Benha Journal of Applied Sciences (BJAS) print: ISSN 2356–9751
Vol. (10) Issue (3) (2025), (47-55) online: ISSN 2356–976x
http://bjas.bu.edu.eg
Applied Agriculture Science

The role of *Zingiber officinale* in alleviating brodifacoum-induced toxicity in male *Mus musculus*

Amr M.Gaafer[2], Gad H.Hassan [1], Abdul mawgoud Abdallah[2], Ghada R.yousef[1] and Moamen A.Elbath[1]
[(1)] Plant Protection Department, Faculty of Agriculture, Banha University.
[(2)] Plant Protection Research institute, Dokki, Giza. Egypt.
E-mail: almohndsamr1@yahoo.com

Abstract

In this study, the focus is on investigating the effects of a specific type of rodenticides on rodents, with an emphasis on chromosomal and histopathological changes. The research also examines the extent of these effects on both the liver and testes of white rats. Additionally, the study explores the role of ginger plant extract as an antioxidant in mitigating these effects. The study was conducted using 65 adult albino mice, which were divided into groups. The first group served as the control group, while groups b & c received ginger extract at doses of 1.5% and 3%. Groups d & e were given a single dose of brodifacoum, with d receiving 1/10 of the lethal dose (LD50) and e receiving 1/20 of the LD50. Groups f to i were treated with brodifacoum followed by ginger extract, while groups j to k received *Zingiber officinale* extract first, then rodenticidesThen, the mice were sacrificed, and their bone marrow cells were analyzed to study chromosomal changes. Then liver and testis samples were collected for histopathological evaluation. The group treated with rodenticides showed a significant increase in chromosomal abnormalities and a reduction in the mitotic index. Additionally, histological changes were observed in the liver and testis. However, in mice treated with both rodenticides and *Zingiber officinale*, chromosomal aberrations decreased, the mitotic index increased, and there were slight improvements in liver and testis histology. These findings suggest that rodenticides has mutagenic effects on chromosomes and causes histopathological changes in organs like the liver and testis, while ginger may serve as a protective agent against the toxic effects of rodenticides.

Key words: Brodifacoum, Anticoagulant Rodenticides, Chromosomal Aberrations

1. Introduction

Chemical pesticides play a crucial role in controlling rodents across various sectors, including agriculture, industry, homes, gardens, and public health worldwide **[13].** Despite their effectiveness, their widespread use has contributed to significant environmental pollution and health risks **[20].** A major concern associated with pesticides is their genotoxic potential, which can lead to genetic disorders, cancers, reproductive issues, and birth defects **[41].** Among the most widely used pesticides are anticoagulant rodenticides (AVKs), which target pests such as roof rats, *Rattus norvegicus*, and *Mus musculus*, also known as commensal rodents **[7].** AVKs are divided into two primary chemical classes: hydroxycoumarins and indandiones. Hydroxycoumarins are further classified into first-generation compounds, such as warfarin and coumatetralyl, and second-generation compounds, including brodifacoum and difethialone **[39].** Brodifacoum is a 4-hydroxycoumarin anticoagulant rodenticide commonly used for rodent control, but it also contaminates food.

Ginger (*Zingiber officinale*), a widely used medicinal plant, is known for its antioxidant properties and its ability to prevent various diseases. According to **[38],** *Zingiber officinale*, derived from the rhizome of ginger, is one of the most commonly utilized members of the Zingiberaceae family. It is a widely used spice in cooking and beverages, with a long history of over 2,500 years in traditional medicine. Ginger has been employed to support digestion and treat conditions such as upset stomach, diarrhea, and nausea. The active compounds in ginger, along with those in other plants from the Zingiberaceae family, which are known for their antioxidant properties. Scientific studies have demonstrated their protective effects against inflammation and cancers, The anticancer efficacy of ginger is attributed to its content of pungent compounds such as (6) gingerol, (6) paradol, as well as other components as shogaols, zingerone.

2. MATERIAL AND METHODS

1-Experimental animals:

A total of 65 adult male albino mice, each weighing between 20-25 grams which are between two to three months old.
, were used in the study. These mice were sourced from the National Research Centre located in Dukki, Giza.

2-Rodenticide and dose used: (KAFR EL ZAYAT PESTICIDES AND CHEMICALS)

The administered doses of brodifacoum were 0.04 mg/kg body weight (B.W) and 0.02 mg/kg B.W, corresponding to 1/10 LD 50 and 1/20 LD 50, respectively. The oral LD50 of brodifacoum for mice is reported to be 0.40 mg /kg B.W (Erickson and Urban, 2004).

