indicate statistical significance.

RESULTS AND DISCUSSION

1-Acute Toxicity of Abamectin:

Data in Table (1), indicated that the LC₅₀ of abamectin to Nile Tilapia fish, *Oreochromis niloticus* L. were calculated after different periods e.g., 24, 48, 72 and 96h.post

treatment. The LC₅₀ after 24h. was 1.38mg/l. whereas the corresponding LC₉₀ after the same period was 4.23 mg/l. On another hand, the LC₅₀ values after 48, 72 and 96 hrs post-treatment were 2.55, 3.20 and 3.69 mg/l to the previous period, respectively. The LC₉₀ values determined for the same period were 8.36, 11.11 and 13.45mg/l. The slop values ranged between 2.44 to 4.63 which refers to the homogeneity of the used fish.

Table 1: Acute toxicity of abamectin to the Nile tilapia fish, *Oreochromis niloticus* L. after different periods of exposure.

Time of exposure In h.	LC ₅₀ mg/l	LC ₉₀ mg/l	SLOP
24h	1.38	4.23	2.44
48h	2.55	8.36	2.94
72h	3.20	11.11	3.97
96h	3.69	13.45	4.63

2-Biochemical Studies:

2.1. Biochemical Studies of Abamectin:

Data in Table (2), show that abamectin treatments increased alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity after 7, 14 and 21 days of treatment the increase was high after 21 days recorded (204.33 and 111 U/l to ALT and AST, respectively as for as recovery period (14 days) the ALT and AST activity was returned to the normal level compare with untreated group (195 and 107.3 U/l)

Data also show an increase in total protein, albumin, creatinine and cholesterol activity after 7, 14 and 21 days of treatment, the increase was high after 21 days, as well as, the recovery period (14 days) the activity of the enzyme returned to be normal level compare with untreated group. Uric acid decreased in the treated group compared with the untreated group (control) recording 1.03 mg/dl after 21 days compared with 1.67mg/dl in the control group.

Table 2: Effect of abamectin on Some biochemical components after exposed *Oreochromis niloticus* L. to sublethal concentration for 21 days.

Sample	Storage Period (Days)	Suger mg/dl	Uric acid mg/dl	Albu mg/dl	ALT u/l	AST u/l	Chole mg/dl	Trigl mg/dl	creat mg/dl	Prot Mg/dl
<i>O. niloticus</i>	Cont	77.00E	1.670A	1.66C	190.0E	100.0 0D	250.0c	157.0 D	0.500B	3.600C
	7d	92.33±2 .08C	1.440 ±0.10B	2.013 ±0.15AB	188.67 ±1.52 C	102.0 ±1.00E	244.3 ±1.52B	154. ±1.00B	0.5300 ±0.10B	3.722 ±0.35BC
	14d	99.00 ±1.0B	1.190± 0.10C	2.110± 0.200A	201.00± 1.00B	106.67 ±1.52A	251.0 ±1.00A	160.0 ±1.00B	0.6133 ±0.057AB	4.011 ±0.11AB
	21d	102.67 ±1.52A	1.033± 0.057D	2.153± 0.208A	204.33± 1.52A	111.0 ±1.00A	254.6 ±1.52A	168.3 ±0.57A	0.7200 ±0.10A	4.255 ±0.057A
	Recov	92.0±1. 00D	1.333± 0.057B	1.830 ±0.10BC	195.00 ±1.00D	107.3±0. 57C	250.6 ±1.52C	152.6± 0.57C	0.5433 ±0.057B	3.765 ±0.057BC

All values represent general mean ± SE; those in the same litters differ significantly (Duncan's multiple-range test, p<0.05).

It is well known that, many avermectin compounds such as abamectin show selective toxicity among fish species (Keizerr *et al.*, 1995). The great range of acute toxicity levels among insecticides for many species or for one compound among species may refer to the differences in inhibitory potency for the target and non-target enzymes and metabolism (Boone and Chambers, 1997). Analyses of plasma constituents proved to be useful in the

detection and diagnosis of metabolic disturbance and disease (Aldriin *et al.*, 1982). Many factors affect the biochemical composition of fish such as fishing area, type of food, water quality and pollution (Wassef and Shehata, 1991; El-Ebiary *et al.*, 1997; El-Ebiary and Mourad, 1998; El-Naggar *et al.*, 1998 and Shakweer *et al.*, 1998).

The present study showed significant changes in plasma total protein, albumin, globulin, total lipids, cholesterol, AST, ALT, uric acid, creatinine and sugar activities in the control one and treated fish. Also, these results introduce the health of the cultured fish at this treatment. Protein plays an important role in the metabolism and regulation of water balance (Heath, 1995). It is the basic building nutrient of any growing animal and is also used as an indicator of their state of health (Alexander and Ingram, 1980 and Lea-Master *et al.*, 1990). Regarding the plasma total protein of the Nile Tilapia fish, *O. niloticus* taken from the different works sites is clear that there is a significant difference in the plasma total protein in the untreated (control group) and treated fish.

Uric acid is formed from the metabolism of nucleic acid. Liver cells metabolize purine nitrogenous bases to uric acid. Serum uric acid is produced by the oxidation of hypoxanthine and xanthine by xanthine oxidase and dehydrogenase enzyme it is less toxic than urea and less soluble. High uric acid is associated with a higher risk of type 2 diabetes independent of obesity, dyslipidemia and hypertension. Increased dietary acids and purine intake increased uric acid formation. Uric acid in high levels in blood can cause solid crystals to form within joints. This causes a painful condition called gout. It can also form crystals or kidney stones that can damage the kidney.

2-2. Biochemical Study of Sewage Water:

1 - Effect of Sewage Water on Some Biochemical Aspects (ALT, AST, Albumin, Total Protein and Glucose) in Nile Tilapia Fish, *Oreochromis niloticus* L.:

Environmental pollution and its effects on the health of aquatic ecosystems is a great problem that has been studied intensely in the last few years. The wastewater treatment plants receive large amounts of compounds from domestic and industrial wastes, which are not totally eliminated during the treatment process.

The present data in Table (3), indicated that increase in ALT and AST activity after 7 days of treatment. The previous parameters recorded 205.6 ± 0.57 to ALT compared with 191.0 ± 0.51 mg/dl to control fish after 7 days, and recorded 111.6 ± 1.52 to AST in treated fish compared with 102.0 ± 0.05 mg/dl to control after 7 days.

Data also show an increase in ALT and AST activity after 21 days of treatment. The previous parameters recorded 210.67 ± 0.57 , and 114.0 ± 1.00 to ALT and AST, respectively.

Data presented in Table (3), show an increase in the level of glucose activity. After 7 and 21 days, the application of sewage caused significant increases in serum glucose concentration (104.0 ± 1.0 and 107.4 ± 0.57 mg/dl, respectively). A slight increase was achieved after 21 days as compared with control (90.40 ± 0.005 and 91.00 ± 0.025) after 7 and 21 days respectively.

Table 3: Effect of sewage treatment on some blood component levels (ALT, AST, albumine, total protein and Glucose) (mg/dL) in Nile tilapia fish, *Oreochromis niloticus* L. after 7 and 21 days.

Targets	Exposure periods			
	7 days		21 days	
	Control	Treated	Control	Treated
ALT	191.0±0.5	205.6±0.51	190.0±0.67	210.67±0.57
AST	102.0 ±0.05	111.6±1.52	101.4 ±1.20	114.0±1.00
Glucose	90.40±0.005	104.0±1.0	91.00±0.025	107.4±0.57
total protein	3.500±0.02	4.00±0.10	3.680±0.05	4.500±0.10
Albumin	1.60±0.2	2.34±0.057	1.73±0.02	2.632±0.057

All values represent means± SE; those in the same row differ significantly (Duncan's multiple-range test, $p < 0.05$)

L.S.D. of treatment means (ALT) = 0.7557

L.S.D. of treatment means (AST) = 1.7722

L.S.D. of treatment means (Glucose) = 1.1948

L.S.D. of treatment means (total protein) = 0.1309

L.S.D. of treatment means (Albumine) = 0.1195

The present data in Table (3), indicated an increase in T.P and ALB activity after 7 days of treatment. The previous parameters recorded 4.000±0.10 to T.P compared with 3.600±0.02 mg/dl to control after 7 days. and recorded 2.333±0.057 to ALB compared with 1.70±0.2 mg/dl to control after 7 days.

Data also indicated an increase in total protein and albumin activity after 21 days of treatment. The previous parameters recorded 4.500±0.10, and 2.633±0.057, respectively.

2- Effect of Sewage Water on Some Blood Components (Uric Acid, Creatinine, Cholesterol and Tryglycerides) in Nile Tilapia Fish, *Oreochromis niloticus* L.

Data presented in Table (4), show a decrease in uric acid levels. After 7 and 21 days, application of sewage water caused significant decreases in serum uric acid concentration (1.266±0.057 and 0.966±0.115 mg/dl, respectively). A slight increase was achieved after 21 days as compared with the control (1.980±0.05).

Table 4: Effect of sewage treatment on some blood component levels (uric acid, creatinine, cholesterol and triglycerides) (mg/dL) in Nile tilapia fish, *Oreochromis niloticus* L. after 7 and 21 days.

Targets	Exposure periods			
	7 days		21 days	
	Control	Treated	Control	Treated
Uric acid	1.900±0.025	1.266±0.057	1.980±0.05	0.966±0.115
Creatinine	0.490±0.025	0.768±0.05	0.540±0.02	0.943±0.057
Cholesterol	251.0±0.57	261.6±0.57	252.0±0.20	265.3±0.57
Tryglycerides	157.6±0.5	164.7±0.57	158.0±0.025	168.3±1.15

All values represent means± SE; those in the same row differ significantly (Duncan's multiple-range test, $p < 0.05$)

L.S.D. of treatment means (uric acid) = 0.1511

L.S.D. of treatment means (creatinine) = 0.0925

L.S.D. of treatment means (cholesterol) = 0.9255

L.S.D. of treatment means (tryglycerides) = 1.3088

Data presented in Table (4), show an increase in the level of creatinine activity. After 7 and 21 days, the application of sewage water caused significant increases in serum

creatinine concentration (0.768 ± 0.05 and 0.943 ± 0.0575 mg/dl, respectively). A slight increase was achieved after 21 days as compared with the control (0.540 ± 0.02).

Data also presented in Table (4), show an increase in the level of triglyceride activity. After 7 and 21 days, the application of sewage water caused significant increases in serum triglyceride concentration (164.7 ± 0.57 and 168.3 ± 1.15 mg/dl, respectively). A slight increase was achieved after 21 days as compared with control (158.0 ± 0.025).

Data presented in Table (4), show an increase in the level of chlostrole activity. After 7 and 21 days, the application of sewage water caused significant increases in serum chlostrole concentration (261.6 ± 0.57 and 265.3 ± 0.57 mg/dl, respectively). A slight increase was achieved after 21 days as compared with control (252.0 ± 0.20).

