Egypt. J. Plant Prot. Res. Inst. (2020), 3 (3): 823 -834

Egyptian Journal of Plant
Protection Research Institute
www.ejppri.eg.net

Effect of garlic oil as a rodenticide for black rats *Rattus rattus* (Rodentia: Muridae)

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ARTICLE INFO
Article History
Received: 5 / 7 /2020
Accepted: 2 /9 /2020

Keywords
Garlic oil, rodenticide, *Rattus rattus*, toxicity and histopathological.

Abstract:

This research focuses on the toxicity study of the garlic oil in *Rattus rattus* (L.) (Rodentia: Muridae). The used of 22 rats has been selected to study acute and chronic toxicity to start a trial in non- choice test. Nine animals were used for 2, 4 and 8 % garlic oil bait for a preliminary experiment, and the 13 number, for 0.5,1 and 1.5% for the main experiment in non-choice laboratory test. Results indicated for a preliminary experiment that 2% of garlic oil highest toxicity concentrations after 48 hrs. was recorded 40% and acceptability 44.45% . It is very clear that the deadly effect of all concentrations according to the histopathology study on kidney and uterus was effective. The main experiment, the high mortality percent of the rat was 60% for 1% garlic oil bait and acceptability 51.57 was recorded while in 1.5% garlic oil bait record 40% mortality and 46.16 acceptability. Histopathological changes in kidney showing glomerular capillaries with very thin basement membrane mesangiolysis and destructed walls, uterus showed degeneration and necrosis of uterine gland and fibrosis in lamina propria of endometrium, the ovaries showed atrophy of the growing follicles and free from any stages of maturation. The testis tubuli lumen exhibited necrosis and degeneration of sprmatogonium cells and epididymis free of spermatozoa and some of lumen full of degenerated necrosed.

Introduction

Rodents are one of the most dangerous pests in Egypt. Among the common types of rodents (Black and Norway rats). Rodents were also considered a serious pest on agricultural crops and stored goods and have behavioral phenomena such as high intelligence and a strong sense of smell, which makes it difficult to control rodents. The repeated use of chemical rodenticides, in agriculture pest control programs, led to the development of many serious problems affecting the control success. From these problems are pest resistance to these rodenticides and the pollution of the environment. Therefore, there is a growing need to find new natural extractions, which have a strong effect against pests but safe and friendly to the environment in order to ensure the future safe of agriculture food required for the world population without destroying the ecosystems. To reach these goals,

researchers began to explore alternative, less expensive and environmentally friendly, pesticides derived from naturally toxic plants.

The researchers study the effect of some different plant products (Garlic oil, garlic extract, garlic powder and drugs). Oils on the control against rats such as garlic oil is a product that can be manufactured at home, does not need many steps and, garlic oil has the properties of disinfectant and the quality deadly bacteria and viruses, and man can eat oil in small quantities and do not constitute harm to health. Chegu *et al.* (2018) showed the daily intake of these aqueous plant extracts may help full to prevent the cardiovascular diseases. And Ozougwu *et al.* (2014) suggested that the administration of *Allium sativum* extracts protected against paracetamol liver damage in rats. Garlic oil significant rise in urea and D-aspartate aminotransferase and inhibition of alkaline phosphatase in serum were observed in rats fed garlic extract (Joseph *et al.*, 1989). But other authors Hammami and El May (2012) which could have another potential negative effect about spermatogenesis. Also, must note the potential interference that may exist between drugs and garlic consumption. Abdelmalik (2011) who described morphological aspects suggesting apoptosis of somatic and myoid cells of adult rat after treatment with 20% of crude garlic for 4 months. Several studies Dixit and Joshi (1982) reported that chronic administration of 50 mg of garlic powder to adult rat over 70 days induced a spermatogenetic arrest at the primary spermatocyte stage. Moreover, aqueous garlic extract have spermicidal effects on adult rats (Chakrabarti *et al*., 2003) .

The aim of this study is to develop a method to control of the rats by using natural plant extracts environmentally safe that have no side effect on non-target (Human , animal and plant) in the environment.

Materials and methods

1. Garlic oil:

Commercially available garlic oil from EL-Captain Company For Extracting Natural Oil Plant and Cosmatic. Info@elcaptain eg.com. The garlic bait was prepared by garlic oil mixing 95% crushed maize, and 5% powder sugar.