Molecular formula

C31H23BrO3

Structural formula:

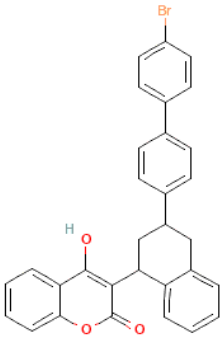

Fig. 1. formula of Brodifacoum

3- Preparation of the aqueous solution of ginger extract:

Ginger (Zingiberaceae) (*Zingiber officinale*) was sourced from alocal markets , two concentrations was prepare extract 1.5% and 3%. To prepare the 1.5% extract, 15 grams of ginger were mixed with 1000 ml of distilled water, boiled for 10 to 15 minutes, then cooled and filtration through filter paper, as described by **[36].** For the 3% extract, the same procedure was followed, using 30 grams of powdered ginger. Each experimental animal was orally administered 1 ml of the final extract **[23]; [32].**

A total of 65 male albino house mouse were assigned to 12 groups in addition to the control group. each consisting of 5 mice. The groups were as follows: Group (a) acted as the control group. Groups (b) and (c) were given ginger extract at concentrations of 1.5% and 3%, respectively. Groups (d) and (e) were treated with brodifacoum at doses of 1/10 LD50 and 1/20 LD50, respectively. Groups f and g (G6 and G7) received brodifacoum at 1/10 LD 50 and 1/20 LD 50, followed by ginger extract at 1.5%. Groups (h) and (i) were administered brodifacoum at 1/10 LD 50, followed by extract at 3%. Group (j) received ginger extract at 1.5%, followed by brodifacoum at 1/10 LD 50. Group (k) was given ginger extract at 1.5%, followed by brodifacoum at 1/20 LD50. Group l received ginger extract at 3%, followed by brodifacoum at 1/10 LD 50, while Group m received ginger extract at 3%, followed by brodifacoum at 1/20 LD50.

Each mouse was orally administered a single dose of brodifacoum and ginger extract, then sacrificed 48 hours after the treatment. Bone marrow cells from the mice were collected for the analysis of chromosomal abnormalities. Metaphase spreads were prepared using the method described by [43] for chromosomal examination.

4-Mitotic index:

A total of one thousand cells were counted from each mouse, and the number of cells undergoing division, including those in prophase and metaphase, was evaluated to determine the level of chromosomal activity. This method helps assess the extent of cell division and any potential chromosomal abnormalities induced by the treatments.

5-Histopathological studies:

Autopsy samples of the liver and testes of albino house mouse were collected and preserved in 10% formalin saline solution for 24 hours. After preservation, the tissues were rinsed in tap water, dehydrated through increasing concentrations of ethyl alcohol, and cleared using xylene. The samples were then embedded in paraffin wax at 56°C using a hot air oven. Tissue blocks, prepared with paraffin and beeswax, were sectioned at a thickness of 5-8 µm using a slide microtome. These sections were mounted on glass slides, deparaffinized, and stained with hematoxylin and eosin **[12].** The samples were then examined under a light microscope for histopathological analysis and photographed.

6-Statistical analysis:

All numerical data were analyzed statistically using a one-way ANOVA test with SPSS software for Windows version 17 (SAS, 2001). Statistical significance was set at $P \leq 0.05$; 0.01, unless otherwise noted. Differences among all treatments were considered statistically significant.

3. RESULTS AND DISCUSION

Chromosomal aberrations:

A range of chromosomal abnormalities were noted in the bone marrow cells of male house mouse exposed to varying doses of brodifacoum (rodenticide). These abnormalities encompassed structural and numerical aberrations, along with chromosomal stickiness. The frequency of these abnormalities was quantified and compared to the control group which was recorded 13.80/1000 metaphase cell that did not receive any treatment. Structural aberrations, such as chromosomal breaks, deletions, and translocations, along with numerical changes like aneuploidy, were all identified, indicating the genotoxic potential of brodifacoum. These findings were assessed to understand the extent of genetic damage caused by exposure to the rodenticide **[13]; [19].**

The mitotic index was observed in the study, alongside the assessment of various structural chromosomal aberrations in the bone marrow cells of treated male house mouse. These aberrations included` chromatid deletions, chromatid gaps, breaks, chromatid fragmentation, centromeric attenuation, centric fusions, chromosomal rings, and end-to-end associations.

Numerical aberrations observed in the study included monosomy and chromosomal stickiness. Table 1 and Figure 2 present the average chromosomal aberrations identified in the bone marrow cells of male house mouse treated with rodenticides. along with those protected by ginger extracts at concentrations of 1.5 & 3%. A statistically significant difference was observed between the control group and the house mouse administered brodifacoum. ($P < 0.05$), indicating that brodifacoum treatment caused chromosomal damage. However, ginger demonstrated a protective effect against this damage, improving the chromosomal integrity of treated animals.