2.3. Biochemical Study of Sex-Hormone:

1- Effect of Growth Hormone on Some Biochemical Aspects (ALT, AST, Albumine, Total Protein and Glucose) in Nile Tilapia, *Oreochromis niloticus* L.:

The present data in Table (5), indicated an increase in ALT and AST activity after 21 days of treatment. The previous parameters recorded 197.3 ± 0.57 to ALT compared with 192.0 ± 0.67 mg/dl to control after 21 days and recorded 105.3 ± 0.57 to AST compared with 101.4 ± 1.20 mg/dl to control after 21 days.

Data also show that ALT and AST activity after 14 days recovery from treatment. 192.3 ± 0.57 mg/dl, and 101.3 ± 0.57 mg/dl to ALT and AST The respectively. present results were similar to that recorded for the level of triglyceride as reported by Wafaa *et al.*, 2011, El-Greisy and El-Gamal, 2012 and Montajami. S., 2012).

Data presented in Table (5), show an increase in the level of glucose activity. After 14 days, the application of hormone caused significant increases in serum glucose concentration (95.00 ± 1.00 mg/dl). A slight decrease after 14 recovery days was recorded (91.66 ± 0.57 mg/dl). The present results were similar to those recorded for the level of glucose as reported by Wafaa *et al.*, 2011).

Table 5: Effect of growth hormone treatment on some blood compounds (ALT, AST, albumin, total protein and Glucose) (mg/dL) in Nile tilapia fish, *Oreochromis niloticus* L. after 21 days and 14 days recovery.

Targets	Exposure periods			
	21 days		14 days recovery	
	Control 00 mg/km	Treated 10 mg/km	Control 00 mg/km	Treated 10 mg/km
ALT	192.0 ± 0.67	197.3 ± 0.57	188.0 ± 1.00	192.3 ± 0.57
AST	101.4 ± 1.20	105.3 ± 0.57	101.00 ± 0.005	101.3 ± 0.57
Glucose	91.00 ± 0.025	95.00 ± 1.00	88.00 ± 0.5	91.66 ± 0.57
total protein	3.680 ± 0.05	3.910 ± 0.10	3.590 ± 0.05	3.643 ± 0.057
Albumin	1.75 ± 0.02	1.876 ± 0.057	1.73 ± 0.1	1.676 ± 0.057

All values represent means \pm SE; those in the same row differ significantly (Duncan's multiple-range test, $p < 0.05$)

L.S.D. of treatment means (Glucose) = 1.5113

L.S.D. of treatment means (ALT) = 1.1948

L.S.D. of treatment means (AST) = 1.7722

L.S.D. of treatment means (total protein) = 0.1511

L.S.D. of treatment means (Albumine) = 0.1195

The present data in Table (5), indicated an increase in T.P and ALB activity after 21 days of treatment. The previous parameters recorded 3.910 ± 0.10 to T.P compared with 3.680 ± 0.05 mg/dl to control after 21 days for TP. and recorded 1.876 ± 0.057 to ALB compared with 1.750 ± 0.02 mg/dl to control after 21 days. as for as recovery period (21 days),

T.P and ALB activity were at a semi-normal level compared with the untreated group. Present results were similar to that recorded for the level of glucose as reported by Wafaa *et al.*, 2011, El-Greisy and El-Gamal, 2012).

2- Effect of Growth Hormone on Some Blood Compounds (Uric Acid, Creatinine, Chlostromole and Tryglycerides) Aspect in Nile Tilapia Fish, *Oreochromis niloticus* L.:

Data presented in Table (6), show a decrease in the level of uric acid activity. After 21 days, the application of sex hormones caused significant decreases in serum uric acid concentration (1.766 ± 0.05). as compared with the control (1.990 ± 0.05). A slight decrease was (1.833 ± 0.057) achieved after 14 days of recovery as compared with control (1.940 ± 0.04) The present results were similar to that recorded for the level of uric acid as reported by El-Greisy and El-Gamal (2012).

Table 6: Effect of growth-hormone treatment on some blood compounds (uric acid, creatinine, chlostromole and triglycerides) (mg/dL) in Nile tilapia fish, *Oreochromis niloticus* L after 21 days and 14 days recovery.

targets	Exposure periods			
	21 days		14days recovery	
	Control 00 mg/km	Treated 10 mg/km	Control 00 mg/km	Treated 10 mg/km
uric acid	1.990 ± 0.05	1.776 ± 0.05	1.940 ± 0.04	1.833 ± 0.057
Creatinine	0.540 ± 0.02	0.567 ± 0.057	0.510 ± 0.10	0.533 ± 0.057
Chlostromole	250.0 ± 0.20	257.6 ± 0.57	251.9 ± 0.20	254.0 ± 1.00
Tryglycerides	156.0 ± 0.025	158.0 ± 1.00	156.7 ± 0.02	158.0 ± 0.57

All values represent means \pm SE; those in the same row differ significantly (Duncan's multiple-range test, $p < 0.05$)

L.S.D. of treatment means (uric acid) = 0.0925

L.S.D. of treatment means (creatinine) = 0.0925

L.S.D. of treatment means (cholesterol) = 1.5113

L.S.D. of treatment means (tryglycerides) = 106.56

Data presented in Table (6), show an increase in the level of creatinine activity. After 21 days, the application of hormone caused significant increases in serum creatinine concentration (0.567 ± 0.057). as compared with the control (0.540 ± 0.02). An increase was (0.533 ± 0.057) achieved after 14 days of recovery as compared with control (0.510 ± 0.10) The present results were similar to that recorded for the level of urea as reported by El-Greisy and El-Gamal (2012) and Montajami (2012).

Data presented in Table (6), show an increase in the level triglyceride activity. After 21 days, application of hormone caused significant increases in serum triglyceride concentration (158.0 ± 1.00). as compared with control (156.0 ± 0.025). The present results were similar to that recorded for the level of urea as reported by (El-Greisy and El-Gamal, 2012 and Montajami, 2012).

Data presented in Table (6), show an increase in the level chlostromole activity. After 21 days, the application of sex hormone caused significant increases in serum chlostromole concentration (257.6 ± 0.057). as compared with control (250.0 ± 0.20). A slight increase was (254.0 ± 1.00) achieved after 14 days of recovery as compared with control (250.9 ± 0.20) The present results were similar to that recorded for the level of urea as reported by El-Greisy and El-Gamal (2012). Montajami (2012).

3- Histopathological Studies:

1. Control Group:

Examined sections of this group revealed a normal liver with preserved morpho-histological structures and a normal hepatic pancreas.

-Histological studies Group A (Control).

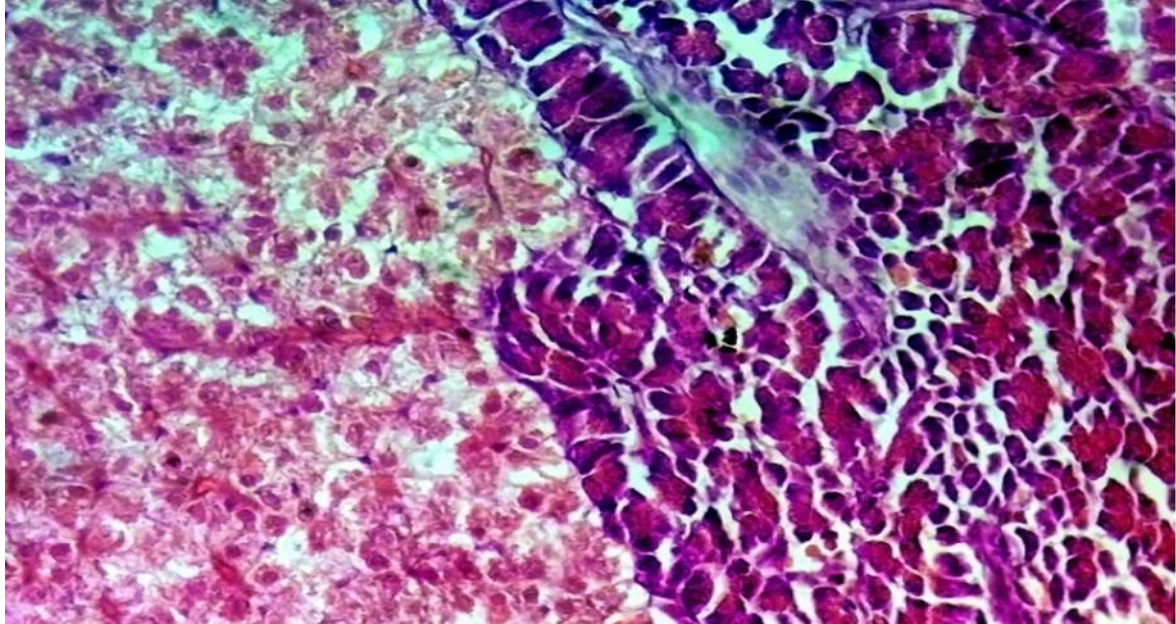


Fig. 1. Liver of group A (Control) with preserved morpho-histological structures and normal hepatic-pancreas (1,2). H&E, X 200.

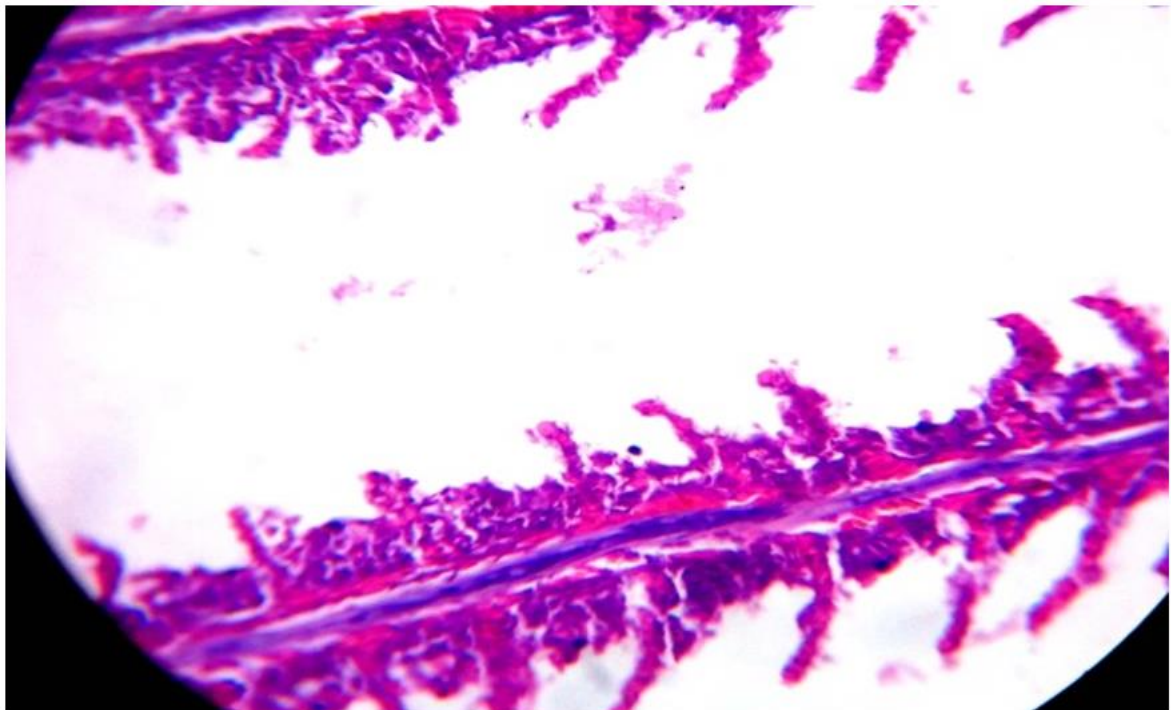


Fig. 2. Gills of Group A (Control) showing normal histological structure. H&E, X 200.