2. Tested animals:

Healthy animals elected black rats *Rattus rattus* (L.) (Rodentia: Muridae), from Abou-Rawash district, Giza Governorate. . Rats were individually caged in wire cages (50 x 30 x 20 cm), acclimatized for at least 2 weeks. Food (dried bread) and water were provided *ad libitum.* Inactive (unhealthy) rats were excluded. The choice of 22 rats has been selected to start trial in non-choice test. Nine animals were used for 2, 4 and 8 % garlic oil bait for preliminary experiment, and the 13 number, for 0.5,1 and 1.5% for main experiment in non-choice laboratory test.

3. Cage tests:

Studies were conducted on rats to test the effect of garlic oil toxicity/bait formulations and its histopathological effect.

4. Preliminary experiment:

A known amount (40g) of crushed maize was placed in a buttery dish, and a known amount (40g) of 2, 4 and 8 % garlic oil mixed with crushed maize were provided daily to each of 3 caged rats for 3 days in pre-treatment and treatment test, respectively. Water was provided to rats *ad libitum*. Consumption have been, number of died rats were daily recorded. The acceptability and mortality of rats were calculated. Finally, observation to period 28 days with plain bait (crushed maize) *ad libitum.* . Rats of each group were sacrificed by an overdose of chloroform, the testes as well as the

epididymides, kidney, uterus and ovary were dissected out for gross as well as for histopathological examination. Rats were sacrificed after diet for all group.

5. Main experiment:

A known amount (40g) of crushed maize was placed in a buttery dish in pre-treatment and a known amount (40g) of 1.5,1 and 0.5 % garlic bait (Garlic oil mixing 95% crushed maize, and 5% powder sugar) were provided daily to each of 5,5 and 3 caged rats for 4 days in treatment test. Water was provided to rats *ad libitum*. Consumption have been, number of died rats were daily recorded. Rats of each concentration were sacrificed by an overdose of chloroform, kidney, uterus, ovary and the testes as well as the epididymides were dissected out for gross as well as for histopathological examination. Males and females of the group were sacrificed after 60 days of treatment, the acceptability and mortality of rats were calculated. Finally, observation to period 4 weeks with plain bait (Crushed maize) *ad libitum*. The acceptability and mortality of rats were calculated. Using the following equation (Mason *et al.*, 1989).

$$Acceptability\ \% = \frac{Average\ daily\ \ consumption\ \ of\ treated\ food\ \ (g)}{Total\ average\ daily\ \ consumption\ \ of\ (treated + untreated)\ food\ (g)} X\,100$$

6. Histopathological examination:

For histopathological examination, the collected specimens were fixed in 10% neutral buffered formalin for at least 24 hrs. and then routinely processed by conventional method and finally stained by heamatoxylene and eosin (Suvarna *et al.,* 2013). The preparations were examined by the light microscope for identifying the presence of histopathological changes for kidney, testis, uterus and ovary for different concentration of garlic oil.

7. Statistical analysis:

The results were statistically analyses using the standard statistical methods LSD- test was applied in the analyzed by SAS (2006).

Results and discussion

1. Preliminary test of the effect of different averages of garlic oil bait for black rats daily consumption, acceptability and mortality:

Table (1) showed that the average daily consumption 2% garlic oil bait were height than 4% and 8%. The acceptability percent was 44.45%, 39.46% and 43.58% for 2%, 4% and 8% garlic oil crushed maize baits, respectively. Rats mortality was observed during the treatment and after 48 hrs. of treatment in case of 2% bait. In case of 4% mortality was after 29 days of treatment, while the mortality in between 44 and 66 days of treatment in case of 8% garlic oil bait. The bait consumption of 2% garlic oil bait in the first second and third day about the same 12.42, 12.13 and 12.68g, respectively. Daily consumption decreased gradually.8.33, 6.82 and 6.75g for 4% bait. From data mention above the effective concentration of garlic oil bait is 2%. Saxena *et al.* (1995) conducted laboratory experiments against *Mus musculus* of 2% garlic pulp and other plants as bait attractants and their palatability was recorded. Garlic pulp almost significantly decreased consumption compared to plain bait. (Roasted bajra). Chegu *et al.* (2018) aimed to evaluate the possible anticoagulant effect of aqueous extract of garlic and were tested for invitro prothrombin time (PT) test. Its results demonstrated that aqueous extract of garlic posse pharmacologically active anticoagulant component which could helpful in prevent blood clot.