The control group recorded an aberration rate of 13.80 cell /1000 metaphase cells, while the ginger 1.5% treated group showed a slight decrease in the aberration rate to 12.80 cell /1000 metaphase cells. Interestingly, the ginger 3% treated group exhibited an aberration rate of 18.40 cell/1000 metaphase cells, suggesting a potential stimulatory effect on cellular proliferation at this concentration.

When the rodents were exposed to brodifacoum at a dose equivalent to (1/10 LD50), a significant increase in chromosomal aberrations was observed, reaching 76.40 cell/1000 metaphase cells. In contrast, exposure to a lower dose of brodifacoum equivalent to (1/20 LD50) resulted in a reduced aberration rate of 53.00 cell/1000 metaphase cells,

indicating a dose-dependent effect of brodifacoum on chromosomal damage.

The protective role of ginger was further highlighted in combined treatment groups. Treatment with brodifacoum (1/10 LD50) followed by ginger 1.5% resulted in a notable reduction in aberrations to 30.80 cell/1000 metaphase cells, while treatment with brodifacoum (1/20 LD50) followed by ginger 1.5% yielded 31.00 cell/1000 metaphase cells. Similarly, treatment with brodifacoum (1/10 LD50) followed by ginger 3% resulted in 55.60 cell/1000 metaphase cells, and treatment with brodifacoum (1/20 LD50) followed by ginger 3% resulted in 53.20 cell/1000 metaphase cells.

Pretreatment with ginger before brodifacoum exposure demonstrated even more pronounced protective effects. Pretreatment with ginger 1.5% followed by brodifacoum (1/10 LD50) resulted in 42.00 cell/1000 metaphasecells, while pretreatment with ginger 1.5% followed by brodifacoum (1/20 LD50) yielded 45.00 cell /1000 metaphasecells. Similarly, pretreatment with ginger 3% followed by brodifacoum (1/10 LD50) resulted in 60.60 cell/1000 metaphase cells, and pretreatment with ginger 3% followed by brodifacoum (1/20 LD50) resulted in 50.80 cell/1000 metaphase cells.

Additionally, the animals exposed to brodifacoum exhibited a decrease in the mitotic index compared to the control group, while those treated with both ginger extract and brodifacoum showed an increase in the mitotic index, suggesting a potential restorative impact of ginger on cellular proliferation.

In conclusion, these findings suggest that brodifacoum causes dose-dependent chromosomal damage, while ginger, particularly at a 3% concentration, may play an effective role in reducing this damage and restoring cellular proliferation. These findings pave the way for further exploration into the potential of ginger as a protective agent against the adverse effects of toxic substances like brodifacoum.

Histopathological results:

The liver:

The liver of control mice is made up of numerous hepatic lobules, each featuring interconnected strands or cords that surround small blood sinusoids. The hepatic cells are large, polygonal in shape, and possess granular cytoplasm. Their nuclei are generally large, vascular, and include one or more distinct nucleoli. The sinusoidal endothelium consists of undifferentiated lining cells and phagocytic Kupffer cells. The undifferentiated cells are flat in shape, with small nuclei that show no intricate structural details.

Kupffer cells in the liver are large, with oval-shaped nuclei, playing a key role in the liver's immune function. These cells are part of the mononuclear phagocyte system, contributing to the clearance of pathogens and debris. In the hepatic tissue, groups of darkly stained cells are frequently observed. These cells include erythroblasts, lymphocytes, and megakaryocytes, which are blood-forming cells. Erythroblasts are immature red blood cells with spherical nuclei, while lymphocytes have large, round nuclei that nearly fill the cell, leaving a thin layer of cytoplasm. Megakaryocytes, responsible for producing platelets, are large cells with polymorphic nuclei, reflecting their complex role in hematopoiesis. These observations help in understanding the cellular dynamics within the liver, especially in relation to blood cell formation and immune function (Figure 2A).

The liver of male house mouse treated with different doses of rodenticides (1/10 and 1/20 LD 50) showed significant histopathological alterations. Groups d and e, which received brodifacoum, displayed congestion in the central vein and blood sinusoids, accompanied by hemorrhaging, pyknotic nuclei, hydropic degeneration, karyorrhexis (nuclear fragmentation), karyolysis (nuclear dissolution), fatty infiltration, and necrosis in hepatocytes, along with fatty changes in the liver tissue (Figure. 2B, C, & D).

However, in groups f, g, h, and i, which received brodifacoum at doses of 1/10 and 1/20 LD 50 followed by ginger extract at 1.5 & 3%, no significant histopathological alterations were observed when compared to the control group. In contrast, groups j, k, l, and m, treated with varying doses of ginger extract (1.5 & 3%) followed by brodifacoum (1/10 and 1/20 LD50), exhibited histopathological changes, suggesting that ginger extract may offer some protective effects, but not completely prevent the damage caused by rodenticides.

