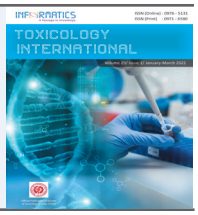
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Carbosulfan Induced Reproductive Toxicity in Male Albino Rats (*Rattus norvegicus*) and Protective effect of *Cissus quadrangularis*

Lokeshkumar Prakash and Muniswamy David*

Department of Zoology, Karnatak University, Dharwad – 580003, Karnataka, India; Lokeshkumarp44@gmail.com, davidkcd@gmail.com

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Abstract

Carbosulfan is the most commonly used carbamate insecticide in the agriculture industry and is known to cause diverse toxicological effects on non-target organisms. Farmers are more susceptible to accidental consumption of insecticides owing to their unscientific use and ease of access. The present investigation was carried out to evaluate reproductive toxicity induced by carbosulfan and its amelioration using traditional *Cissus quadrangularis* plant extract. The rats were divided into six groups; (E_1) control treated with 1% DMSO/kg bw, (E_2) plant extract alone (1000 mg/kg bw), (E_3) low dose of carbosulfan (3.4 mg/kg bw), (E_4) high dose of carbosulfan (6.37 mg/kg bw), (E_5) low dose (3.4 mg/kg bw) and (E_6) high dose (6.37 mg/kg bw) carbosulfan co-treatment with *Cissus quadrangularis* extract (1000 mg/kg bw) for 60 days. The reproductive parameters including histology of testes, sperm count, motility, morphology, and hormonal assay (FSH, T, LH) were performed. Carbosulfan treated groups (E_3 & E_4) were manifested histopathological alterations including tubular degeneration, disorganisation of cells, vacuolisation, and sloughing of germ cells into the tubular lumen. The hormonal level, sperm count, and motility were significantly decreased and the sperm abnormality rate was increased in the E4 group compared to the control group. The groups (E_5 & E_6) co-treated with plant extract were significantly recovered compared to insecticide-treated groups (E_3 & E_4). The histological and hormonal changes were attributed to the reprotoxic effects of the insecticide. However, co-treatment of plant extract significantly recovered the toxic effects caused by low concentration (E_5) than the high concentration (E_6) of carbosulfan.

*Author for correspondence

1. Introduction

Pesticide self-poisoning has been a widespread alternative suicidal method used by most people in rural Asia. Pesticides were initially marketed for the elimination of pests in the agriculture field, unfortunately, the toxic effect of these chemicals so intensive that even humans were suffered from many physiological disorders[1]. Considering the pesticide consumption statistics released by the ministry of agriculture and farmers welfare, Government of India, a total of 60,599 Metric Tonnes (MT) of technical grade chemical pesticides were consumed in India. Karnataka was found to utilize 1,608 MT of chemical pesticides for agriculture purposes in the year 2019-20. Overall consumption of 28 MT of technical grade carbosulfan was recorded in 2019-20 in India[2].

Carbosulfan (2, 3-dihydro-2, 2-dimethy l-7-benzofuranyl (dibutylamino) thio) methylcarbamate is one of the carbamate derivatives which is widely used as an insecticide in many parts of the world. It is effective against a wide range of pests including green leafhopper, caterpillars, brown planthopper, gall midge, stem borer, and leaf folder of paddy, white-backed planthopper, white aphids of chillies, and it was also found to be potent on pyrethroid-resistant mosquitoes[3]. Degradation of carbosulfan in natural water is very slow and can persist in natural sand soil for 40 days[4,5]. Carbosulfan was known to induce physiological deformities in non-target organisms[6,7]. Acute doses of carbosulfan were able to induce genotoxic effects in germ cells of mice[8]. Pesticide-induced gene mutation can cause developmental malformations through the expression of the wrong genetic code. In a previous study, it was shown that the treatment of carbosulfan increased sister chromatid exchange frequency and induced cell cycle delay in the bone marrow cells of mice[9]. Accumulation of stress markers and altered antioxidants in the kidney & liver were significant in rats treated with carbosulfan. Nonetheless, co-administration of N-acetylcysteine improved biochemical and antioxidant status[10]. The same laboratory in another study explained the anti-inflammation property of N-acetylcysteine against carbosulfan induced liver impairments in rats[11]. Biochemical parameters such as Lactate De-Hydrogenase (LDH), Succinate De-Hydrogenase (SDH), ASpartate Transaminase (AST), and histological changes were observed in the liver of albino mice exposed to carbosulfan[12]. Pesticide exposure was not only restricted to terrestrial animals but also aquatic animals, recent study observed changes in catalase, superoxide dismutase, glutathione-S-transferase, and malondialdehyde content in zebrafish liver exposed to carbosulfan[13]. Oxygen deprivation and behavioral anomalies were evident in *Cyprinus carpio* exposed to sublethal concentrations of carbosulfan[6]. The freshwater fish *Channa punctatus* was also affected and shown genotoxic and mutagenic properties of the insecticide[14].

