EGYPTIAN ACADEMIC JOURNAL OF

BIOLOGICAL SCIENCES

TOXICOLOGY & PEST CONTROL

F

ISSN
2090-0791

WWW.EAJBS.EG.NET

Vol. 14 No. 1 (2022)

www.eajbs.eg.net

Citation: *Egypt. Acad. J. Biolog. Sci.* (F.Toxicology& Pest control) *Vol.14(1)pp35-46(2022)*
DOI: 10.21608/EAJBSF.2022.215132

Egypt. Acad. J. Biolog. Sci., 14(1):35-46(2022)

Egyptian Academic Journal of Biological Sciences
F. Toxicology & Pest Control
ISSN: 2090 - 0791
http://eajbsf.journals.ekb.eg/

Can Acetylsalicylic Acid be used As a Rodenticide?

Randa A. Kandil, Fatma M. Elgohary and Soha A. Mobark
Plant Protection Research Institute, ARC. Dokki, Giza, Egypt
E-mail* :randa.kandil@yahoo.com

ARTICLEINFO
Article History
Received: 3/12/2021
Accepted: 7/1/2022
Available:12/1/2022

***Keywords*:**
Acetylsalicylic acid – *Rattus rattus*- Rodent control- Clothes storage

ABSTRACT

This study was conducted to evaluate the effect of Acetylsalicylic Acid (ASA) against the black rat, *Rattus rattus*, under laboratory and field conditions. ASA at 0.04 % mixed with crushed maize was tested as bait using non-choice and free-choice feeding tests. Moreover, the bleeding time was measured after different treatment periods, and the histopathology of some organs was studied as well. The efficiency of ASA bait was evaluated in clothes storage at El-Mansouria village, Giza Governorate. The laboratory results revealed that ASA bait achieved 80% mortality in non-choice feeding test and 60% mortality, with 48.5% acceptance, in free-choice feeding test. The bleeding time was enhanced after all treatment periods with different values. In addition, ASA bait caused severe histopathological changes in the tissues of some organs (brain, heart, lung, liver, kidney and ovaries). Regarding the efficiency of the ASA bait in the clothes storage, it achieved a 72% rat population reduction. Therefore, ASA (0.04 %) bait can be used as a safe and cheap compound to reduce the rodent population in the fields.

INTRODUCTION

Rodents damage a variety of agricultural crops throughout most regions of the world. In the developing countries, where the economy depends mainly on agriculture, a rodent infestation can pose a serious threat, not only by reducing human income but also by spreading dangerous diseases to man and his domestic animals (Singla &Babbar, 2012 and Desoky, 2015). Rodents acquired resistance in many countries due to the repeated use of anticoagulant rodenticides, The development of resistance to anticoagulant poisons by rodents has been well documented (Witmer *et al.*, 2007). Therefore, scientists start to look for other safe and cheap compounds that can be used against rodents. Aspirin (Acetylsalicylic acid, ASA) is an easily available, cheap and widely used non-steroid anti-inflammatory drug that is useful as an analgesic to reliefe minor aches and pains (Gociong, 2003). Aspirin irreversibly inactivates cyclooxygenase (COX)-1 and suppresses the generation of prostaglandin H_2 (a precursor of thromboxane A_2). It achieves this effect through its acetyl group, which becomes covalently attached to Ser 529 of the active site of the cyclooxygenase enzyme (Tóth, *et al*., 2013 and Mekaj, *et al.*, 2015). Safety Data Sheet (2015) reported that acute toxicity of Acetylsalicylic acid was 1500 mg/kg orally for rats**.** Acute oral LD_{50} values have been reported as over 1.1 g/kg in mice and1.19 g/kg in albino rats, (Yildiz *et al.*, 2019). Administration of anti-platelet drugs such as aspirin can reduce mortality caused by CVD. This antiplatelet therapy is said to be effective in treating severe vascular disease with both short-term and long-term administration (Trialists' Collaboration,