Testis:

The testis is composed of two main components: The stroma and parenchyma comprise the testicular structure. The stroma consists of the tunica albuginea and the tunica vasculosa. The tunica albuginea is a dense fibrous capsule enveloping the testis, with increased thickness at the posterior region, forming the mediastinum testis. From this mediastinum, fibrous septa extend, partitioning the testis into interconnected lobules. These structural divisions support the testis in its organized functions of spermatogenesis and hormone production.

The tunica vasculosa is a vascularized layer of loose connective tissue that lines the tunica albuginea and the fibrous septa within the testis, extending between the seminiferous tubules.supplying them with blood vessels that are essential for nutrient exchange during spermatogenesis. The parenchyma of the testis consists of the seminiferous tubules and the interstitial cells .

The seminiferous tubules are rounded or oval structures, each encased by a thin basement membrane and lined by spermatogenic epithelium. These tubules are the sites of sperm production and contain Sertoli cells and spermatogenic cells. Sertoli cells, which are tall, pyramidal cells with large oval nuclei, provide structural and metabolic support to the spermatogenic epithelium, playing a crucial role in the development and nourishment of developing sperm cells **[16].**

The majority of the cells are spermatogenic, spanning from the periphery to the lumen. These consist of spermatogonia, primary spermatocytes, secondary spermatocytes, and spermatids. The interstitial cells are found either individually or in clusters, Located in the interstitial spaces between the seminiferous tubules and surrounded by the blood capillaries of the tunica vasculosa, these cells are large and oval-shaped, featuring one or two rounded, vesicular nuclei. (Figure. 3A). Mice treated with ginger at doses of 1.5 & 3% (Group b &c) exhibited normal testicular structure without histological changes, similar to the control house mouse (Group a) (Figure. 3A). In contrast, house mouse treated with different doses of rodenticides (1/10 and 1/20 LD 50) in all groups (d & e) showed degeneration and atrophy of the surrounding tubules, with irregular peripheral outlines,

loss of spermatogenesis, and the appearance of giant spermatogonial cells. Additionally, and the most seminiferous tubules exhibited a diffusion of their components, with no clear distinction between different spermatogenic cell types, nuclei of these cells were pyknotic Figure 3 (B & C).

The examination of the testes of male house mouse treated with different doses of rodenticides (1/10 & 1/20 LD 50) followed by *Zingiber officinale* extract (1.5 & 3%) in groups f, g, h and i showed almost complete restoration of the histopathological damage, resulting in a nearly normal testicular appearance. In contrast, the testes of male house mouse treated with Zingiber officinale extract (1.5 & 3%) followed by brodifacoum (1/10 & 1/20 LD 50) in groups j, k, l and m showed the presence of giant spermatogonial cells in the lumen of seminiferous tubules, along with the accumulation of cellular debris in the lumen of some seminiferous tubules (Figure. 3 D).

4. Discussion:

The study highlights the genotoxic and histopathological effects of brodifacoum, a rodenticide, on male house mice, as well as the potential protective role of ginger (*Zingiber officinale*) and *Origanum majorana* in mitigating these effects. The findings are discussed in two main sections: chromosomal aberrations and histopathological alterations in the liver and testes.

Chromosomal Aberrations:

The study revealed that brodifacoum exposure induced significant chromosomal abnormalities in bone marrow cells, including structural aberrations (e.g., chromosomal breaks, deletions, translocations) and numerical aberrations (e.g., aneuploidy, chromosomal stickiness). These findings align with previous research demonstrating the genotoxic potential of rodenticides [13]; [19]. The dose-dependent increase in chromosomal aberrations, particularly at higher doses of brodifacoum (1/10 LD50), underscores the compound's capacity to cause genetic damage.

Ginger extract, however, demonstrated a protective effect against brodifacoum-induced chromosomal damage. While the 1.5% ginger concentration slightly reduced aberrations, the 3% concentration showed a more pronounced protective effect, particularly in combined treatment groups. This suggests that ginger may possess antioxidant properties that counteract the oxidative stress induced by brodifacoum, thereby preserving chromosomal integrity. Interestingly, the 3% ginger concentration alone resulted in a higher aberration rate, possibly due to its stimulatory effect on cellular proliferation, which could increase the likelihood of mitotic errors.

The mitotic index, a marker of cellular proliferation, was reduced in brodifacoum-treated groups but improved in groups co-treated with ginger, further supporting its restorative potential. These findings suggest that ginger, particularly at higher concentrations, may play a significant role in mitigating the genotoxic effects of brodifacoum, although further research is needed to elucidate the underlying mechanisms.