In case of emergency of pesticide suicide or accidental consumption, it is necessary to first aid the patient before hospitalization. It could be achieved via home remedies; medicinal plant extract is one of such home remedies that an ordinary village person can prepare and save a life. It is evident from previous studies that plant extracts could provide necessary health benefits against pesticide poisoning[15–17]. *Cissus quadrangularis* is a perennial climber that belongs to the family Vitaceae and is found widespread in tropical regions of India. It is well known for its medicinal uses and used for the treatment of anorexia, colic, epistaxis, asthma, seizures, tumours, flatulence, abnormal menstrual disorders, antibacterial infections, inflammation, and was also found to be effective in inhibition of lipid accumulation in 3T3-L1 cells[18–21]. Studies regarding toxicological profiles indicate that the extract of *Cissus quadrangularis* does not induce any adverse effect[22]. A recent study on quinalphos (an organophosphate insecticide) induced alteration in the antioxidant profile of mice was found to be efficiently recovered upon co-treatment of aqueous extract of *Cissus quadrangularis*[23]. Methanolic extract of *Cissus quadrangularis* on diabetes-induced rats revealed the pronounced antidiabetic activity through the regulation of carbohydrate metabolic enzyme activities[24]. It was found to demonstrate antidiabetic activity in nicotinamide/streptozotocin-treated rats[25].

Apart from few studies, there is meagre literature regarding ameliorative prospective on pesticide intoxication. Several studies were conducted on plant extract mediated ameliorative effects on pesticide induced toxicities, which deals with most real situations and relates daily exposure of human life to these toxic chemicals. Using natural extracts instantly at the time of unintentional or intentional pesticide consumption makes more sense

than finding a suitable single compound medicine that is time consuming, labour intensive and moreover unrealistic to the situation. Considering the aforementioned facts, the present study was aimed to investigate the ameliorative effect of *Cissus quadrangularis* on reproductive aspects of rats exposed to different concentrations of carbosulfan.

2. Materials and Methods

2.1 Collection of Plant Material

The plant material (stem) *Cissus quadrangularis* was collected from the local habitats of Nelamangala Taluk, Bengaluru District, Karnataka State, India (13°3′43′N & 77°20′32′E). The specimen was shade dried for 30 days and powdered. The finely sewed powder was subjected to Soxhlet apparatus containing ethanol and distilled water (75:25 ml v/v). Final ethanolic extract was obtained by rotary evaporation (BUCHI rotavapor R-210) followed by 10 hours of continued extraction. The dosing concentration of 1000 mg/kg body weight (bw) of the rat was prepared using distilled water and DMSO (Dimethyl sulfoxide) (99:1 ml v/v) as a vehicle.

2.2 Chemicals

Carbosulfan (Marshall 25% EC, 27.18g of active ingredient) was procured from FMC Corporation India Pvt., Ltd. Mumbai, Maharashtra. Benzene, Eosin (water-soluble), Xylene, paraffin wax, and all other chemicals were analytical grade & purchased from SRL Pvt. Ltd.