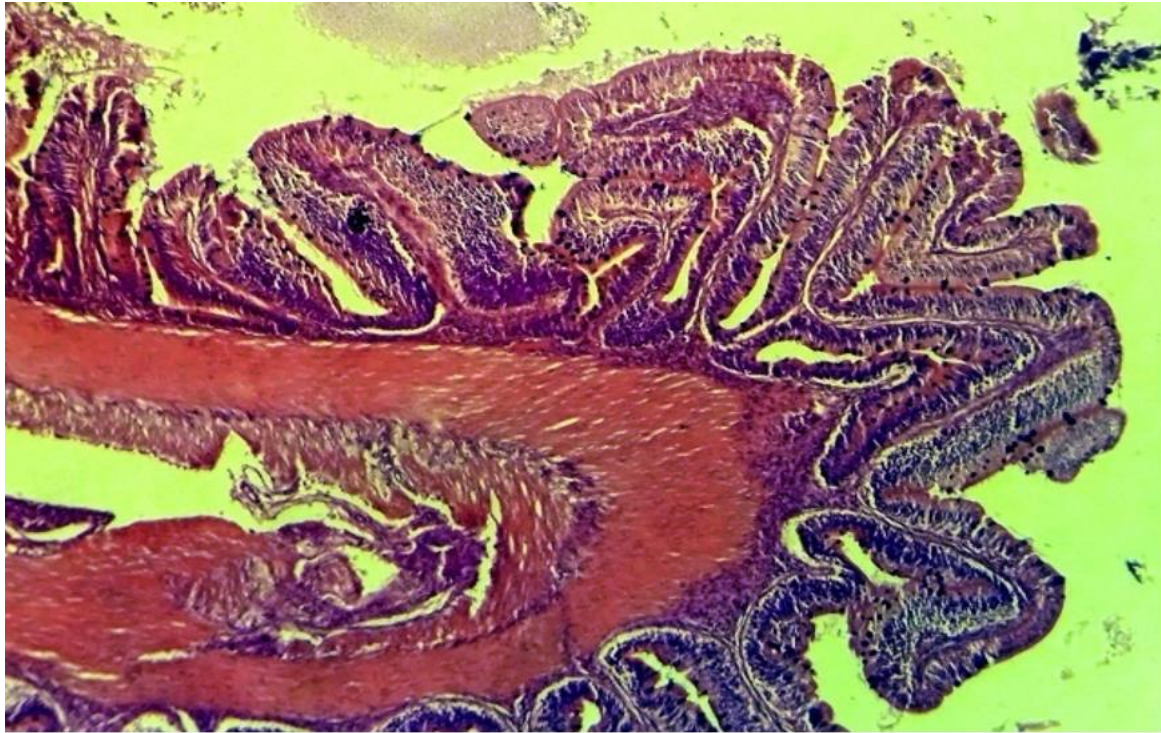


Fig.3. Intestine of Group A (Control) showing normal histological structure. H&E, X 200.

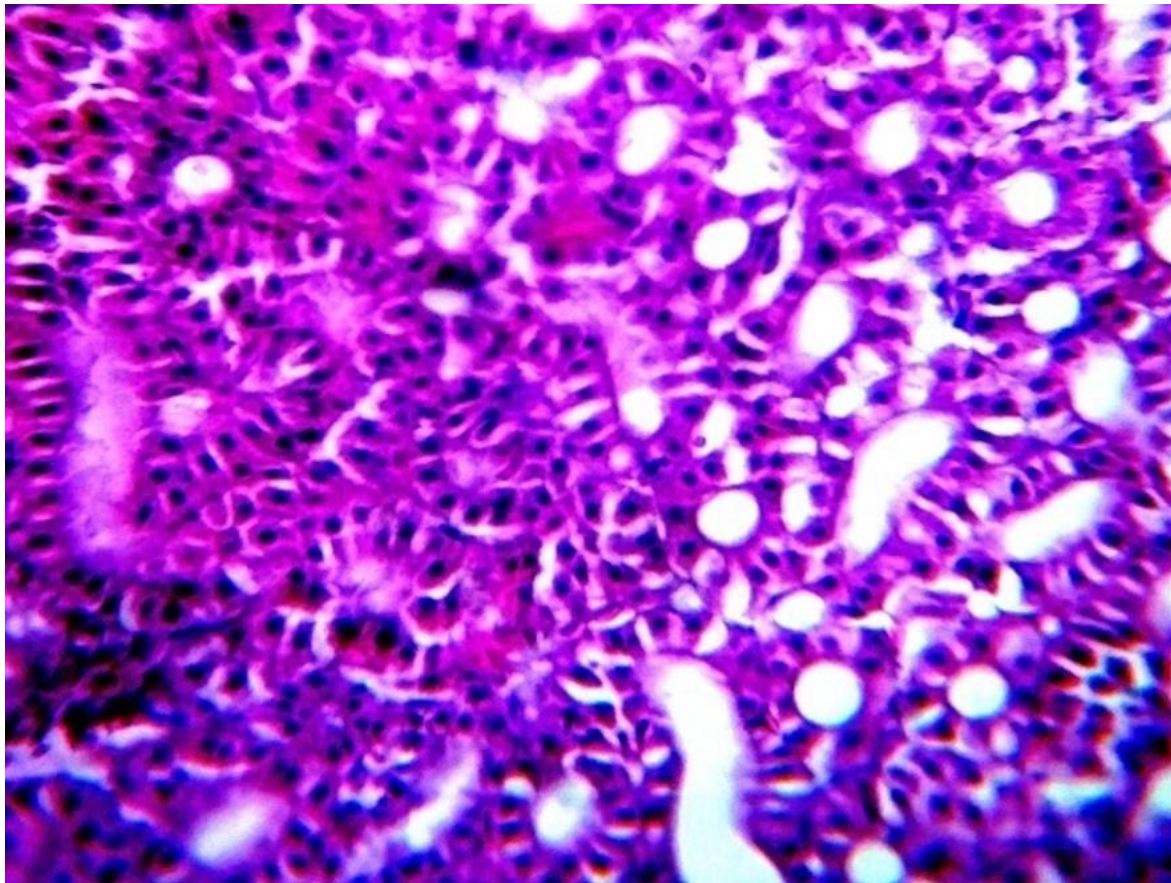


Fig.4. Kidney of Group A (Control) showing normal histological structure. H&E, X 200.

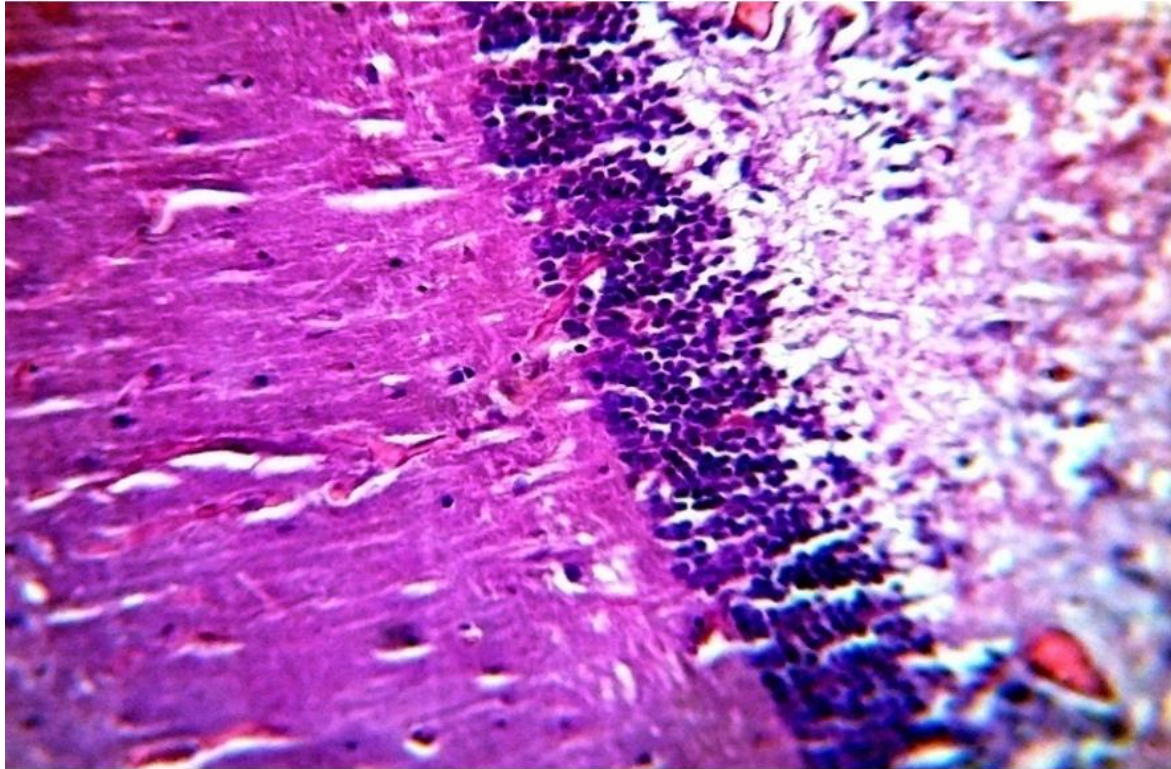


Fig.5. Brain of Group A (Control) showing normal histological structure. H&E, X 200.