2002). Aspirin (Acetylsalicylic acid-ASA) works by inhibiting the synthesis of thromboxane in platelets and prostacyclin in blood vessels by irreversibly inhibiting cyclooxygenase enzymes (Rosmiati & Gan, 1995). However, the dose of aspirin used as an antiplatelet drug in clinical trials is not equivalent (Patrono *et al.*, 2004). Excessive administration of single-dose aspirin, as well as long-term, can cause the risk of poisoning (Litovitz, *et al.,* 2001). The severity of complications that occur with aspirin depends on the dose and duration of treatment. Complications can include bleeding and perforation of the gastrointestinal system (Farrugia, 1999). Salicylate poisoning causes a variety of metabolic disorders. Direct stimulation of the cerebral medulla causes hyperventilation and respiratory alkalosis. As it is metabolized, it causes an uncoupling of oxidative phosphorylation in the mitochondria leading to hemodynamic instability and end-organ damage (Patel, 2013 and Runde & Nappe, 2020). Boyd (1959) and Charles *et al.*, (2018) mentioned that varying degrees of gastroenteritis, hepatitis, nephritis, pulmonary edema, and lesser toxic changes in the salivary glands, ovaries, skin, adrenals, thymus, mesentery, spleen, cardiac muscle, and skeletal muscle after using repeated dose (1 g/kg) of acetylsalicylic acid on albino rats. Aspirin causes a small increased risk of hemorrhagic stroke, which is a concern as it could potentially worsen a compromised blood-brain barrier (Tsau *et al.,* 2015). Jain *et al*., (2012) found that the effect of oral administration of aspirin drug on female albino rat, *Rattus norvegicus* caused significant histopathological variations in reproductive organs such as ovary and uterus by modulating certain enzyme metabolism in female albino rats. Nuha *et al.,* (2016) showed that demonstrated mild hyperplasia and degeneration in the epithelial cells lining the uterus, and there are few numbers of uteri glands. Also, it marked hyperplasia and vacuolation of the epithelial cells which line the uterus. In ovaries showed markedly few follicular growths wave characterized by primary, secondary follicles and there is congestion and thrombi in the ovarian stroma. Also, an over dose of aspirin stimulates corticosteroid secretion by the adrenal cortex (Luigi *et al*., 2001). The present study was conducted to evaluate the toxic effect of Acetylsalicylic acid (ASA) on black rat, *Rattus rattus*, one of the most common and harmful rodents in Egypt, and the histopathological changes in some organs under laboratory and clothes storage conditions.

MATERIALS AND METHODS

Tested Compound:

Acetylsalicylic acid (ASA), is a pure white powder (2-Acetoxy Benzoic acid). Linear Formula C8H8O2 was obtained from Oxford Laboratory Reagent. LD50 for rats was 200 mg/kg (Deichmann *et al*., 1969). It was used as bait mixed with crushed maize at 0.04%.

Experimental Animals:

Adult individuals of black rats, *Rattus rattus* were trapped from houses and storage of Al- Mansouria village, Giza Governorate. Thirty life traps (30×20×15 cm) were used to catch rats. Traps were provided with fresh bait (tomatoes, cucumbers or falafel). The animals were collected every day for two weeks and transferred to the laboratory of the Plant Protection Research Institute, Agriculture Research Center (ARC), Giza, Egypt. Animals were caged individually in standard laboratory metal cages (50× 30× 30cm). They fed on a free crushed maize and water *ad libitum*. Rats were held at 12 h., light / 12 h dark cycles at 25C^{o} conditions. Animals were acclimatized for two weeks before treatment. The unhealthy, young and pregnant rats were excluded. Thirty adult animals (110-150 g) were divided into three groups (each of 10 rats), two groups for treatments and one as a control.

Laboratory Experiments:

1. Non-Choice Feeding Test:

One group of rats was fed on 40g / rat of ASA (0.04%) bait for seven successive days. The consumed amount of bait was weighted daily. Then, the bait was removed and the animals fed on untreated crushed maize. Animals were observed for up to 28 days. During this period mortality was recorded (Shefte *et al.,* 1982).

2. Free Choice Feeding Test:

The free-choice feeding test is important to determine the acceptability of ASA (0.04%) bait to black rats in the laboratory in comparison with the consumption of free crushed maize, according to Palmateer (1974). One group of animals fed on ASA bait and untreated crushed maize in two separate bowls for each rat (40 gm/rat). The positions of the two bowls were switched daily during the test period of the seven successive days, to avoid side preference. Daily bait consumption and mortality were recorded. The acceptance % was calculated using the following equation (Mason *et al.,* 1989).

$$\text{Acceptance \%} = \frac{\text{Treated bait consumption (g)}}{\text{Treated bait consumption} \quad + \text{Untreated bait consumption}} \times 100$$

3. Bleeding time.

The bleeding time is the time when the animal started to bleed, from its tail after being pricked by a needle and wiped with a filter paper until the bleeding stopped. The bleeding time was measured at 1, 2, 3, 7, and 10 days after treatment by the method of Duke (1910).