2.3 Animal Maintenance

Thirty-six adult male albino rats of Wistar strain (*Rattus norvegicus*) weighing 200-250g (7-8-week-old) were obtained from the animal house facility, Department of Zoology, Karnatak University, Dharwad (No. 693/ GO/Re/S/02/CPCSEA). Animals were maintained in polypropylene cages with access to feed (VRK nutritional solutions, Sangli, Maharashtra) and water *ad libitum*. The rats were allowed to acclimatize to a laboratory environment of temperature 26 ± 2 °C, 12/12h day and night cycle, and relative humidity of 55 ± 7%.

2.4 Experimental Design

In the present investigation, a plant extract concentration of 1000 mg/ kg bw and the carbosulfan concentrations of high dose (6.37 mg/ kg bw), & low dose (3.4 mg/ kg bw) was selected based on No Observed Adverse Effect Level (NOAEL) as depicted in the present study following OECD guidelines[26]. The selected doses were administered daily during daylight for 60 days. The animals were randomly selected and divided into 6 groups containing 6 animals in each group. The first group E_1 was the control group with the administration of 1% DMSO/ kg bw, the second group E_2 was doused with plant extract only (1000 mg/ kg bw), third group E_3 received a low dose (3.4 mg/ kg bw) and fourth group E_4 received a high dose (6.37 mg/ kg bw) of carbosulfan, fifth group E_5 administered with plant extract plus carbosulfan low dose (1000 mg + 3.4 mg/ kg bw) and sixth group E_6 received plant extract and carbosulfan high dose (1000 mg + 6.37 mg/ kg bw).

2.5 Histology

The rats were sacrificed on completion of the 60th experimental day. The testes were dissected out and fixed in formalin, fixed organs were then subjected to dehydration process with different gradients of alcohol and embedded in paraffin wax[27]. A 5µm thin sections of testes were taken using an automated microtome (Leica RM 2255). The sections were stained (Hematoxylin & Eosin) and photographed using a Magnus phase-contrast microscope (Magnus MLXi plus, India) with an attached photographic camera (MagCam DC 5).

2.6 Hormone Assay

The blood was collected by cardiac puncture and was subjected to centrifugation (3000 rpm, 10 minutes), then the serum was separated. The hormonal assay was performed in a fully automated analyser (Cobas e411) using Electro-Chemi-Luminescence Immune-Assay (ECLIA) method. The serum was analysed to determine the concentrations of Testosterone (T), Follicle-Stimulating Hormone (FSH) and Luteinizing Hormone (LH).

2.7 Sperm Count and Motility Assay

In the present study, a previously published reproductive toxicity protocol was followed with slight modifications to examine the sperm count and motility[28]. Briefly, the cauda epididymis was separated, weighed, and immersed in a glass petri dish containing 10 ml of prewarmed BSA/ PBS diluent (1% BSA). The cauda epididymis was sliced several times with a sharp sterile scalpel blade to liberate the sperm and the petri dishes were incubated at 34 ºC for 5 minutes to allow sperm to swim out. To evaluate the sperm count, an aliquot of sperm was diluted 1:10 in fixative (1% formalin in PBS) and counted using Neubauer chamber.

The sperm motility was determined according to WHO laboratory manual[29]. An aliquot of 20 µl of sperm sample was placed on a clean glass slide and examined. At least 200 sperm were analyzed and categorized into motile and immotile sperm. The result was expressed as percentage of sperm motility.

2.8 Sperm Morphology

For the evaluation of sperm morphology, the samples were mixed with Eosin Y 0.5% (w/v) and smeared on a clean microscopic glass slide. The smears were then observed in high magnification of a phase-contrast microscope (Magnus MLXi plus, India). Two hundred sperm were assessed for head and tail abnormalities and expressed as a percentage of sperm abnormality.

2.9 Ethical Approval

All experiments performed in the present study followed the guidelines of the Institutional Animal Ethics Committee (IAEC) (No. 693/GO/Re/S/02/CPCSEA). The experimental animals used in the study were handled with care according to the guidelines provided by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

2.10 Statistical Analyses

In the present study, Origin statistical software package ver. 2019b was used for statistical analyses. All the data presented in the study were subjected to One Way Analysis of VArience (ANOVA) with Tukey's post hoc test. Results were expressed as mean plus or minus standard deviation ($x \pm SD$) and $p < 0.05$ was considered statistically significant.