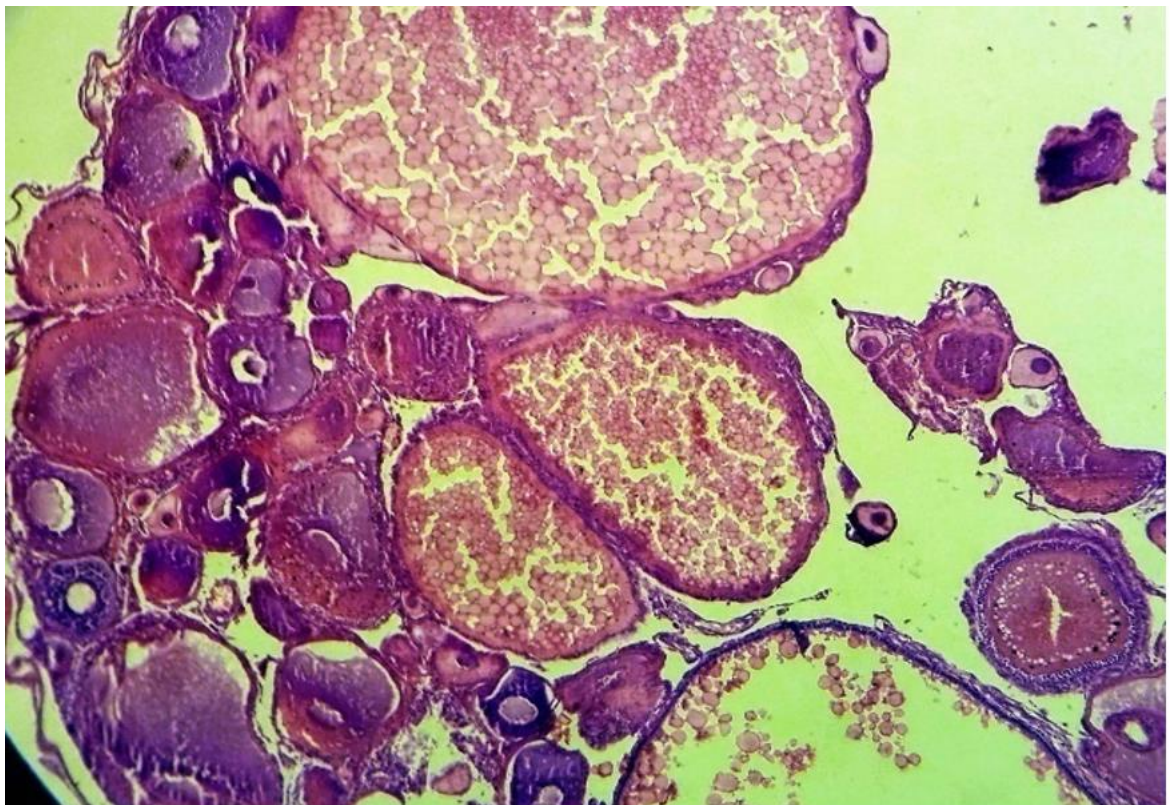


Fig. 6. Ovary of Group A (Control) showing normal histological structure . H&E, X 200.

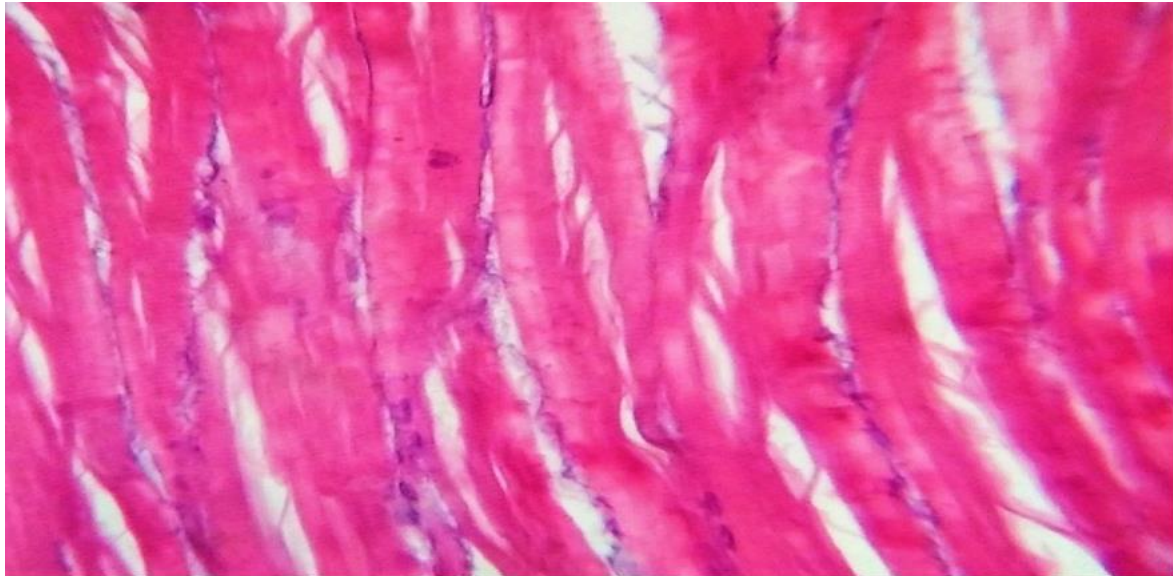


Fig. 7. Muscles of Group A (Control) showing normal histological structure. H&E, X 200.

2-Histopathological Study of Abamectin:

-Group B (Treated with Abamectin):

Examined sections treated with Abamectin revealed extensive hydropic degeneration of hepatocytes, congestion of blood vessels and mild to moderate pancreatic changes represented by hyperemia and infiltration of round cells. Gills showed desquamation and sloughing of the lining epithelium of the primary and secondary gill filament, infiltration of lymphocytes and excess mucous production. Kidney: Showed glomerular shrinkage, perivascular edema, hemorrhage, necrosis and degenerative changes. The intestine revealed lymphocytic enteritis “lymphocytic aggregation in the mucosa and submucosa” and dilatation of blood vessels. Muscles showed minimal interstitial edema with apparently normal morpho-histology of muscle fibers.

-Group C (Abamectin Recovery):

Liver examined sections revealed congestion of blood vessels, extensive hydropic degeneration and mildly inflamed hepato-portal pancreas with inactivated pancreatic acini. Gills were apparently normal with minimum degenerative and exfoliative changes. Testis rete testes were cystically dilated. The seminiferous tubules showed activated spermatogonia but few numbers of sperms were seen in their lumina. The intestine revealed mild lymphocytic infiltration in the lamina propria. Kidneys showed shrink glomeruli, hemorrhage, perivascular edema and degenerative changes in the renal tubules. Muscles showed normal morpho-histology of muscle fibers.

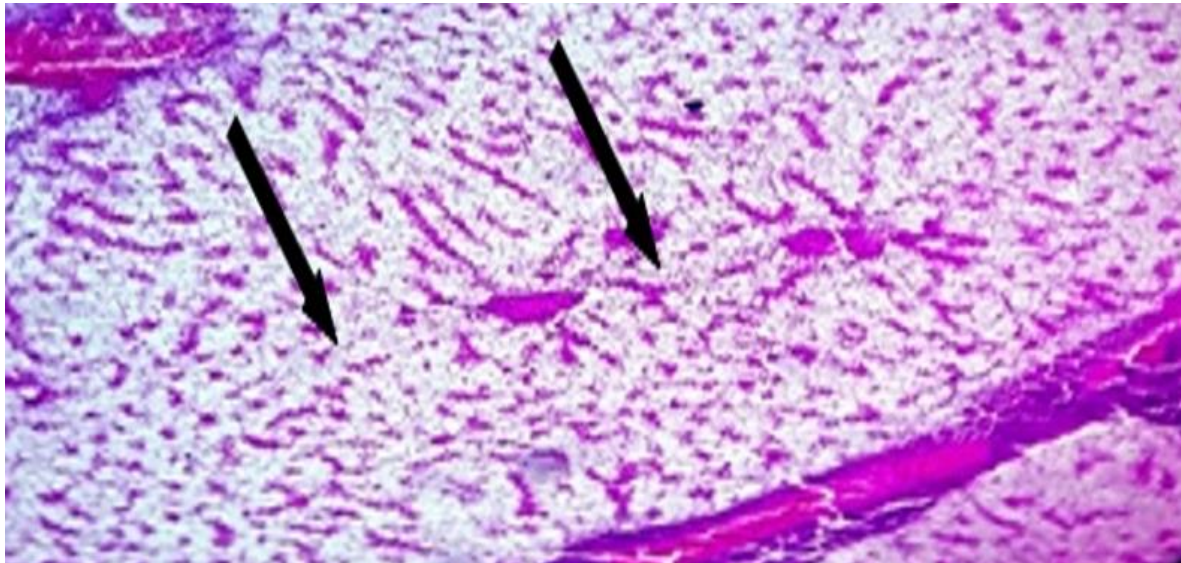
Group B (Treated with Abamectin)

Fig. 8: The Liver of group B treated with Abamectin revealed extensive hydropic degeneration of hepatocytes, and congestion of blood vessels.

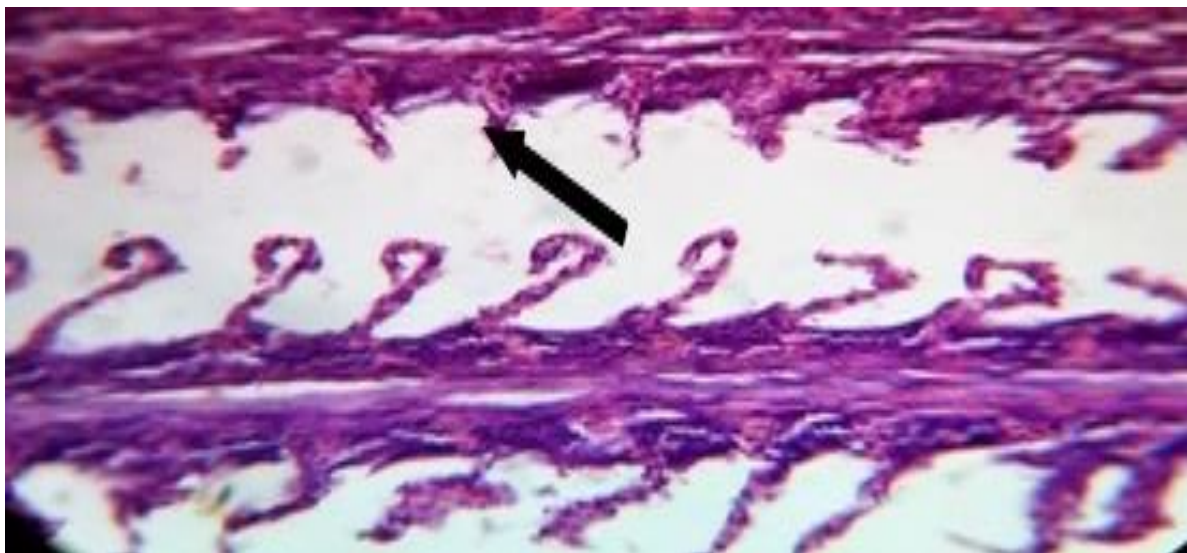


Fig. 9: Gills of group B treated with Abamectin revealed desquamation and sloughing of the lining epithelium of the primary and secondary gill filament.

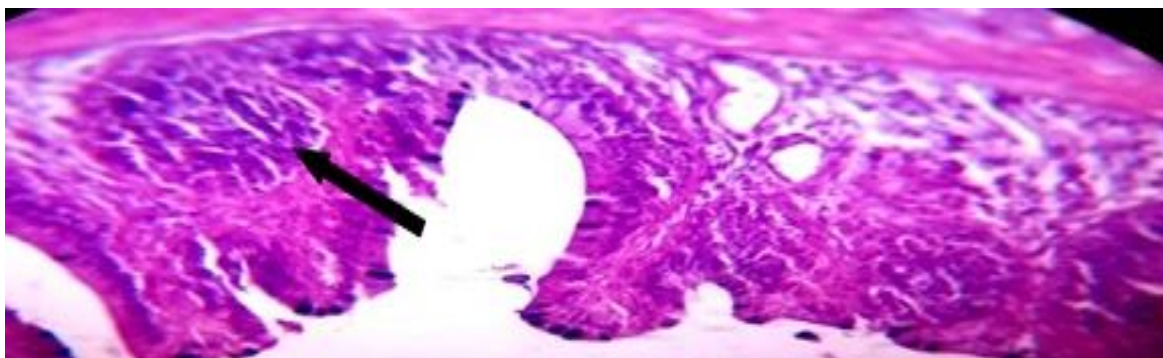


Fig. 10: The intestine of group B treated with abamectin revealed lymphocytic enteritis “lymphocytic aggregation in the mucosa and submucosa” and dilatation of blood vessels.