Histopathological Results of Liver:

The liver of control mice exhibited normal histological architecture, with well-defined hepatic lobules, sinusoidal endothelium, and Kupffer cells. In contrast, brodifacoum-treated mice showed severe histopathological alterations, including congestion, hemorrhage, hydropic degeneration, karyorrhexis, karyolysis, and fatty infiltration. These changes are consistent with the hepatotoxic effects of rodenticides, which likely result from oxidative stress and lipid peroxidation.

Ginger extract, particularly at 3%, significantly reduced these histopathological changes, suggesting its hepatoprotective potential. The antioxidant properties of ginger may help neutralize reactive oxygen species (ROS) generated by brodifacoum, thereby preserving hepatic structure and function. However, the incomplete prevention of damage in some groups indicates that ginger's protective effects may be dose-dependent and influenced by the timing of administration.

Testis:

The testes of control mice displayed normal histology, with intact seminiferous tubules, spermatogenic cells, and interstitial cells. Brodifacoum exposure caused severe testicular damage, including tubular atrophy, loss of spermatogenesis, and the appearance of giant spermatogonial cells. These findings are consistent with the vulnerability of testicular tissue to oxidative stress due to its high mitotic activity and unsaturated fatty acid content.

Ginger extract, particularly at 3%, nearly restored normal testicular histology in groups treated with brodifacoum, highlighting its potential to protect against testicular damage. However, pretreatment with ginger followed by brodifacoum exposure still resulted in some histopathological changes, such as the presence of giant spermatogonial cells and cellular debris. This suggests that while ginger offers significant protection, it may not completely prevent the adverse effects of brodifacoum on testicular tissue.

5. Conclusion:

The study demonstrates that brodifacoum induces dose-dependent chromosomal and histopathological damage in male house mice, while ginger and Origanum majorana exhibit protective effects against these alterations. The antioxidant properties of these plant extracts likely play a key role in mitigating oxidative stress and preserving cellular integrity. However, the extent of protection varies with the concentration and timing of administration, highlighting the need for further research to optimize their use as protective agents against toxic substances like brodifacoum. These findings open new avenues for exploring natural compounds as potential therapeutic interventions against chemical-induced toxicity.

Table (1) Mean of chromosomal aberration/50 metaphase cell observed in the bone marrow cells of male house mouse treated with brodificoum and protected with ginger extract (1.5 & 3%)