3. Results

3.1 Histology

Histology provides the best possible internal pathological observation of organs that were exposed to toxicants. Histological examination of control (E_1) and plant extract treated group (E_2) rat testes were revealed well-organized epithelium in the seminiferous tubules. The cells of spermatogenesis were present with all successive stages (spermatogonia, primary spermatocytes, and secondary spermatocytes) without disruption in their regular structure and arrangement. Sertoli cells were normal and interstitial cells were intact with Leydig cells. The spermatozoa were abundant at the centre of the tubule and appeared normal (Figure 1). The rats treated with carbosulfan low dose (E_3) were manifested mild testicular alterations. Histology of testes was prominent with intercellular vacuole formation in the seminiferous tubules as well as in the interstitial cells. The sloughing of germ cells into the tubular lumen was also noticed in the E_3 groups. However, all cells of the spermatogenesis stage were arranged concentrically in the seminiferous tubule. Carbosulfan high dose (E_4) treated group showed severe alterations in the histoarchitecture of the rat testes. The pathological observations such as sloughing of germ cells into the lumen, absence of spermatozoa, or tubular degeneration were noticeable. Vacuolisation was frequent, disorganization and displacement of the spermatogonial cells were pronounced in the E_4 group.

Protective treatment of *Cissus quadrangularis* on carbosulfan exposed group of rats exhibited improvement in the histoarchitecture of testes. The low dose carbosulfan and plant extract treated group (E_5) was noticed with a few histopathological changes. Vacuolisation of seminiferous tubules, interstitial cells, and sloughing of germ cells into the lumen were less frequent compared to carbosulfan treated groups (E_3 & E_4 groups). Histoarchitecture of epithelial tissue was appeared normal but the interstitial cells were found with few vacuole formations and normal Leydig cells. Rats treated with high dose carbosulfan and plant extract (E_6) were noticed with tubular degeneration, sloughing of germ cells into the lumen, and vacuolisation. However, apart from these alterations, the epithelial tissue