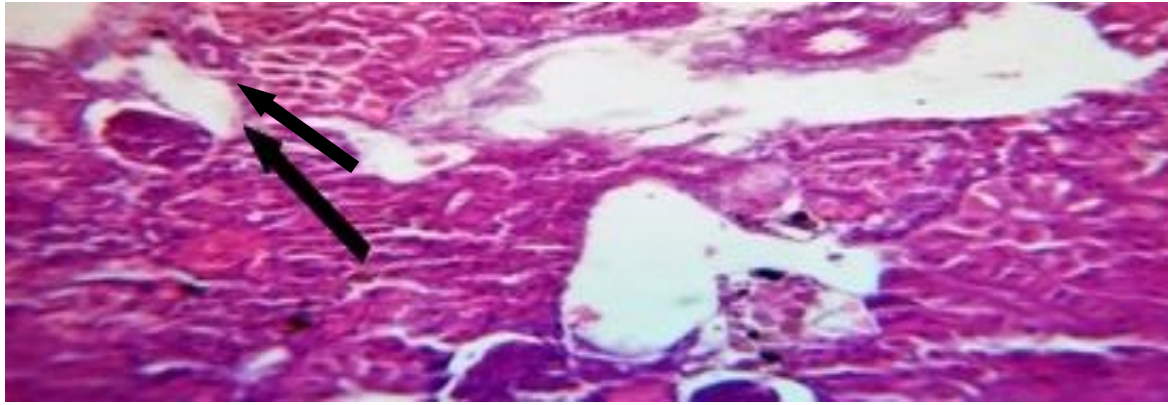


Fig. 11: The kidney of group B treated with abamectin revealed glomerular shrinkage (red arrow), perivascular edema, hemorrhage, necrosis and degenerative changes.

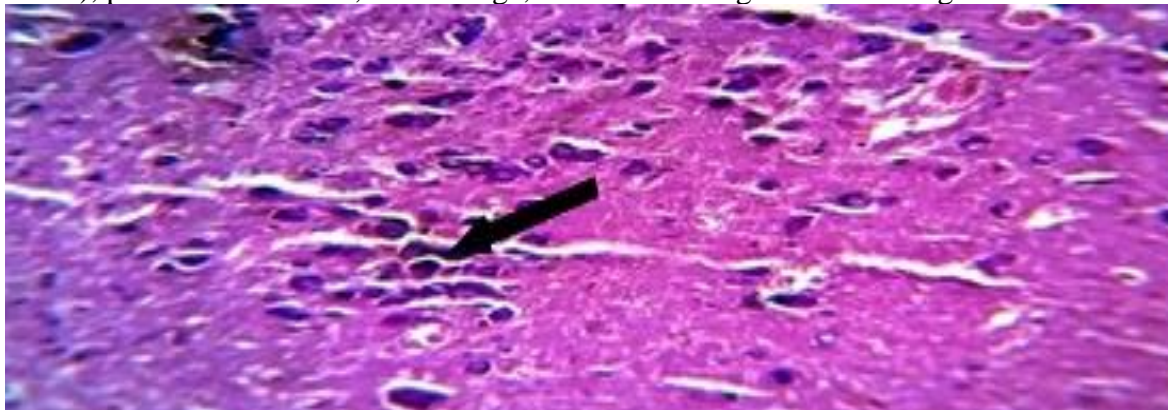


Fig. 12: The brain of group B treated with abamectin showed tissue revealed congested cerebral blood vessels, focal coagulative necrosis infiltrated and surrounded by inflammatory cells and astrocytes. Some nerve fibers revealed axonal degeneration and demyelination.

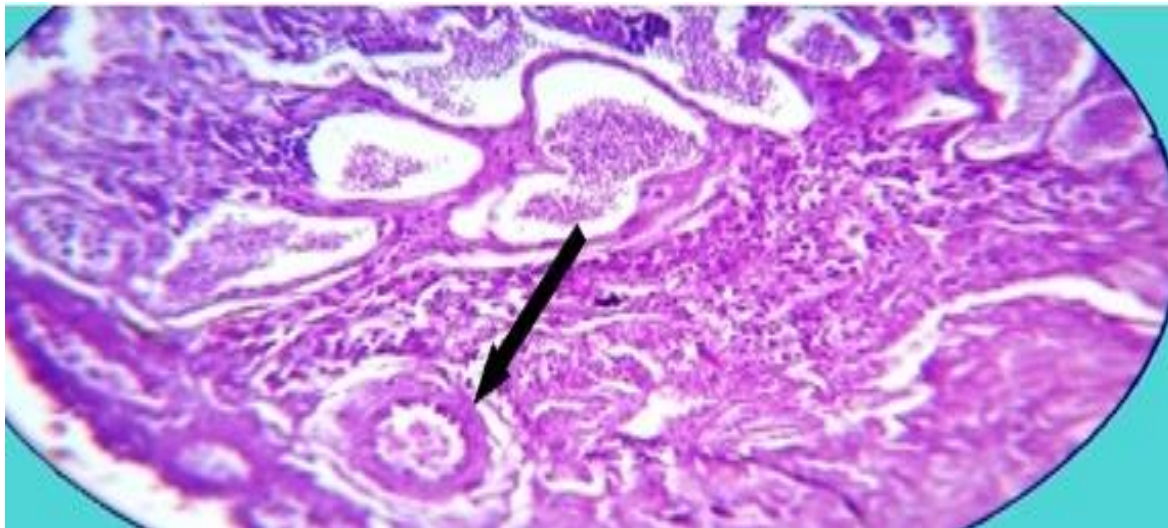


Fig. 13: The testis of group B treated with abamectin showed most of the seminiferous tubules revealed mildly active and contained spermatozoa.

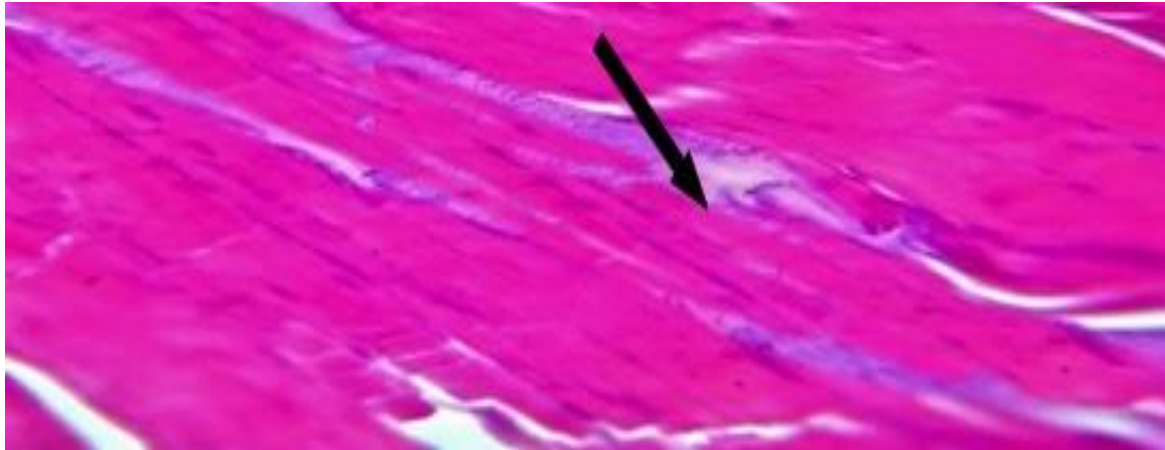


Fig. 14: Muscles of group B treated with abamectin revealed minimal interstitial edema with apparently normal morpho-histology of muscle fibers.

Group C Abamectin Recovery

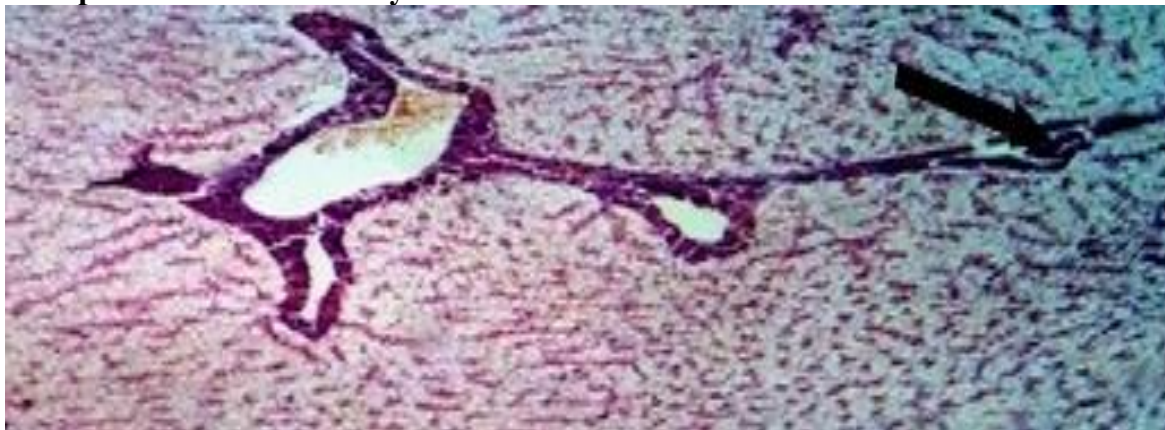


Fig.15: Liver of group C the abamectin recovery group showed revealed congestion of blood vessels, extensive hydropic degeneration and mildly inflamed hepatopancreas.

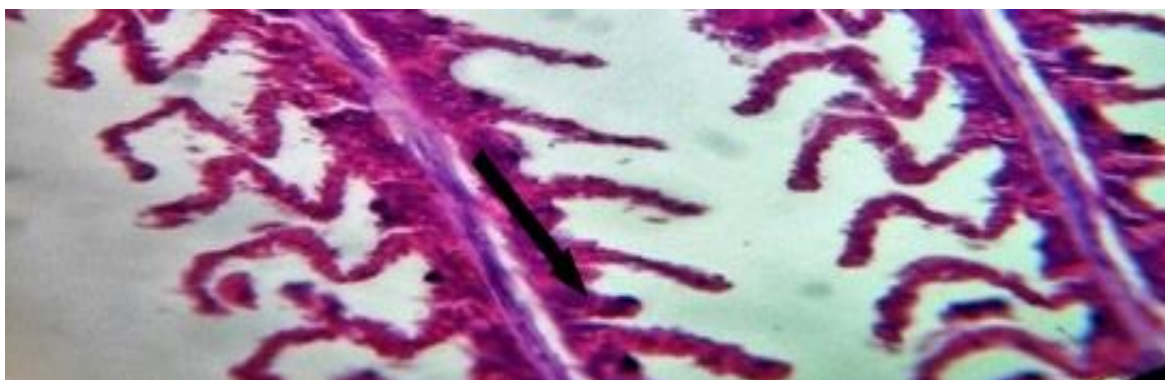


Fig. 16: GILLS of group C the abamectin recovery group showed revealed within the normal with minimum degenerative and exfoliative changes.