Group	The Tested	No. of cell with aberration/50 metaphase cell										S.D	Total	Total ± S.E
		deletion	fragment	ring	attenuation	fusion	End to End	breaks	gap	monosomy	stickness			
a	Control	4.4±0.510	0.0±0.0	1.2±0.210	1.0±0.00	4.4±0.497	0.6±0.249	0.6±0.236	0.4±0.231	0.2±0.200	1.0±0.452	1.63	13.80	13.80±0.517
		dehijklm	defghijklm	dehijklm	dehilm	dehilm	defhijlm	dfghijklm	dehijklm	deghijklm	dehilm			
b	Ginger (1.5 %)	4.6±0.583	0.0±0.0	1.0±0.318	0.6±0.400	3.2±0.354	0.8±0.367	0.8±0.367	0.6±0.246	0.2±0.200	1.0±0.389	1.46	12.80	12.80±0.460
		dehijklm	defghijklm	deghijklm	dehilm	dehijlm	defhijlm	deghijlm	dehijlm	deghijklm	dehilm			
c	Ginger (3 %)	4.8±0.663	0.2±0.198	2.2±0.198	1.2±0.202	4.6±0.379	1.2±0.190	1.8±0.349	0.6±0.245	0.4±0.249	1.4±0.0.416	1.63	18.40	18.40±0.515
		dehijklm	abc	dehim	dehlm	dehil	defhijlm	dhil	dehijlm	deghiklm	dehilm			
d	B (1/10 LD50)	22.6±1.882	3.6±0.397	4.8±0.381	4.8±0.376	12.6±0.833	7.2±0.379	4.4±0.574	4.4±0.512	4.4±0.289	7.6±0.499	5.89	76.40	76.40±1.864
		abcefghijklm	abc	abcfgijk	abcfghijkm	abcefghilm	abcefgijkm	abc	abcfgk	abcefgijkm	abcfgjk			
e	B (1/20 LD50)	14.8±1.463	3.0±0.449	3.8±0.201	3.6±0.247	8.0±0.764	4.6±0.382	3.2±0.389	3.8±0.482	2.8±0.364	5.4±0.587	3.67	53.00	53.00±1.161
		abcdfg	abc	abcf	abcfg	abcdgk	abcdg	abl	abcfg	abcdf	abcgjk			
f	B (1/10 LD50) G (1.5%)	7.0±0.707	2.4±0.511	2.2±0.200	1.6±0.298	5.4±0.420	3.2±0.537	2.6±0.398	1.8±0.369	1.2±0.215	3.4±0.560	1.82	30.80	30.80±0.574
		dehijklm	abc	dehim	dei	di	abdi	al	dehil	deghi	dhi			
g	B (1/20 LD50) G (1.5%)	7.8±0.665	3.0±0.318	2.6±0.247	2.6±0.247	3.8±0.278	2.0±0.346	3.2±0.249	1.8±0.376	1.8±0.228	2.4±0.273	1.77	31.00	31.00±0.560
		dehilm	abc	bdi	abcd	dehilm	dehilm	abl	dehil	abcdf	dehilm			
h	B (1/10 LD50) G (3%)	13.8±1.020	2.6±0.245	3.8±0.376	3.0±0.318	7.8±0.592	5.4±0.841	4.2±0.389	4.2±0.371	3.6±0.266	7.2±0.594	3.36	55.60	55.60±1.062
		abcdfg	abc	abcf	abd	abcdgk	abcgk	abc	abcfgk	abcdfjkm	abcfgjk			
i	B (1/20 LD50) G (3%)	16.0±1.225	3.2±0.379	3.2±0.194	2.8±0.467	8.2±0.342	4.4±0.494	3.8±0.497	3.6±0.249	2.4±0.234	5.6±0.543	4.12	53.20	53.20±1.301
		abcdfg	abc	abd	d	abcdgk	abcdg	abc	abcfg	abcd	abcgjk			
j	G (1.5%) B (1/10 LD50)	13.6±1.030	2.2±0.374	3.0±0.314	2.4±0.0.296	6.4±0.637	3.6±0.522	3.2±0.464	3.4±0.297	1.8±0.281	2.4±0.510	3.54	42.00	42.00±1.120
		abcdfg	abc	abd	d	bdi	abcdi	abl	abc	abdlh	dehilm			
k	G (1.5%) B (1/20 LD50)	12.2±0.865	12.2±0.865	2.8±0.190	2.2±0.364	4.4±0.0.614	2.2±0.309	2.6±0.272	2.2±0.248	2.6±0.249	1.6±0.543	4.12	45.00	45.00±1.304
		abcdf	abc	abd	abcfg	dehilm	dhlm	al	ah	abcdhl	dehilm			
l	G (3%) B (1/10 LD50)	15.8±0.739	3.4±0.412	4.2±0.564	3.8±0.275	9.4±0.965	6.4±0.627	4.2±0.435	3.8±0.352	3.6±0.248	6.0±0.708	3.89	60.60	60.60±1.231
		abcdfg	abc	abcfg	abcfg	abcdfgjk	abcfgjk	abcefg	abcfg	abcdfjlm	abcfgjk			
m	G (3%) B (1/20 LD50)	14.6±0.926	3.0±0.319	3.6±0.221	3.2±0.364	7.2±0.682	5.2±0.591	3.4±0.384	3.0±0.329	2.2±0.211	5.4±0.521	3.66	50.80	50.80±1.158
		abcdfg	abc	abcf	abcd	abdgk	abcdgk	abi	abc	abcdhi	abcgjk			

(a): Significant compared to the control group. (b): Significant compared to GINGER (1.5%). (c): Significant compared to GINGER (3%). (d): Significant compared to Brodificoum (1/10 LD50). (e): Significant compared to Brodificoum (1/20 LD50). (f): Significant compared to Brodificoum (1/10 LD50) and GINGER (1.5%). (g): Significant compared to Brodificoum (1/20 LD50) and GINGER (1.5%). (h): Significant compared to Brodificoum (1/10 LD50) and GINGER (3%). (i): Significant compared to Brodificoum (1/20 LD50) and GINGER (3%). (j): Significant compared to GINGER (1.5%) and Brodificoum (1/10 LD50). (k): Significant compared to GINGER (1.5%) and Brodificoum (1/20 LD50). (l): Significant compared to GINGER (3%) and Brodificoum (1/10 LD50). (m): Significant compared to GINGER (3%) and Brodificoum (1/20 LD50).