4. Histopathological Studies:

Autopsy samples were taken from the brain, lung, heart, liver, kidney, and ovaries of treated and untreated rats, after two weeks of treatment with ASA (0.04%) bait in different groups samples were fixed in 10 % formalin saline for 24 hours. Washing was done in tap water then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 degrees, in a hot air oven for twenty- four hours. Paraffin bee wax tissue blocks were prepared for sectioning at 4 microns thickness by sledge microtome. The obtained tissue sections were collected on glass slides, deparaffinized and stained by hematoxylin and eosin stain for examination through the light electric microscope (Banchroft *et al*., 1996). The preparation was examined by the light microscope to identify the presence of histopathological changes in the tested organs.

Field Experiments:

Field efficiency of ASA (0.04 %) bait was tested in clothes storage at El- Mansoria village, Giza Governorate. The storage area of 4600 m^2 infested with black rat, *Rattus rattus*, was chosen and divided into four replicates, three for treatment and one as a control. The population density of rats was estimated pre- and post-treatment using the food consumption method (Dubock, 1984). An amount of 6400 g of crushed maize, used for pre and post-treatments, was divided into sacks (50 gm), each was put inside a plastic tube bait station (50 cm in length and 12 cm in diameter) for five successive days. The average consumption was estimated on the fourth and fifth days. After that, the treated bait was placed in each bait station, weekly and removed after the third week. The bait stations were left, empty in place, for another week. Then, untreated crushed maize was placed inside each bait station, as mentioned above, for one more week. The average consumption was estimated, and the population reduction percent was calculated as follow:

$$\text{Population reduction \%} = \frac{\text{Pretreatment consumption (g) - post treatment consumption (g)}}{\text{Pre-treatment consumption (g)}} \times 100$$

Statistical Analysis:

The experimental design was completely randomized with different replicate. The obtained results were statistically analyzed by one-way ANOVA and Least Significant Difference (LSD) at ($p \leq 0.05$) using the Costat program (Cohort, 2005).

RESULTS AND DISCUSSION

Laboratory Studies:

1-Non -Choice Feeding Test:

Data in Table (1) showed the result of the non-choice feeding test of Acetylsalicylic acid (ASA0.04%) bait against *Rattus rattus*. The average bait consumption was 9.0 g compared with 11.7g for the control. Acetylsalicylic acid achieved 80% mortality with 7.4 days' time to death with a range between 8to 15 days. There was a significant difference between the treated bait consumption compared with the control. ASA caused mortality for rats due to repeated feeding which led to the toxic effect and death. These results agreed with Boyd, (1959), who recorded that death within the first 24 hours was due to tonic-clonic convulsions and respiratory failure. Deaths on the second and third days were attributed to cardiovascular shock after using the oral lethal dose of Acetylsalicylic acid (0.045g /kg b. w.) in albino rats. Mortality has been observed following convulsions or cardiovascular shock due to using the acute toxic dose of acetylsalicylic in cats and dogs (Authors unspecified, 2015 and Runde & Nappe., 2020). Death commonly occurs after using a toxic dose of aspirin attributed to cardiopulmonary edema secondary to pulmonary edema (Arif*and* Aggarwal., 2020).

Table 1: Effect of Acetylsalicylic acid (0.04%) bait against black rats, *Rattus rattus,* for seven days using non-choice feeding test.

Group	Average consumption (g) Mean ± SE	Mortality %	Time of death (days)	
			Mean	Range
Treated	9.0 ± 0.2^{b}	80.0	7.4	8.0- 15.0
Control	11.7 ± 0.2^{a}	0.0	0.0	0.0
LSD	2.25			

Values are expressed as means (10 rats) ± standard errors ^{ab} values in Column with different letters are significantly different at ($P < 0.05$). LSD: Least Significant Difference

2-Free Choice Feeding Test:

Data in Table (2) illustrated the effect of ASA 0.04% bait against *Rattus rattus* using a free-choice feeding test. Results revealed that ASA gave 60% mortality with the time of death ranging between 25-30 days and a mean of 22.33 days. It also achieved 48.5% acceptance, whereas the consumption of the average bait was 7.58g for treated bait and 8.16g for untreated bait in the case of treated animals compared with 10.5g food consumption for control. There was a significant difference between the treated and untreated bait consumption compared to the control group. This result may be due to that ASA bait has an acceptable taste. Kandil, *et al.* (2015) recorded that diphacinone 0.005% anticoagulant rodenticide caused 73 % mortality for *Rattus rattus* after three days. Also, Kandil *et al.* (2019) recorded that mortality percent in the free-choice test was 80% after using the furosemide 2.1% drug (40g) and palatability was 51% for the black rat, *Rattus rattus*.