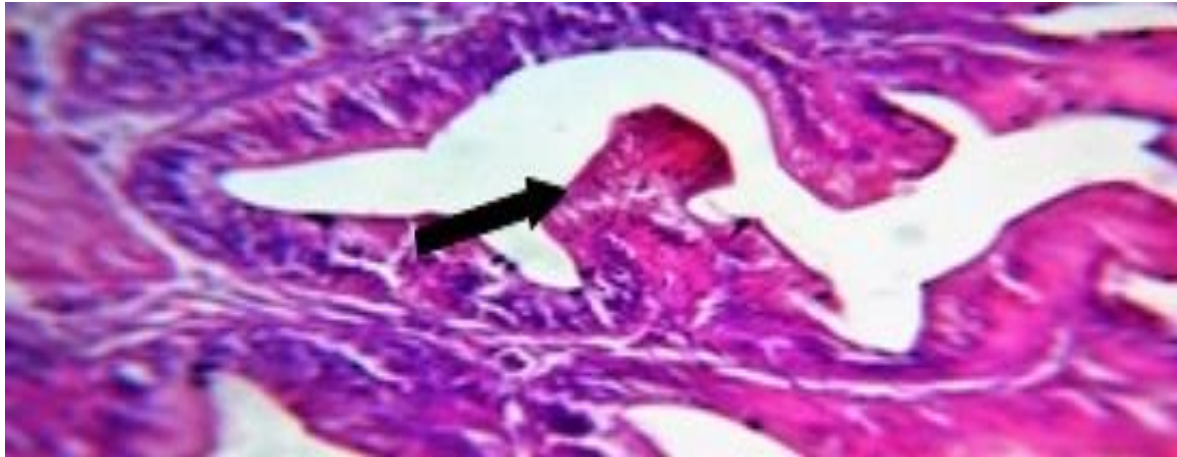


Fig. 17: The intestine of group C the abamectin recovery group revealed mild lymphocytic infiltration in the lamina propria.

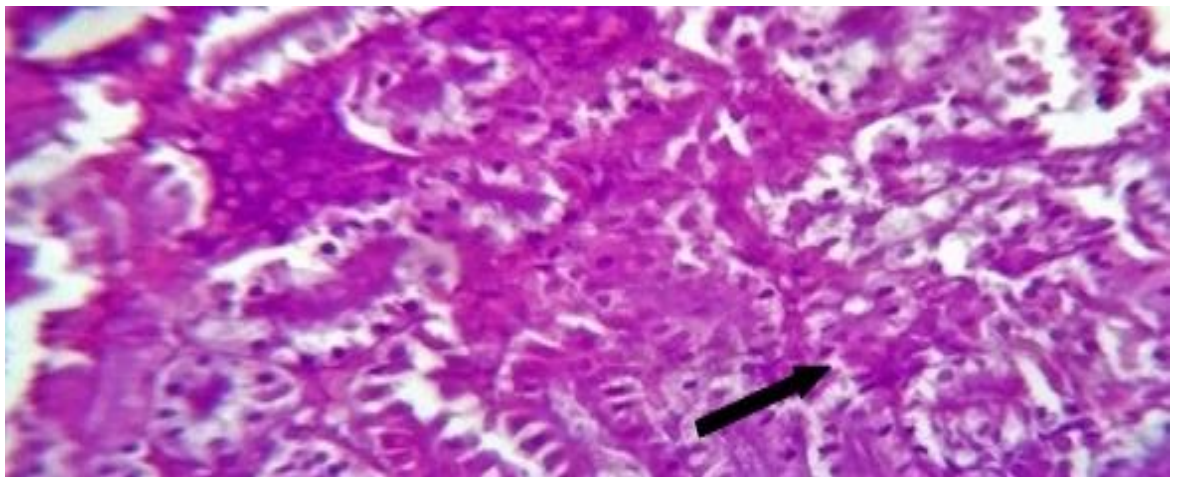


Fig. 18: The kidney of group C the abamectin recovery group revealed glomeruli are hemorrhage, perivascular edema and degenerative changes in the renal tubules.

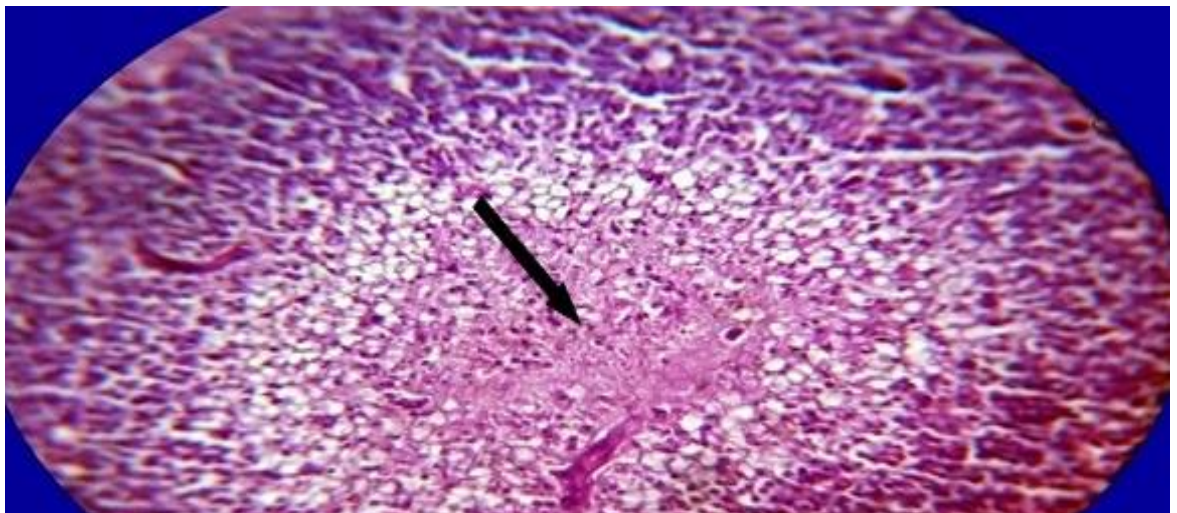


Fig. 19: The brain of group C the abamectin recovery group showed tissue revealed nodules formed from “central caseation followed by vacuolated neuropil and a zone of lymphocytes.

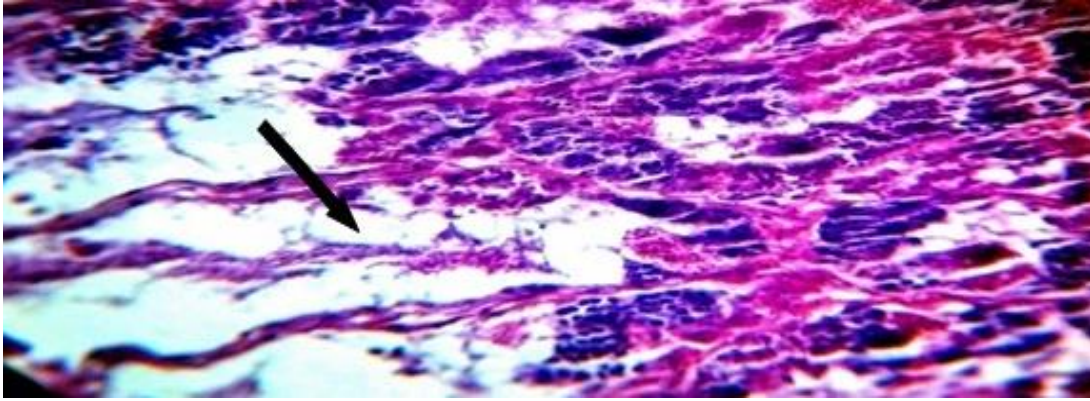


Fig. 20: Testes of group C the abamectin recovery group revealed cystically dilated. The seminiferous tubules showed activated spermatogonia but few numbers of sperms were seen in their lumina.



Fig. 21: Muscles of group C the abamectin recovery group revealed normal morphology of muscle fibers.

2-Histopathological Study of Sewage Water: Group D Treated with Sewage Water:

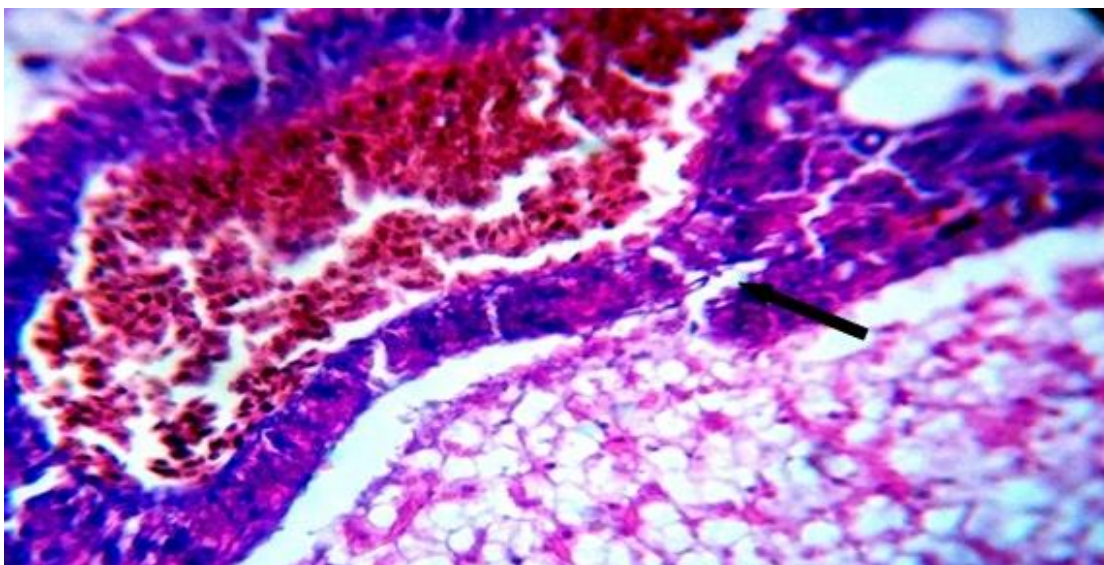


Fig.22: Liver of group D revealed congestion of most hepatoportal blood vessels, hydropic degeneration and necrotic changes in a large number of hepatocytes with the presence of degenerated and distorted pancreatic acini H&E X 400.