Table (1): Preliminary test of the effect of average 2%, 4% and 8% of garlic oil bait for black rats daily consumption (g±SD), acceptability and mortality under laboratory conditions.

C.	R.	Average daily consumption of rat (g)								M.	A.
		Pre-treatment				Treatment					
		1st day	2nd day	3rd day	A.	1st day	2nd day	3rd day	A.		
2%	5	15.49 ±5.21	12.57 ±5.22	14.10 ±7.76	14.11± 5.67^{A}	12.42± 7.37	12.13 ±4.51	12.68 ± 8.99	11.29± 6.83^{A}	40	44.45
4%	2	11.18 ±0.21	11.44 ±1.13	9.62± 0.83	10.75± 0.75^{B}	8.33± 3.91	6.82± 0.48	6.75± 1.51	7.30± 1.97^{B}	0	39.46
8%	2	11.31 ±2.29	9.33± 2.30	6.90+ ±0.50	9.18± 0.17^{B}	9.22± 1.09	5.92± 3.53	6.14± 1.26	7.09± 1/96^{B}	0	43.58

C.: Cocentration R. : Replecates A. : Average M. : Mortality A.: Acceptability.
The vertical columns marked with the same letters are not significantly different by SAS (2006).

2. Histopathology studies:

It is very clear that the deadly effect of all concentration according to the histopathology study on kidney (Figures 1-3) and uterus (Figures 4-6) was effective, but the occurred early death in a 2% garlic bait be the optimal concentration of treatment. There was histopathologyical findings observer in ovary, testes which probable cause in main is pyelonephritis.

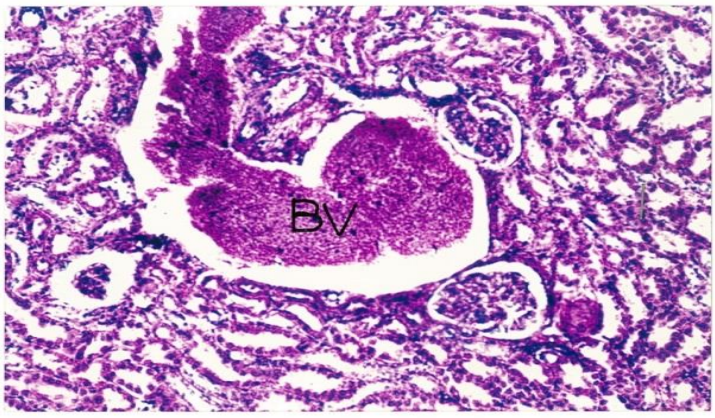


Figure (1): Kidney of rat 2% showing sever dilation and congestion of the intertubular blood vessels (BV) FE X40.

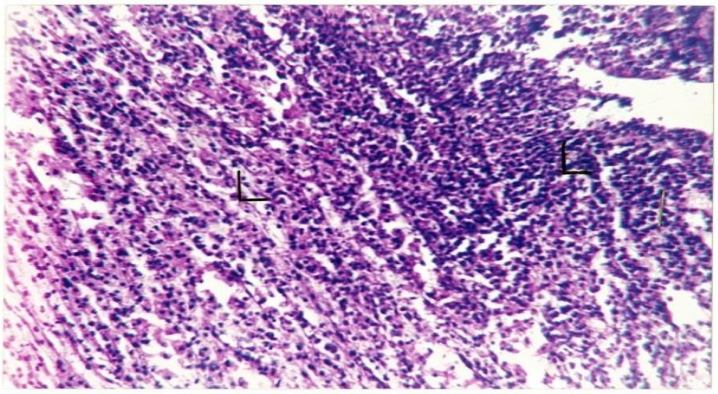


Figure (2): Kidney of rat 4% identify the pus formation and liquefaction in the renal pelvis and medulla HE X40.