Table 2: Effect of Acetylsalicylic acid (0.04%) bait against black rats, *Rattus rattus*, for seven days using free-choice feeding test.

Diet type	Average consumption (g) Mean ± SE	Acceptance%	Mortality %	Time of death(days)	
				mean	range
Treated bait	7.58 ± 0.4^{b}	48.5	60.0	22.23	20.0 – 25.0
Untreated bait	8.16 ± 0.5^{b}	-	-	-	-
Control	10.5 ± 0.5^{a}	100.0	0.0	0.0	0.0
LSD	2.66				

Values are expressed as means (10 rats) ± standard errors [a]values in column with different letters are significantly different at ($P < 0.05$). LSD: Least Significant Difference.

3-Bleeding Time:

Data in Table (3) presented the effect of ASA 0.04%bait on the bleeding time of the black rat, *Rattus rattus*. The results revealed that the average bleeding time was 2.6, 4.42, 6.78, 9.5, and 11.4 min for treated rats, and 2.5, 2.8, 2.6, 2.6, and 2.8 min., for untreated rats after 1, 2, 3, 7, and 10 days, respectively. There was a significant increase in bleeding time with increasing time in treated bait compared with control. These results indicated that ASA 0.04% bait increased the bleeding time because it breaks down the platelets. This result agreed with Martinal *et al.*, (2019). They recorded that aspirin caused an increase in the bleeding time in mice compared with control. Also, Stilla *et al.* (1975) observed that oral administration of Aspirin at various dosages gave no significant variations of bleeding time in rats after treatment. Dejana *et al*., (1979) reported that Aspirin 200mg/ kg caused bleeding for maximal one hour in treated rats compared with untreated. Kandil *et al*., (1991) found that brodifacoum anticoagulant enhanced the bleeding time of albino rats from 3.5 to 10.0 min after three days of administration. El-Mahrouky Fatma, (1984) mentioned that bleeding time was greatly increased to about 15 times when albino rats were treated with LD_{50} from brodifacoum in comparison with untreated ones.

Table 3: Bleeding time of black rat, *Rattus rattus*, treated with Acetylsalicylic acid (0.04%) bait for seven successive days.

Group	Bleeding time (min.) after				
	Day 1	Day 2	Day 3	Day 7	Day 10
Treated	2.6 ± 0.09^{a}	4.42 ± 0.5^{a}	6.78 ± 0.39^{a}	9.50 ± 0.2^{a}	$11.4 \pm 0.45b^{a}$
Control	2.5 ± 0.68^{a}	2.8 ± 0.08^{a}	2.6 ± 0.06^{b}	2.8 ± 0.39^{b}	2.8 ± 0.05^{b}
LSD	1.57	1.93	0.93	1.03	1.68

[a]values in columns with different letters are significantly different at ($P < 0.05$).

Histopathological Studies:

The histopathological effect of Acetylsalicylic acid (ASA) 0.04% bait was studied on the liver, heart, lung, brain, kidney, fallopian tube and ovaries of the black rat, *Rattus rattus.* The results were illustrated in Table (4). The changes in the liver and portal area of rats' liver may be due to the toxic effect of ASA compound. Similar results were reported by Mossa, *et al.*, (2016) and Charles, *et al.*, (2018). They found that the liver of rats treated with 70 mg and 105 mg of aspirin showed increased cell basophilia with congestion and severely increased amount of cell basophilia. The increased cell basophilia could develop into hepatotoxicity, leading to hemodynamic instability and end-organ damage.

Moreover, results also showed that ASA compound caused high injury in cells of lung, brain and heart due to repeated consumption of ASA bait for four days. These results confirmed with Patel (2013) and Runde& Nappe., (2020). They mentioned that Salicylate poisoning caused hyperventilation and respiratory alkalosis in the cerebral medulla. Also, it caused uncoupling of oxidative phosphorylation in the mitochondria, when hyperventilation

worsens, in an attempt to compensate for the metabolic acidosis. A single dose of ASA administrated intravenously or orally, caused necrosis in the tubular kidney of rats (D'Agati, 1996). The histopathological alteration in ovaries and fallopian tubes in these studies may be due to paralysis in all bodies and acidophilic necrosis of neurons in the brain cortex with acidophilic cytoplasm and axons degeneration of the white matter (destructed axons) with digestion chambers. These results agree with Jain *et al.,* (2012), who studied the histopathological changes in both ovaries and uterus of treated rats with Aspirin. This is maybe due to the vascular properties of aspirin as well as its induced vasoconstriction and smooth muscle atrophy via inhibition of the synthesis of different prostaglandins. Aspirin also caused histopathological variations in reproductive organs such as the ovary and uterus by modulating certain enzyme metabolism in female albino rats. Nuha *et al.,* (2016) mentioned that the microscopic examination of the histopathological sections of uteri of all the treated and control groups showed that demonstrated mild hyperplasia and degeneration in the epithelial cells lining the uterus and there are few numbers of uteri glands compared with control.