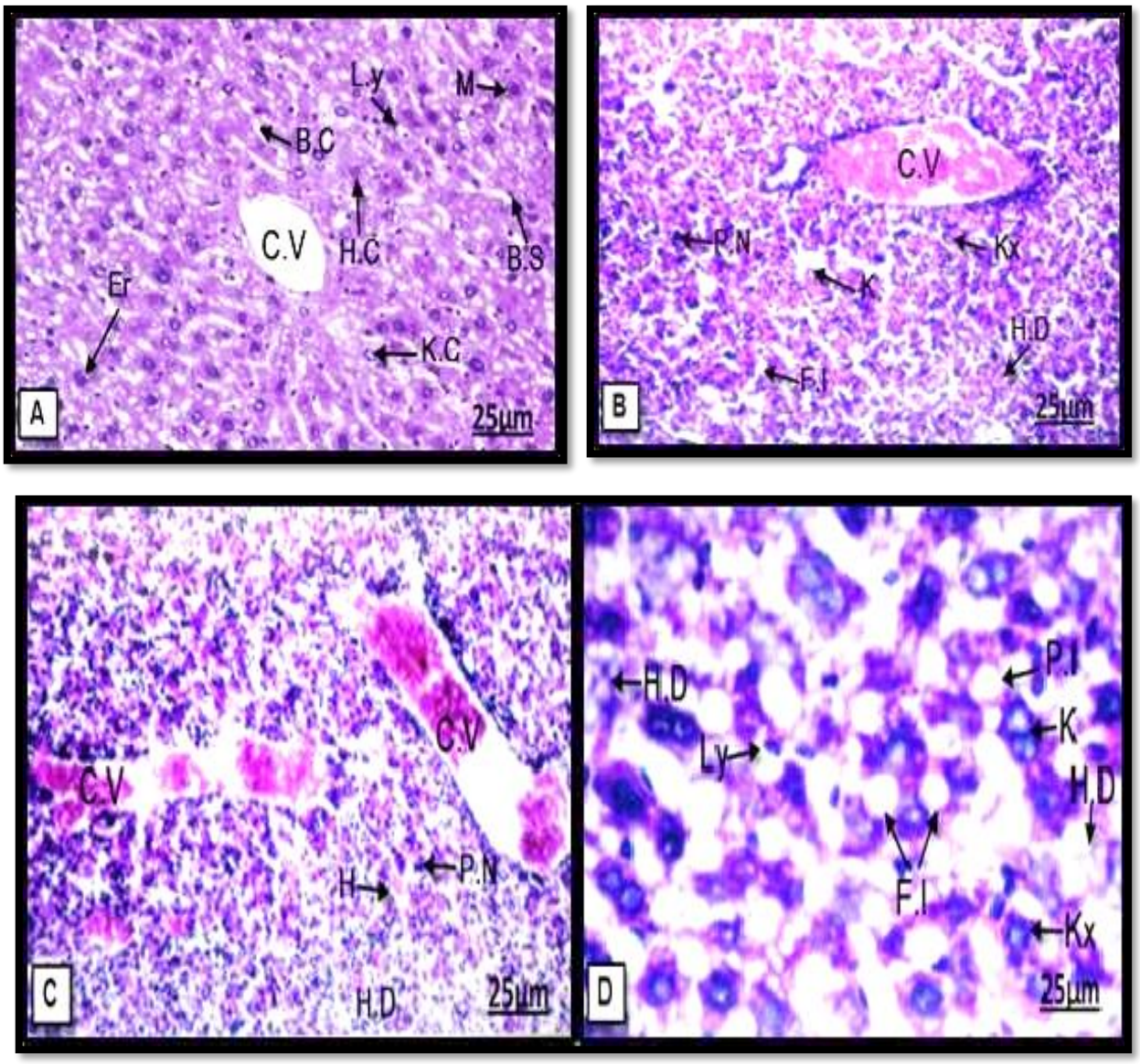

Fig.2(A,B,C and D). Microscopic image of a cross-sectional view of the liver in control mice.

(Fig 2A) Illustrating the typical histopathological architecture of the central vein. (C.V), blood sinusoid (B.S), Surrounding liver cells (hepatocytes). (H.C), erythroblasts (Er), megakaryocytes (M) and Kupffer cell (K.C). liver sections from house mouse treated with varying doses of rodenticides (1/10 and 1/20) LD50 (d & e).

(Fig 2B-D) Demonstrated congestion in the Central vein (C.V). and Blood sinusoid (B.S) with Haemorrhage (H), Pyknotized nuclei (P.N), hydropic degeneration (H.D), karyorrhexis (Kx), karyolysis (K), fatty infiltration (F.I), necrosis in hepatocytes (N), lymphocytes (Ly) and fatty change (F.C). The sections of the liver of male house mouse, administrated ginger extract at doses (1.5 & 3%) followed by rodenticides at different doses (1/10 &1/20 LD 50) (Groups j, k, l and m) demonstrated histopathological changes compared to the control group.