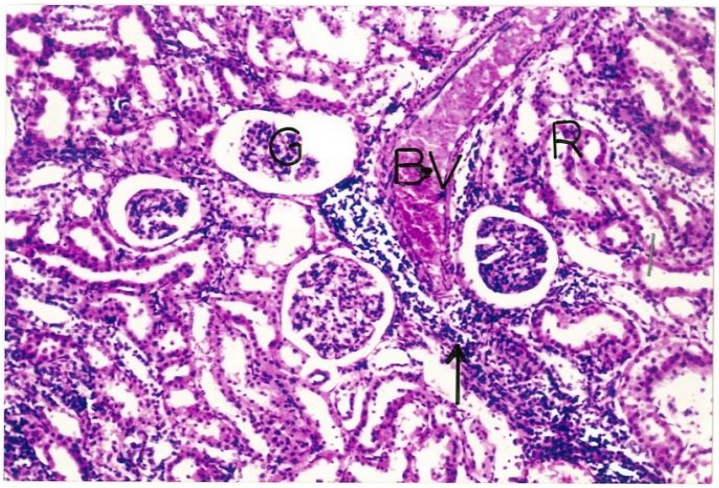


Figure (3): 8% Kidney showing inflammatory cells infiltration surrounding the dilated blood vessels (BV).

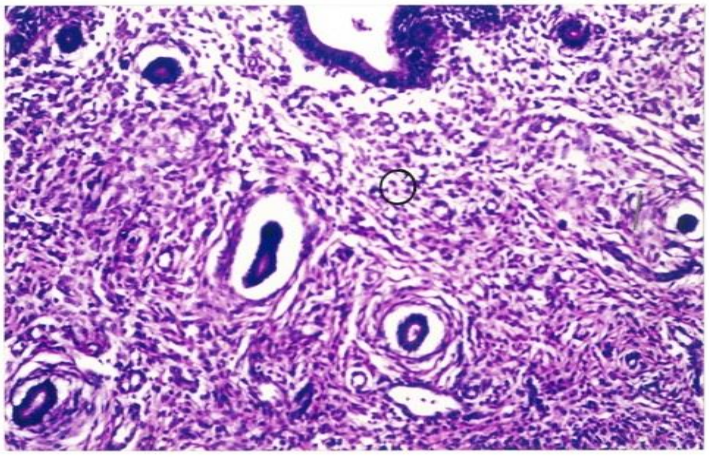


Figure (4): Uterus female rat showing oedema in lamina propria (o) HE X 40 2%.

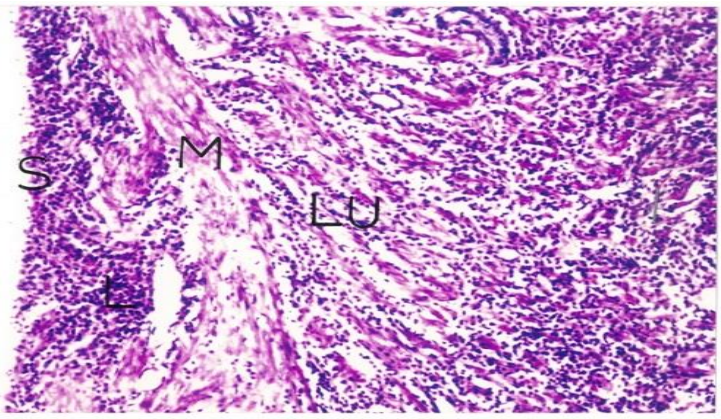


Figure (5): Uterus of rat showing massive number of inflammatory cells infiltration (LU) in the mucosnl, muscular (M) and serosol layer(S) HE X 40 8%.

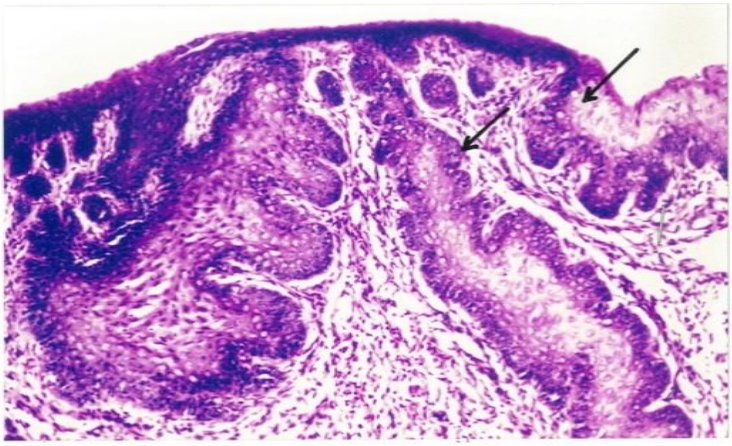


Figure (6): Uterus of rat showing hyperplastic and metaplastic of the mucosal liming epithelium 8% (HE X 40).