Table 4: The histopathological effect of acetylsalicylic acid (0.04%) bait on different organs of black rat, *Rattus rattus*, after two weeks of treatment**.**

Organs	The observation in untreated	The observation in treated
Liver	Fig. (1). Normal histological structure of the central vein and portal area with the surrounding hepatocytes	Fig (2). Showing necrobiotic changes of hepeatocytes, coagulative necrosis of hepatocytes (long arrow) and pyknotic nuclei (short arrows) severe loss of hepatocytes and vacillations. Also, the portal area of liver showing dilated portal-vein with hyalinized wall and heamolysed blood in the lumen with per- vascular proliferation of fibrosis with vacuolar degeneration of the surrounding hepatocytes.
Heart	Fig (3). Normal histological structure of the epicardium and vaccuolations of the heart muscle.	Fig. (4). Showing calcification of the epicardium and vaccuolations of the heart muscle.
Lung	Fig. (5). Normal histological structure of the bronchiol and surrounding air alveoli as well as the blood vessels.	Fig. (6). Showing Intravascular papillary endothelial hyperplasia with pseudo-epitheliomatus hyperplasia.
Brain	Fig. (7). No histopathological alteration in the neurons of brain.	Fig. (8). Showing acidophilic necrosis of neurons, the neurons showing shrinkages of the neurons nuclei with acidophilic cytoplasm, degeneration of the white matter (destructed axons) with digestion chambers (the space of autolysed axons).
Kidney	Fig. (9). Normal histological structure of the glomeruli and tubules at the cortex.	Fig. (10). Showing severe hyalinosis of the renal glomerular blood capillaries (thickened esinophilic) thickening with necrotic nuclei of the endothelial cells, and necrobiotic changes of the renal tubule.
Fallopian tube	Fig. (11). Normal histological structure	Fig. (12). Showing shortening and necrosis of the fallopian tube villi
Ovaries	Fig. (13). Normal of different stages of ovarian follicles (f), corpus luteum (c) and interstitial stromal cells.	Fig. (14). Showing necrosis of the oocytes (long arrow) and necrosis of the cellular structure of the medulla of ovary.

Field Performance:

Data in Table (5) showed the efficiency of Acetylsalicylic acid (ASA 0.04%) bait application, against black rat, *Rattus rattus,* in the clothes storage. The average consumption of untreated crushed maize during the pre-treatment was 523.6 g while it was 103.3gduring the post-treatment. There is a significant difference between the consumption of ASA bait and untreated crushed maize (pre-and post-treatment) compared to the control treatment. The ASA bait achieved 72% population reduction in rats, therefore, it has high efficiency on the reduction of the rat population in storage. The consumed amount of treated bait was 291.7g. The field results agreed with those obtained from the laboratory. Some drugs were

used as poison baits for rodent control in the fields, such as furosemide drug 40mg bait (2.1%), which achieved 89% population reduction against *Rattus rattus,* in an animal production farm (Kandil, *et al.* 2019). From previous results, ASA bait gave satisfactory results in reducing rat populations in the storage.

Table 5: Efficiency of Acetylsalicylic acid (0.04%) bait against black rat, *Rattus rattus,* underclothes storage conditions**.**

Average consumption of bait (g) Mean ± SD				LSD	Population Reduction %
Control	Pre- Treatment	Treatment	Post treatment		
504.0 ±5.14 [a]	523.6 ± 22.9 [a]	291.7 ± 3.12 [b]	103.13 ± 6.25 [c]	124.7	72.0

Values are expressed as means ± standard division. [a]values in columns with different letters are significantly different at (P < 0.05). LSD: Least Significant Difference

Conclusion

Our study revealed that Acetylsalicylic acid 0.04% bait caused bleeding effect and severe damage to different organs of the black rat, *Rattus rattus,* which led to the death of individual animals in the laboratory, as well as a reduction in rat numbers in cloth storage. Therefore, it is recommended to be used in integrated rodent control programs against rats under field and storage conditions.

Acknowledgment.

I would like to express my deepest gratitude to Dr. Aly El-Sherbiny, Professor of Vertebrate Ecology, at the Harmful Animal Research Department, Plant Protection Research Institute, ARC., for editing the manuscripts, and for the scientific review.