Fig.23: Gill of group D showed atrophied primary and secondary filaments with necrosis of the lamellar epithelium also revealed venous dilatation and capillary telangiectasis were also detected H&E X 400

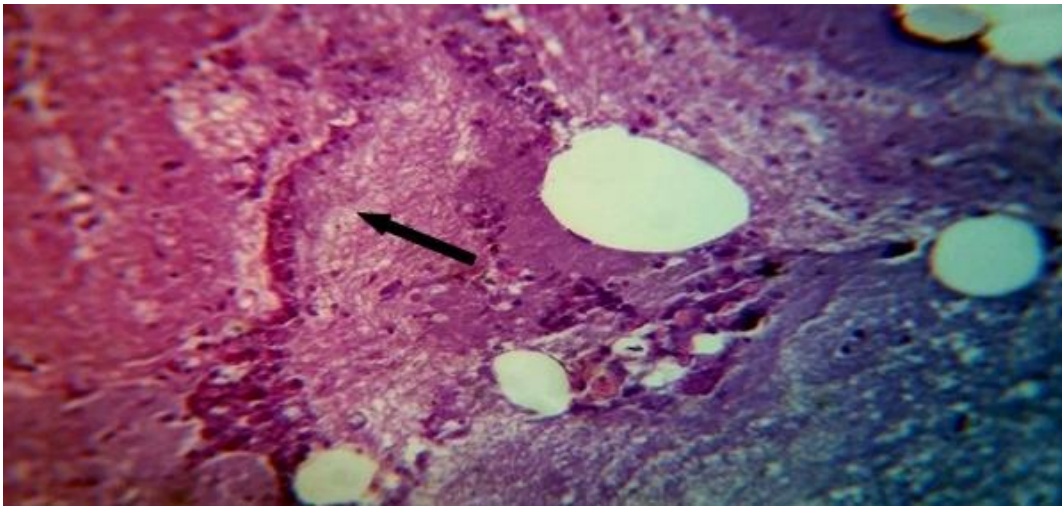


Fig. 24: Brain of group D revealed focal neuronal vacuolation, malacia, cystification and demyelination together with Lymphocytic meningitis H&E X 200.

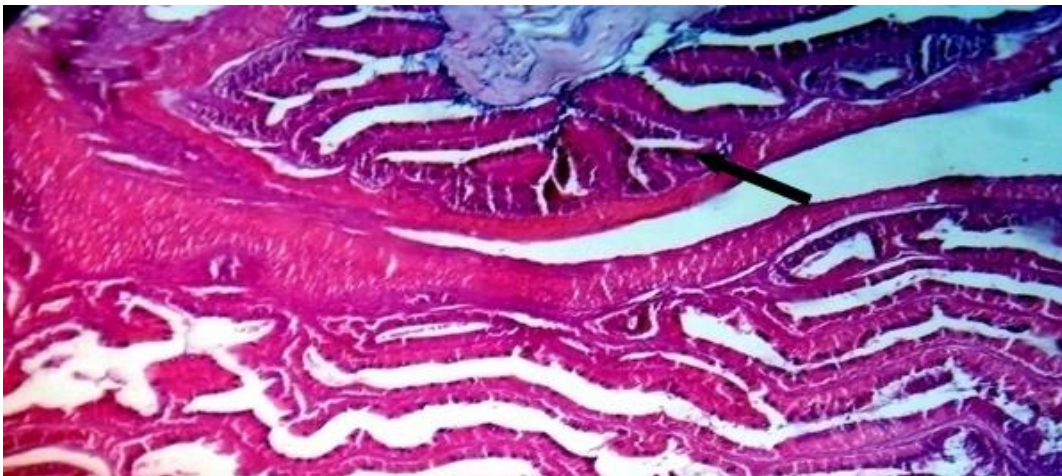


Fig. 25: Intestine of group D showed an increased number of goblet cells, increased amount of mucus secretion and round cells infiltration in the lamina propria could also be detected H&E X 200.

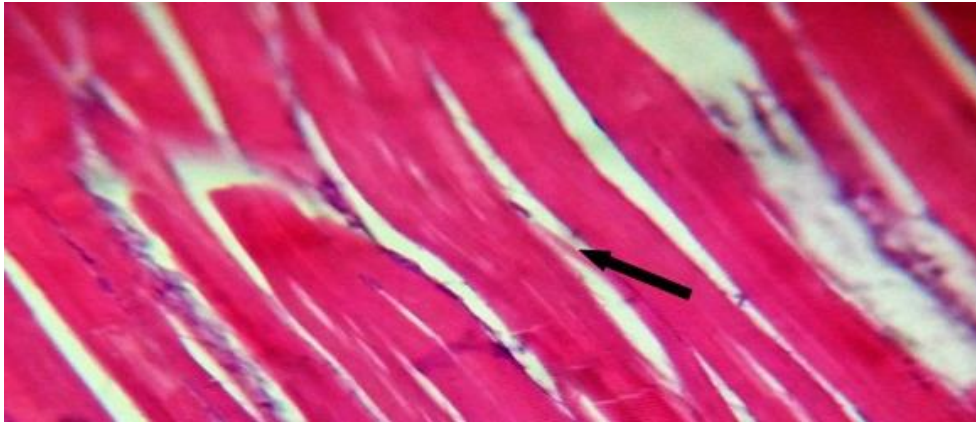


Fig. 26: Muscles of group D revealed within the normal, however, some muscle fibers showed hyaline degeneration and interstitial edema H&E X 400

**3-Histopathological Study of Hormone:
Group E Treated with Groth Hormone:**

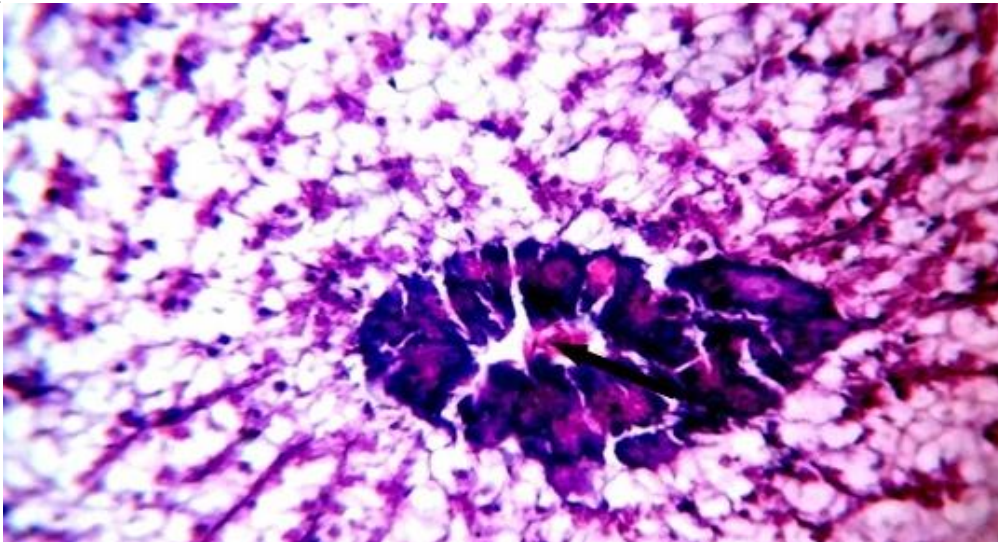


Fig.27:Liver of group E revealed a hydropic degeneration in a moderate number of hepatic cells with activated hepatic-pancrase, activated pancreatic acini (thick arrow), H&E, X 200.

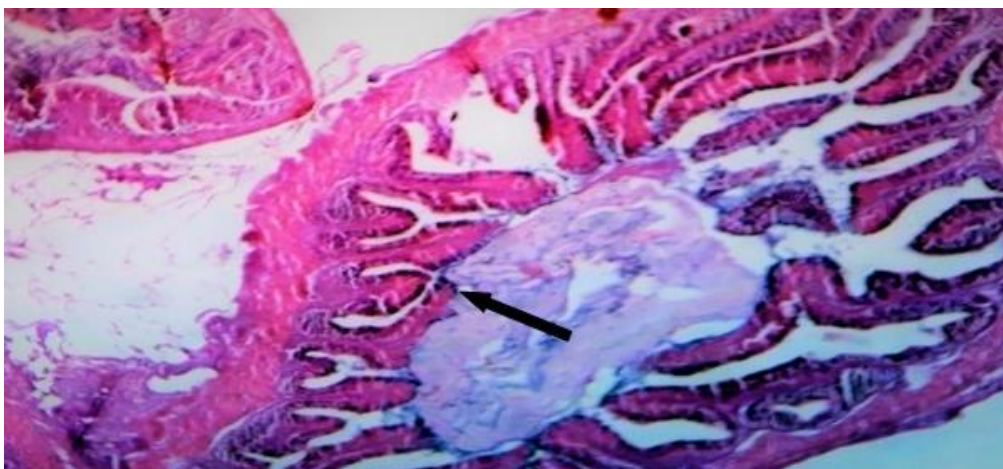


Fig.28: Intestine of group E revealed the presence of actively proliferated villi which appeared elongated and branched with the presence of excess intraluminal mucus secretion (arrow). The villus epithelial cells appear large in size and pseudostratified in some parts, H&E, X 200

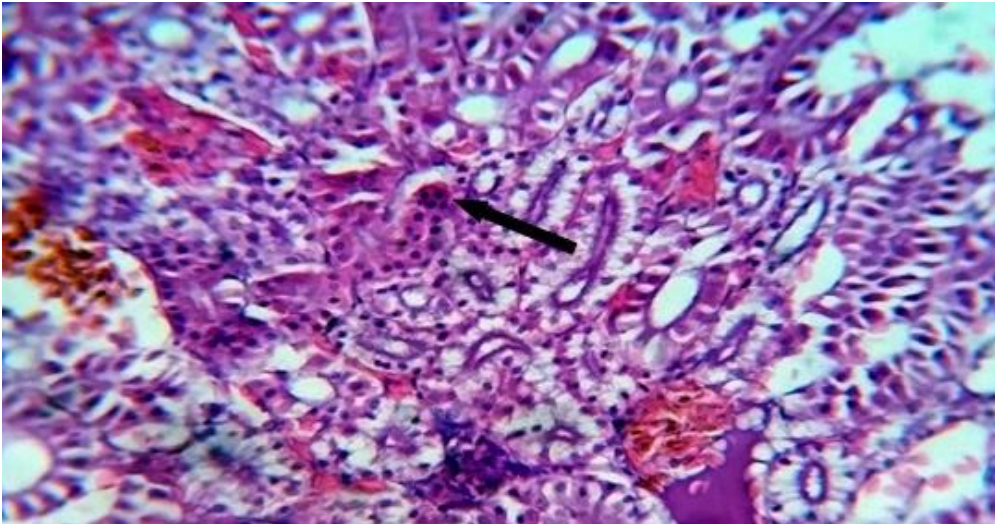


Fig. 29: Kidney of group E has moderate dilatation of renal blood vessels, mild 14 perivascular and interstitial edema and cystically dilated tubules “hydronephrotic”, (arrow) H&E, X200.

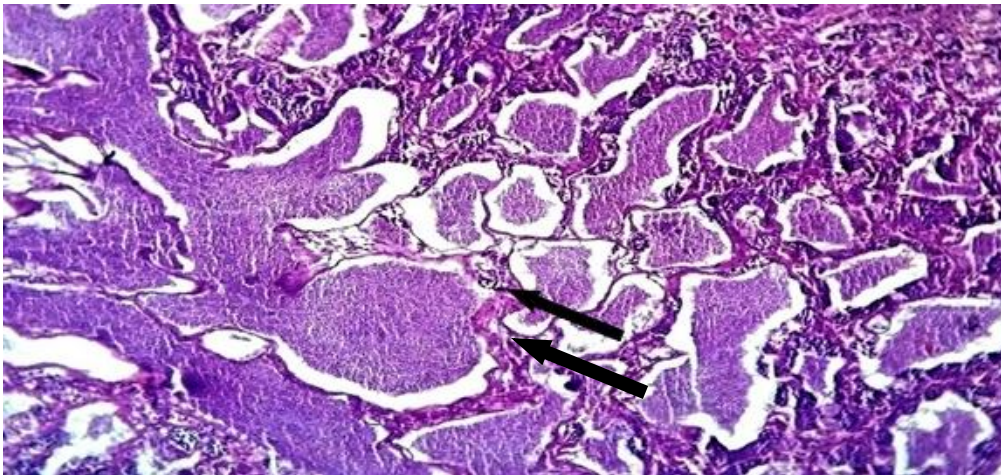


Fig.30: Testis of group E Testis revealed highly activated seminiferous tubules with actively proliferated spermatogonia and an increased number of sperms inside the seminiferous tubules and rete testis, H&E, X200.