Histopathological changes in kidney, uterus, ovary, testis and epididymis in survived rat fed garlic oil crushed bait during 4 days in non-choice test after 60 days of treatment in the following.

2.1. 0.5% garlic oil bait:

The histopathological changes in kidney showing glomerular capillaries with very thin basement membrane mesangiolysis and destructed walls (Pink arrows), very thin basement membrane cells(Green arrow) and loss of glomerular tuft with empty glomerular corpuscle (Yellow arrow) (Figure 7). Joseph *et al.* (1989) who recorded significant rise in urea and D-aspartate aminotransferase and inhibition of alkaline phosphatase in serum were observed in rats fed garlic extract (2 ml/100 g body wt., intragastrically) for 10 days. Richard *et al.* (2000) impaired reproductive function accompanies chronic renal insufficiency (uremia) in experimental animal. Clinical hypogonadism occurs in both genders. Anti-ovulatory effects of uremia in the female rat was record. The ovaries showed regressed atrophied fibrosed ovaries (Yellow arrow) (Figure 8). The uterus revealed mucoid degeneration of the of endometrial lamina propria (Figure 9). That results supported by Musk and Clapham (1997) who found that daily sulfide (DAS) and daily disulfide (DDS) from garlic oil, were tested for cytotoxic and genotoxic effects in a Chinese hamster ovary cell line who found both compounds were found to induce both chromosome aberrations and sister chromatid exchanges (SCEs) with DDS but DDS was found to be more cytotoxic than DAS. Mucinous metaplasia is an uncommon type of endometrial epithelial metaplasia, often associated with hyperestrogenic states or hormone replacement therapy.

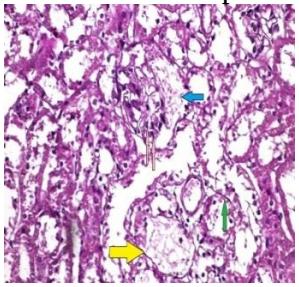


Figure (7): Kidney (H& EX200).

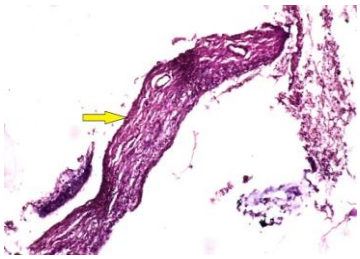


Figure (8): Ovarian (H& EX100).

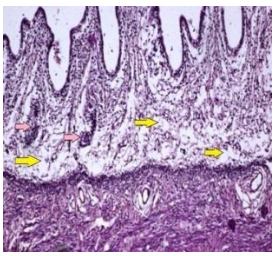


Figure (9): Uterus (H& EX200).

Complete loss of spermatogonium cells and the basement membrane of testis tubuli appear bar of the cells, severe vaccuolations of sertoli cells (Pink arrows) thrombus blood vessel (Blue arrow) degeneration, necrosis in leydig cells. In other words, the severely stiffed wall of testis tubuli fibrosed increase hyalinazation of the basement membrane with severely damaged wall of testis tubuli and disruption of blood testis barrier and loss of all spermatogonium cells and thrombi in the blood capillatries (Figure 10). Uhrin *et al.* (2000) who found protein C inhibitor (PCI) is a nonspecific, heparin-binding serpin (serine protease inhibitor) that inactivates many plasmatic and extravascular serine proteases by forming stable 1:1 complex. Proteases inhibited by PCI include the anticoagulant activated protein C, the plasminogen activator urokinase, and the sperm protease acrosin. PCI circulates as a plasma protein but is also present at high concentrations in organs of the male reproductive tract. Infertility was apparently caused by abnormal spermatogenesis due to destruction of the sertoli cell barrier, perhaps due to unopposed proteolytic activity. The resulting sperm are malformed and are morphologically similar to abnormal sperm seen in some cases of human male infertility .Epididymis free of spermatozoa and some of lumen full with degenerated necrosed cells showing honey comb cellular contents in the epididymal lumen (Yellow arrow) (Figure 11).This concentration is sterile for rat and not cause death

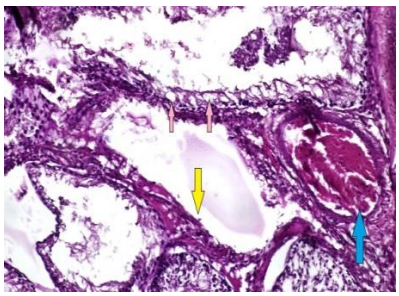


Figure (10): Testis (H& EX400).