Great and special thanks to Dr. Waheed Gabr, Professor at the Harmful Animal Research Department, Plant Protection Research Institute, ARC., for the scientific correction of the manuscript.

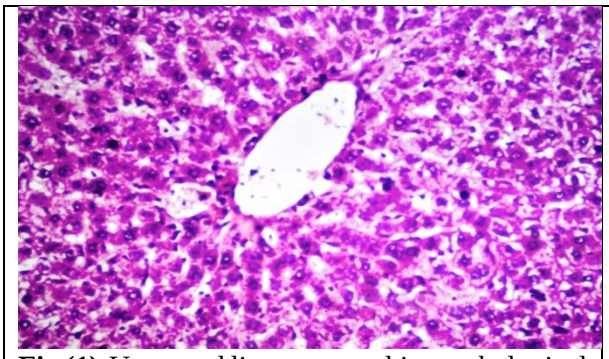

Fig (1): Untreated liver was not histopathological alteration and the normal histological structure of the central vein and portal area with the surrounding hepatocytes

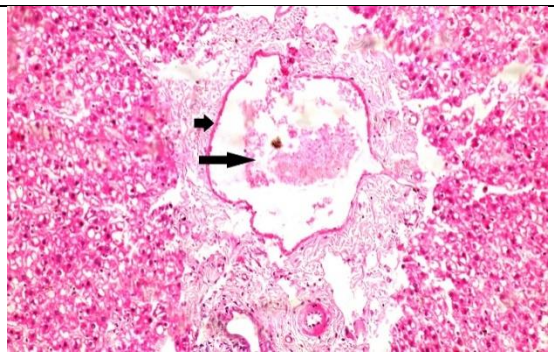

Fig (2): Treated liver showing necrobiotic changes of hepeatocytes, coagulative necrosis of hepatocytes (long arrow) and pyknotic nuclei (short arrows) severe loss of hepatocytes and vaccuolations. Treated portal area of liver showing dilated portal-vein with hyalinized wall (short arrow) and heamolysed blood in the lumen (long arrow) with peri-vascular proliferation of fibrosis with vacuolar degeneration of the surrounding hepatocytes (StainH&EX200

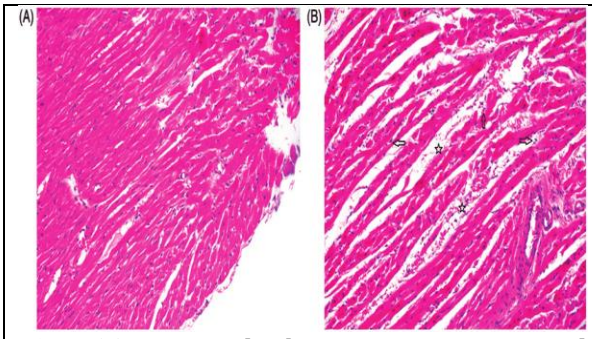

Fig (3): Untreated heart was a normal histological structure of the epicardium and vacuolations of the heart muscle.

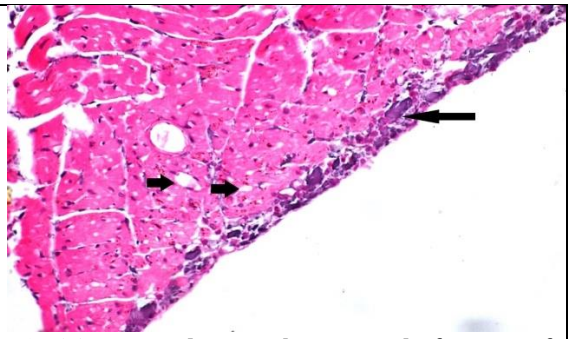

Fig (4): Treated Heart showing calcification of the epicardium (long arrow) and vacuolations of the heart muscle (short arrows) (StainH&EX200).

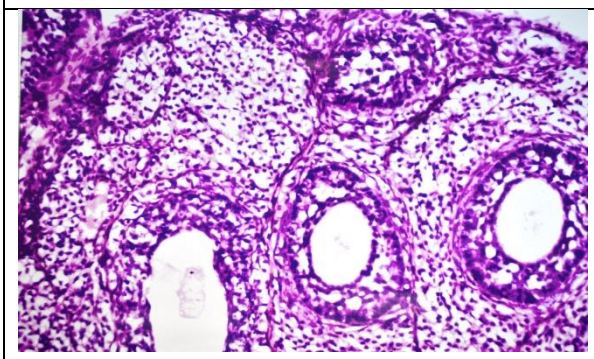

Fig (5): Untreated lung was a normal histological structure of the bronchiol and surrounding air alveoli as well as the blood vessels