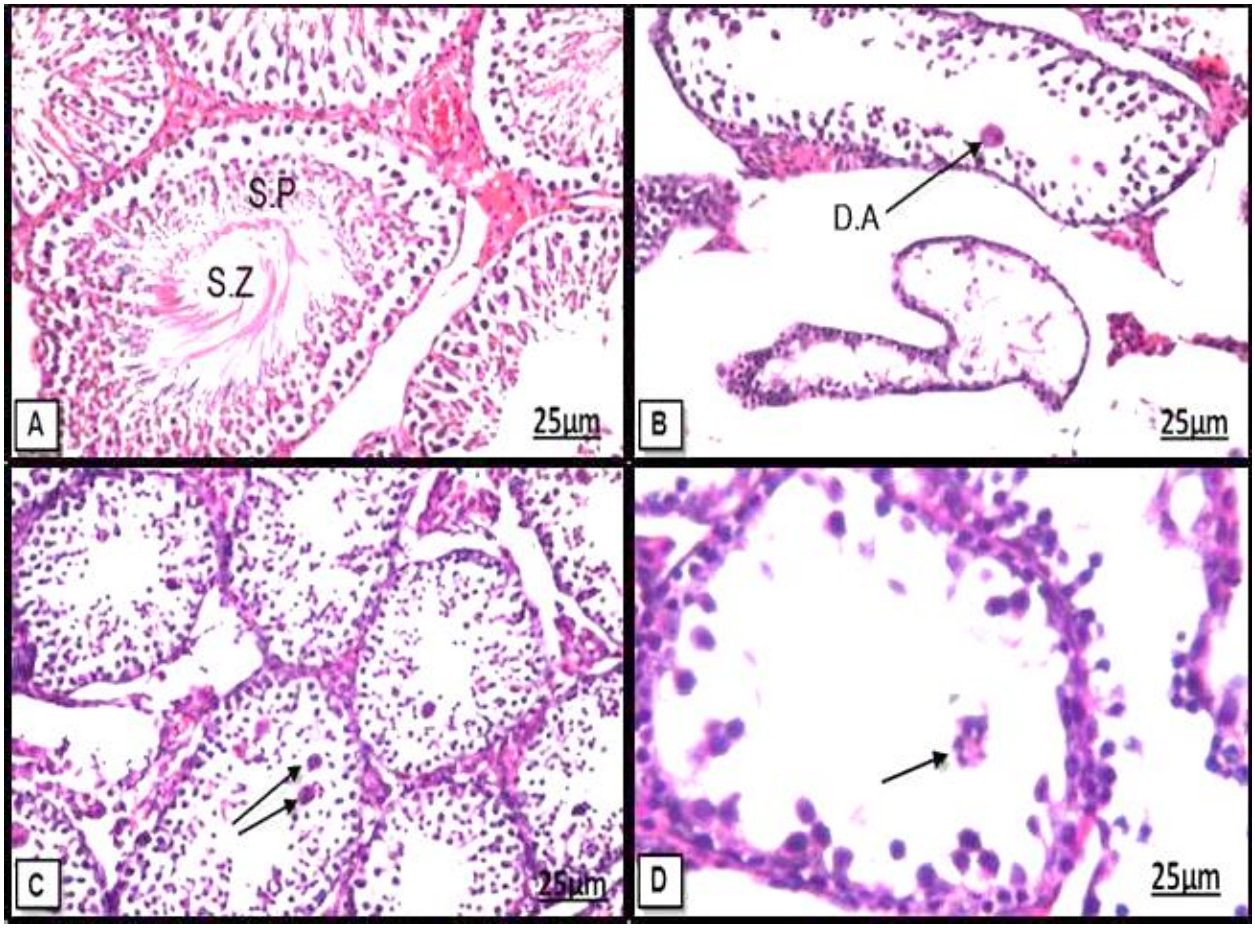

Fig. 3 (A - D): Microscopic image of a cross-sectional view of the testis in control mice.

(Figure. A) Illustrates seminiferous tubules; i.e. spermatogonia (Sg), primary spermatocyte (S.P), secondary spermatocyte (SE.SP), spermatid (SP) and spermatozoa. (SZ). The sections of testis of male mice treated with different doses of (1/10 & 1/20 LD50) of rodenticides (group d & e).

(Figure. B) Illustrates degeneration and atrophy of surrounding tubules with irregular peripheral out line (D.A) and lose of spermatogenesis and appearance of giant spermatogonial cell (arrow) and, most of the seminiferous tubules showed diffusion of its constituents without differentiation between various types of spermatogenic cells.

(Figure. C) Demonstrating magnification to highlight the presence of a giant spermatogonial cell. (arrow), pyknotized nuclei (P.N). The sections of the testis of male mice, treated with ginger extract at doses (1.5 & 3%) followed by different doses (1/10 & 1/20 LD50) of rodenticides (Groups j to m).

(Figure. D) Illustrating the presence of giant spermatogonial cells within the lumen of certain seminiferous tubules.

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