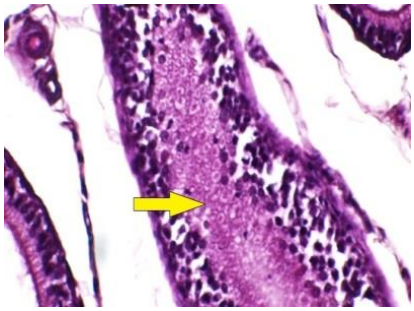
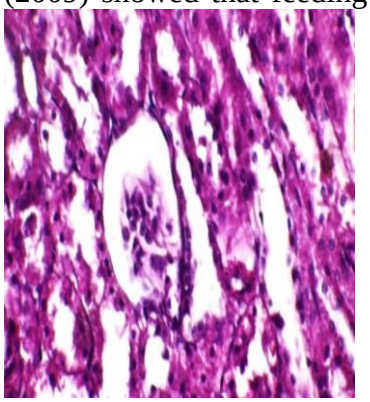


Figure (11): Epididmys (H& EX400).

2.2. 1% garlic oil bait:

Which showing changes in kidneys, atrophy of the glomerular tufts was recorded in male kidneys with necrosis and degeneration of the glomerular capillaries walls (Figure 12). That results agreed with Muralidaran *et al.* (2018), who found a remarkable attenuation in the mesangial expansion and proliferation, glomerular and tubular basement membrane thickening, and the tubular lipid deposits. And found that garlic oil blend significantly promoted renal podocin gene expression by 3.98-fold ($p < 0.001$) and attenuated increased urinary podocin level by 2.92-fold ($p < 0.01$). The changes recorded in testis with garlic oil appeared in 1% as edema (Stars) in between testis tubuli (Blue arrow) and severe shrinkage in basement membrane of testis tubuli with destruction of myoid cells (Yellow arrow) and the testis tubuli lumen exhibited necrosis and degeneration of sprmatogonium cells with loss of abundant number of spermatogonium cell (Green arrow) with vacuuolations and necrosis of sertoli cells and atrophy of leydig cells (Figure 13). Abnormal germ cells present in the lumen of the epididymis (Figure 14). This concentration caused kidney failure and sterile for male rats and cause death for female rat". According to Dixit and Joshi (1982) treated male rats with garlic powder (50 mg garlic powder for 45 days or 70 days by oral route). After

oral administration of 50 mg of garlic powder for 45 days, the testes showed degenerative changes but in most of the tubules normal stages from spermatogonia to spermatids have been seen; seminiferous tubule and leydig cells nuclei were shrunken. After oral administration of 50 mg of garlic powder for 70 days, severe testicular lesions were seen. Spermatogenesis was arrested at the primary spermatocyte stage; sertoli cells also showed degenerative changes (Hammami *et al.*, 2008). The treatment resulted in a significant decrease in testosterone serum levels (at 10, 15 and 30%) associated with a significant increase in LH serum levels. Testicular histology showed a dose-dependent increase in the percentage of empty seminiferous tubules. Moreover, testicular function was affected and Hammami *et al.* (2009) showed that feeding with crude garlic inhibited leydig steroidogenic enzyme expression and sertoli cell markers. These alterations might induce germ cell death (spermatocytes and spermatids) via an apoptotic process.to and added that crude garlic-feeding induced apoptosis in testicular germ cells (Spermatocytes and spermatids). This cell death process was characterized by increased levels of active CASP3 but not CASP6. Expression of the caspase inhibitors BIRC3 and BIRC2 was increased at all doses of as while expression of XIAP and BIRC5 was unchanged. Moreover, expression of the IAP inhibitor DIABLO was increased at doses 10% and 15% of As. The germ cell death process induced by as might be related to a decrease in testosterone production because of the reduced expression of steroidogenic enzymes.