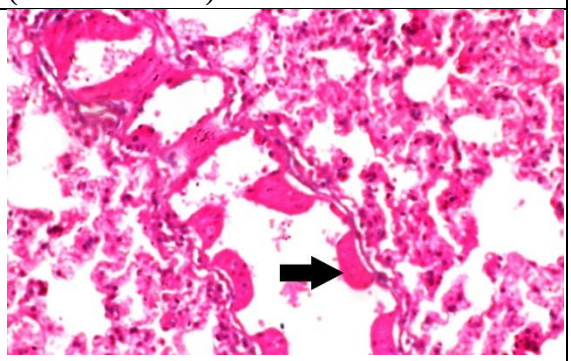

Fig (6): Treated lung showing Intravascular papillary endothelial hyperplasia with pseudoepitheliomatus hyperplasia (arrow) **(**StainH&EX400)

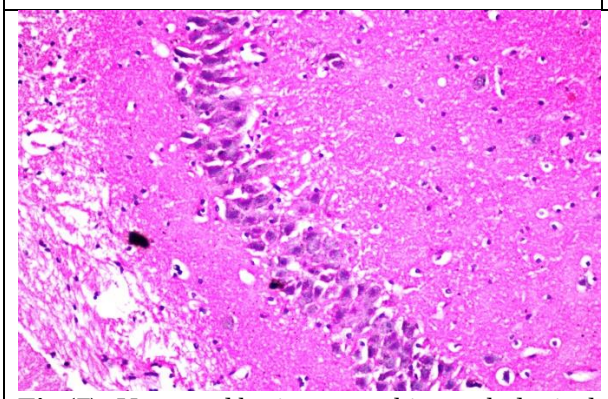

Fig (7): Untreated brain was no histopathological alteration in the neurons of the brain.

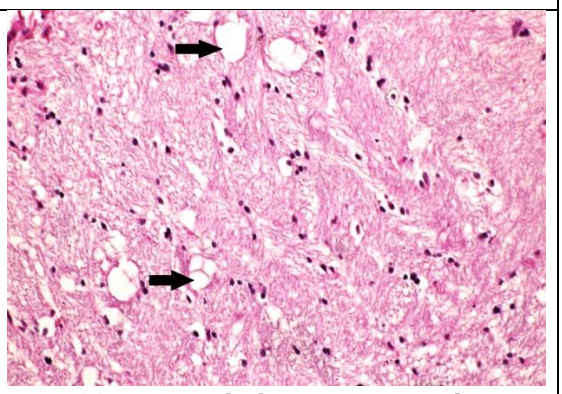

Fig (8): Treated brain cortex showing acidophilic necrosis of neurons, the neurons showing shrinkages of the nuclei of the neurons with acidophilic cytoplasm (arrows) degeneration of the white matter (destructed axons) (long arrows) with digestion chambers (the space of autolysed axons) (StainH&EX400)

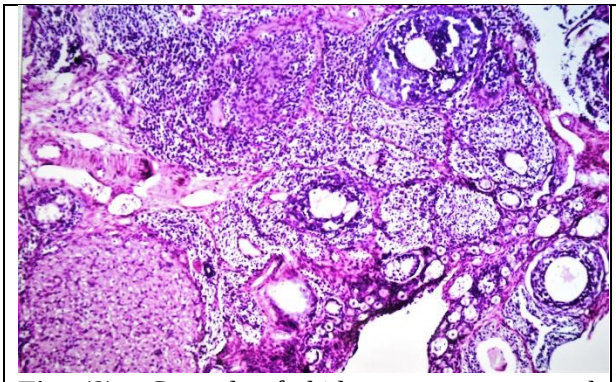

Fig (9): Control of kidney was a normal histological structure of the glomeruli and tubules at the cortex

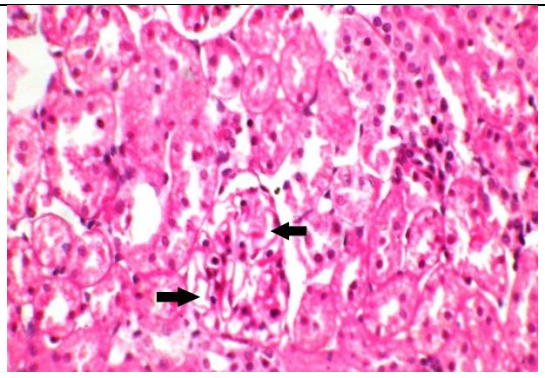

Fig (10): Treated kidney sever hyalinosis of the renal glomerular blood capillaries (thickened eosinophilic thickening with necrotic nuclei of the endothelial cells (short arrows) and necrobiotic changes of the renal tubuli (long arrows (StainH&EX600)