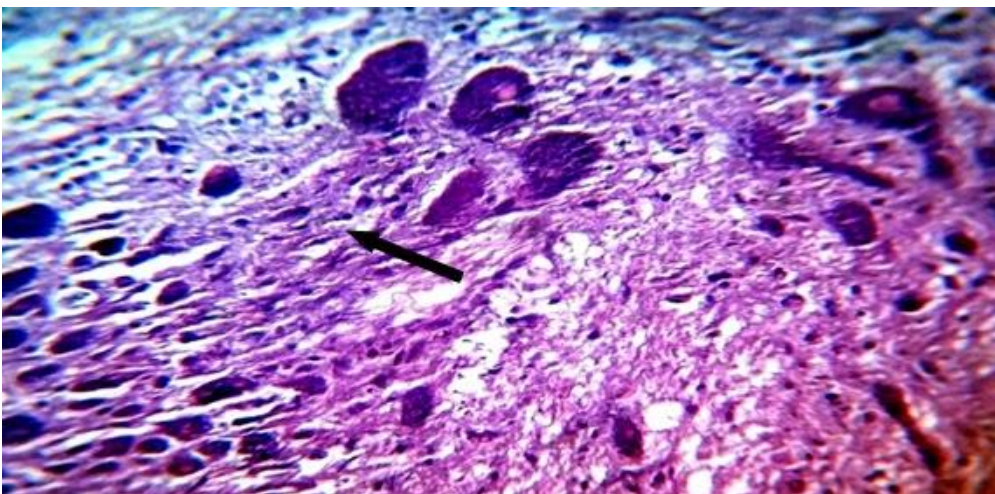


Fig.31: Brain of group E revealed focal neuronal degeneration and focal demyelination, (arrow) H&E, X400

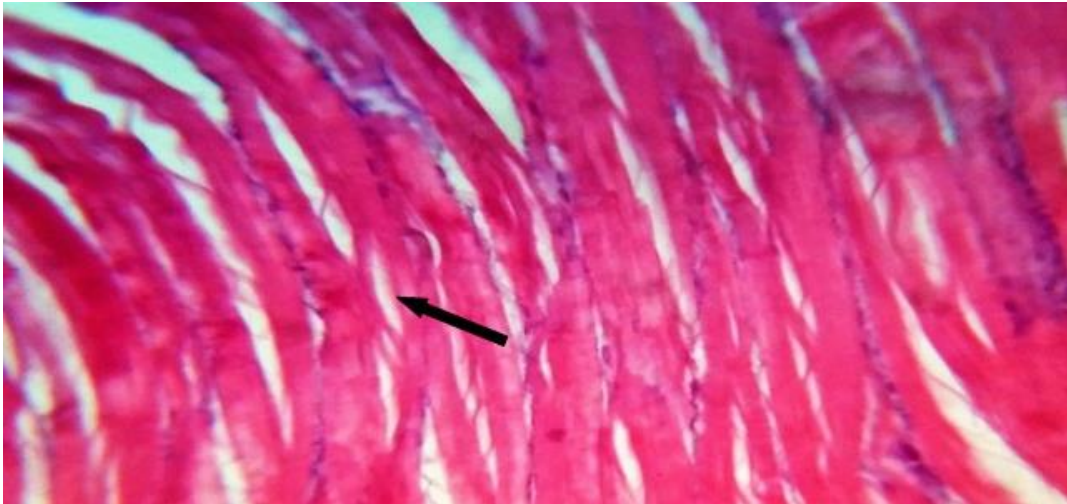


Fig.32: Muscles of group E revealed apparently normal fibers, including the characteristic longitudinal and cross striations, however, sarcolemma hypertrophy and hyperplasia were seen in some muscle fibers (arrow) H&E, X400

Group F Growth Hormone Recovery:

Gills, Muscles and brain showed normal morpho–histological structures. The liver revealed moderate hydropic degeneration of the hepatocytes and activation of the hepato-pancreatic acini. Kidney: showed changes nearly similar and comparable to the aquagen-treated group. Testis: demonstrated moderate activation of the seminiferous tubules, particularly the spermatogenic and the primary and secondary spermatocyte cellular layers, with the presence of a moderate number of sperms in the tubular lumina.

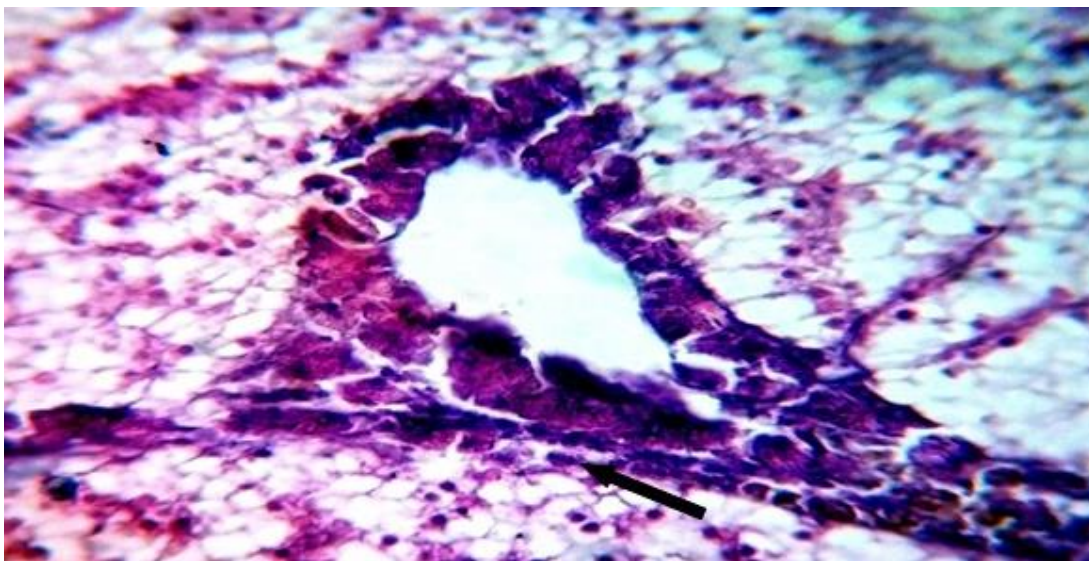


Fig.33: Liver of group F revealed moderate hydropic degeneration of the hepatocytes and activation of the hepato-pancreatic acini H&E, X 400.

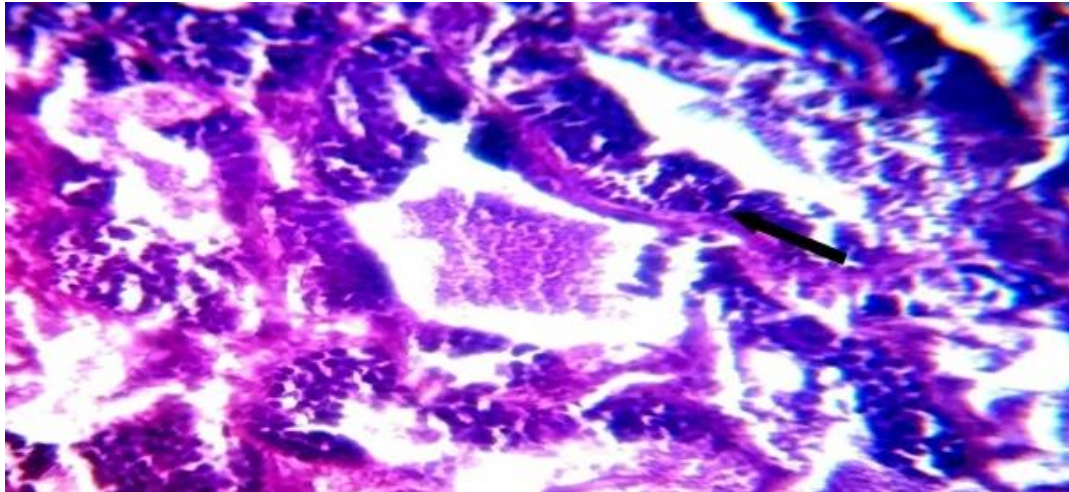


Fig.34: Tests of group F revealed demonstrated moderate activation of the seminiferous tubules, particularly the spermatogenic and the primary and secondary spermatocyte cellular layers, with the presence of a moderate number of sperms in the tubular lumina, H&E, X 200

Declarations:

Ethical Approval: Ethical Approval is not applicable.

Competing interests: The authors declare no conflict of interest.

Authors Contributions: I hereby verify that all authors mentioned on the title page have made substantial contributions to the conception and design of the study, have thoroughly reviewed the manuscript, confirm the accuracy and authenticity of the data and its interpretation, and consent to its submission.

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Availability of Data and Materials: All datasets analysed and described during the present study are available from the corresponding author upon reasonable request.

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ARABIC SUMMARY

التأثيرات الضارة لمبيد ابامكتين , ماء الصرف الصحي وهرمون النمو علي اسماك البلطي النيلي

محمد عبد العال هنداوي –سالوناز السيد عوض – احمد محمد علي الديب
قسم وقاية النبات كلية الزراعة جامعة الزقازيق

تم دراسة التأثيرات البيوكيماوية والهستولوجية الضارة للمبيد ابامكتين,وماء الصرف الصحي وهرمون النمو علي السمك البلطي النيلي وزن 60 جرام .اظهرت الدراسات البيوكيماويه انه عند تعرض السمك البلطي النيلي لتركيزات غير مميته من مبيد الابامكتين ,ماء الصرف الصحي (عند نسبة تخفيف 50% بماء نظيف) وهرمون النمو عند تركيز 6 مللجرام \ كلبيوجرام لمدة 21 يوم سبب ذلك العديد من التغيرات البيوكيماوية للسمك البلطي النيلي . تسبب ماء الصرف في زياده مستويات كل مكونات الدم خلال فترة المعاملة , كما تسبب هرمون النمو في زياده مستويات انزيمات الكبد والجلوكوز والبروتين الكلي والالبومين وحمض اليوريك والكرياتينين والكلوستيرول خلال فترة المعاملة . علي الجانب الاخر فان الدراسات الهستولوجية اظهرت العديد من التأثيرات الضارة علي العديد من الاعضاء المعامله (الخياشيم – الكبد – الكليه – الامعاء – العضلات – المخ – الخصيه – المبايض) مقارنة بالكنترول غير معامل .