Figure(12): Kidney (H&EX600)

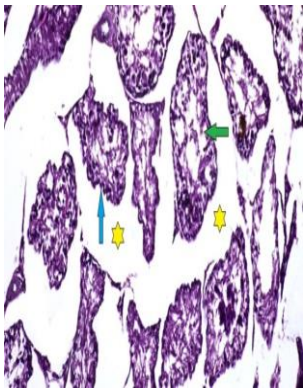


Figure (13): Testis (H&EX200)

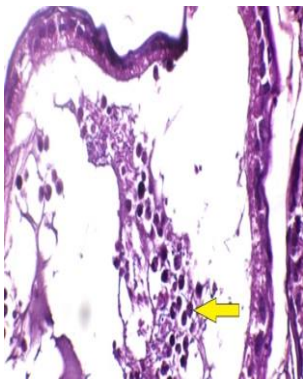


Figure (14): Epididymis lumen (H&EX600)

2.3.1.5% garlic oil bait:

Remain of remnant of glomerular capillaries was highly recorded in this group with presence of erythrocytic casts in the glomerular corpuscle space and in the lumen of renal tubules (Blue arrows) (Figure 15).That was supported by Joseph *et al.* (1989) who significant rise in urea and D-aspartate aminotransferase and inhibition of alkaline phosphatase in serum were observed in rats fed garlic extract (2 ml/100 g body wt., intragastrically) for 10 days (Fowotade *et al*., 2017), who found pronounced degeneration of the tubular epithelial cells lining of the bowman's capsule and enlargement of the bowmann's space due to garlic toxicity.

The uterus showed degeneration and necrosis of uterine gland and fibrosis in lamina propria of endometrium (Figure 16). Two the ovaries showed atrophy of the growing follicles and free from any stages of maturation (Figure 17).

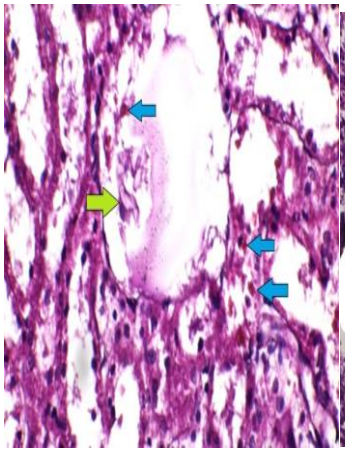


Figure (15): Kidney (H&EX600)

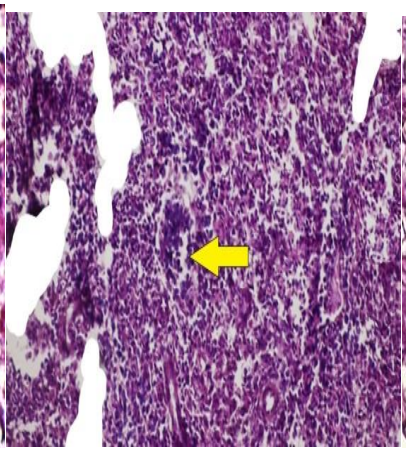


Figure (16): Ovarian (H&EX100)

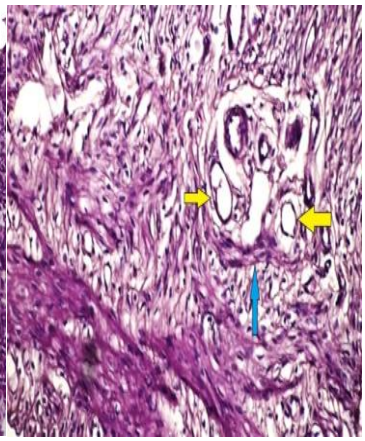
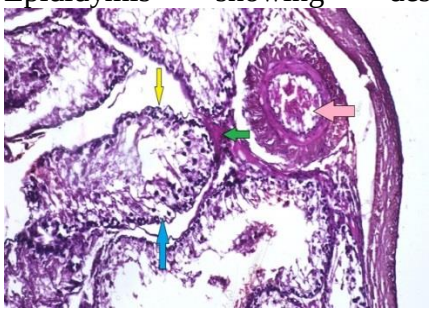


Figure (17): Uterus(H&EX600)

Showed severely edematous testis tubuli and severely degenerated necrosed testis tubuli , the basement membrane appeared bar of the spermatogonium cells (Blue arrow) with sever loss of myoid cells (Yellow arrow) ,degeneration and necrosis of leydig cells (Green arrow) start of accumulation of coagulated blood (Pink arrow), in rete testis (Figure 18). Epididymis showing destructed degenerated immature spermatozoa with reduction in number **(**Figure 19). We also find effect like the above and that no deaths occurred. From this study, we can consider the concentration of garlic oils as a substance that affects the death and sterility of rats for both sexes and it preferable to use a 1% for deadly and sterile enrichment at the same time.