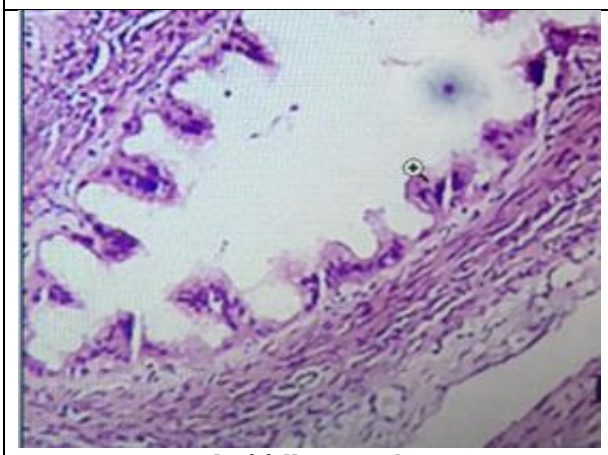

Fig (11): Control of fallopian tube was no histopathological alteration and the normal histological structure of the fallopian tube.

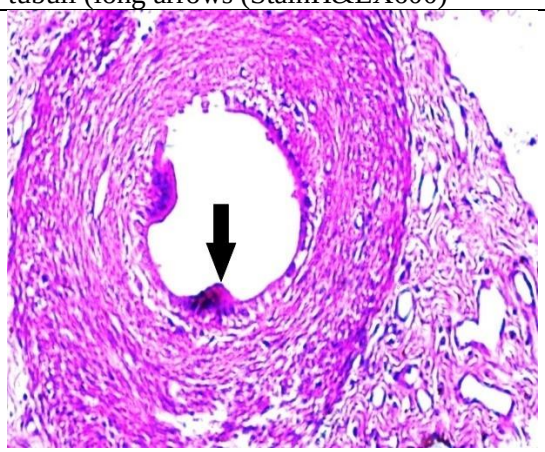

Fig (12): Treated fallopian tube showing shortening and necrosis of the fallopian tube villi (Arrows) and shortening and necrosis of the fallopian tube villi (Arrows) (StainH&EX400)

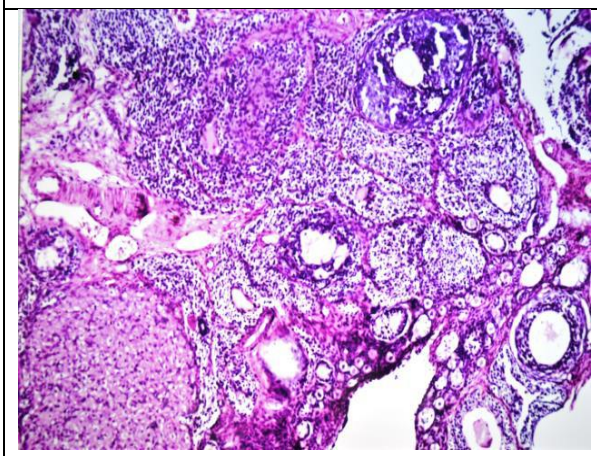

Fig (13): Ovary of rat in control group showing normal of different stages of ovarian follicles (f), corpus luteum (c) and interstitial stromal cells (H&E* 16)

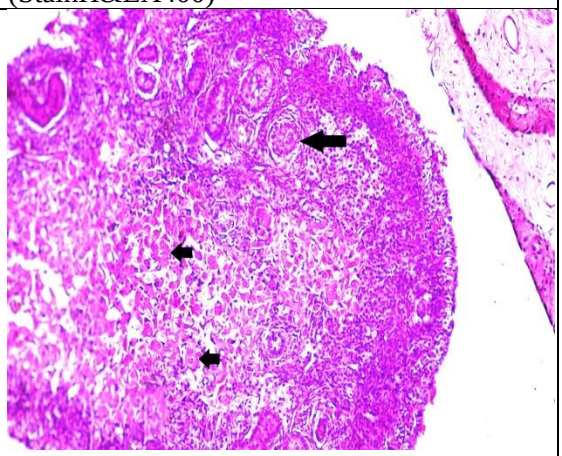

Fig (14). Treated ovary showing necrosis of the oocytes (long arrow) and necrosis of the cellular structure of the medulla of ovary (short arrows) (StainH&EX200)

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