Figure(18): Testis (H&EX 400).

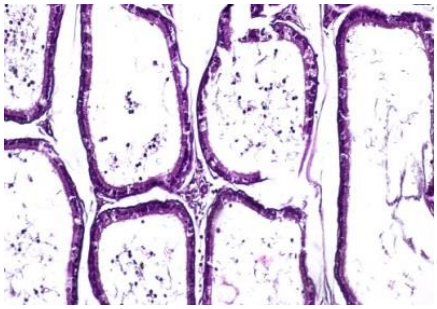


Figure (19): Epididymis(H&EX200).

3. Main test of the effect of different averages of garlic oil bait for black rats daily consumption, acceptability and mortality:

This discrepancy of the studies could be linked of differences in garlic concentration and the period length of treatment. Table (2) showed that the high average daily consumption and acceptability of rats was 0.5% of garlic oil bait, 11.19g and 54%, respectively. The high mortality percent of rat was 60% for 1% garlic oil bait. Non-significant change between the average daily consumption in pre-treatment and treatment test for rats in all concentration. In 0.5% the average daily consumption in pre-treatment and treatment test were 9.48g and 11.19g, respectively with increased 1.18-fold. The acceptability and mortality percent's were 54.14% and 0%, respectively. In 1% garlic oil bait the average daily consumption in pre-treatment and treatment test were 7.69 g and 8.19 g, respectively with increased 1.07-fold in crushed maize . The acceptability and mortality percent's for rats were 51.57 and 60%, respectively. While than 1.5% garlic oil bait the average daily consumption in pre-treatment ant treatment test for rats

were 10.72 g and 9.19 g respectively with decreased 0.009-fold in crushed maize. The acceptability and mortality percent's for rats 46.16 and 40%, respectively. From the data mention above the acceptability decreased when increased concentration and not in mortality. The one percent (1%) garlic oil bail is the effective concentration, (60% mortality) for rats control than 0.5 and 1.5%.

Table (2): Effect of average 0.5%, 1% and 1.5% of garlic oil bait for black rats daily consumption (g±SD), acceptability and mortality under laboratory conditions.

C.	R.	Average daily consumption										M.	A.
		Pre- treatment					Treatment						
		1st day	2nd day	3rd day	4th day	A.	1st day	2nd day	3rd day	4th day	A.		
0.50%	5	11.45 ± 4.85	9.33±3.32	8.53±3.29	8.53±3.29	9.48±3.51^{B}	11.15±4.21	11.41±3.52	12.00±4.51	10.19±1.50	11.19±3.33^{B}	0	54.14
1%	5	8.58±1.35	7.89±1.52	7.59±1.92	6.72±1.42	7.69±1.31^{B}	8.20±0.85	8.95±1.25	7.69±1.73	7.9±1.50	8.19±0.83^{B}	60	51.57
1.50%	3	11.73±2.66	11.05±1.33	9.80±1.82	10.32±2.10	10.72±1.65^{B}	9.31±2.14	8.99±1.73	9.32±1.67	9.14±1.77	9.19±1.55^{B}	40	46.16

C.: Cocentration R. : Replecates A. : Average M. : Mortality A.: Acceptability.
The vertical columns marked with the same letters are not significantly different by SAS (2006).

This study summaries that different garlic oil concentration has different histopathology properties and the impact of 1% garlic oil bait is the most consistent in atrophy of leydig cells of testes, atrophy of the glomerular tufts was recorded in male kidneys and all female is died. This review may be a good reference on relations between male and female sexual function for rat.

Other studies, Arla *et al.* (2004) found that product containing garlic oil show considerable promise as inexpensive, environmental benign and non-lethal bird repellents. Evaluation of acute toxicity of essential oil of garlic and its selected major constituent compounds against overwintering *Cacopsylla chinensis* (Yang and Li) (Hemiptera: Psyllidae) was studies by Zhao *et al.* (2013). His results, the two main constituent compounds , diallyl trisulfide and diallyl disulfide, exhibited strong acute toxicity against overwintering *C. chinensis*, with LC50 values of 0 .64 and 11.04 ug per adult, respectively.

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