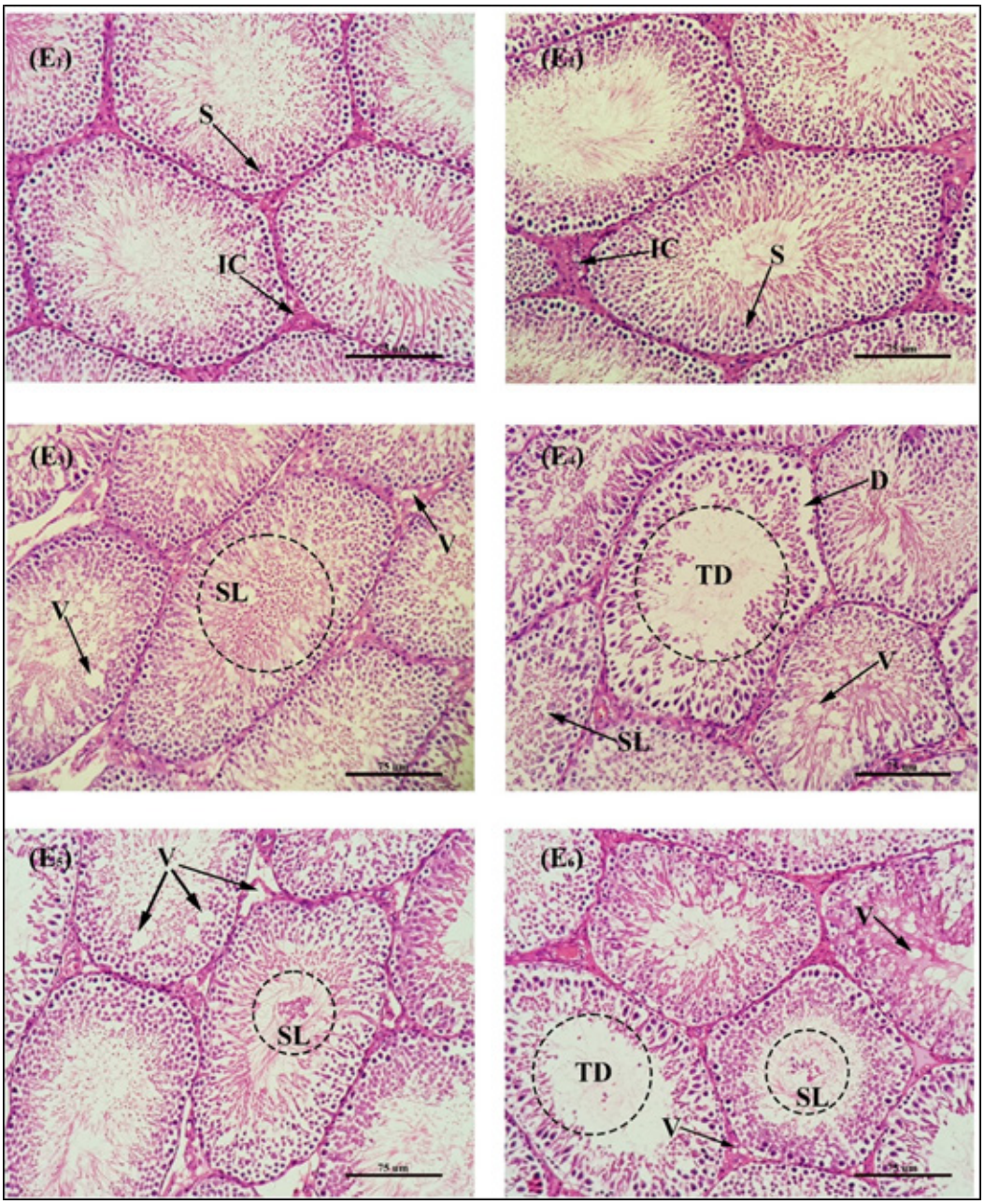


Figure 1. Photomicrographs of histology of 60 days exposed rat testes. (E_1) Control group showing normal histology: (S) Spermatocytes; (IC) Interstitial cells. H&E. (E_2) Plant extract alone treated group: (S) Spermatocytes; (IC) Interstitial cells. H&E. (E_3) Carbosulfan low dose treated group: (SL) Sloughing of germ cells into the tubular lumen; (V) Vacuolisation. H&E. (E_4) Carbosulfan high dose treated group: (TD) Tubular degeneration; (D) Disorganisation of cells; (V) Vacuolisation; (SL) Sloughing of germ cells into the tubular lumen. H&E. (E_5) Carbosulfan low dose & Plant extract-treated group: (V) Vacuolisation; (SL) Sloughing of germ cells into the tubular lumen. H&E. (E_6) Carbosulfan high dose & Plant extract-treated group: (TD) Tubular degeneration; (V) Vacuolisation; (SL) Sloughing of germ cells into the tubular lumen H&E.

was arranged in a regular pattern and the interstitial cells were attained a normal cytoskeletal structure with few vacuole formations. The normal histoarchitecture in the E_6 group was slightly recovered compared to the carbosulfan high dose treated group (E_4).

3.2 Hormone Assay

In the present study Testosterone (T) Follicle Stimulating Hormone (TSH) and Luteinizing Hormone (LH) were evaluated and summarised in Figure 2. The Testosterone levels (Figure 2A) were significantly decreased in E_3 (81.87 ± 1.68 SD) and E_4 (65.38 ± 1.75 SD) compared to control group E_1 (94.48 ± 3.16 SD). In contrast, a significant recovery was observed in plant extract treated groups E_5 (83.31 ± 3.07 SD) and E_6 (70.15 ± 2.67 SD). Luteinizing hormone was found to be significantly decreased in the E_4 group (0.59 ± 0.08 SD) and exhibited recovered hormonal levels in E_5 (1.16 ± 0.14 SD) & E_6 (0.86 ± 0.05 SD) groups compared to the control group. Follicle Stimulating Hormone (FSH) in E_3 (2.15 ± 0.22 SD) and E_4 (1.63 ± 0.30 SD) exhibited a significant decrease in the hormonal level (Figure 2B) compared to the control group (2.71 ± 0.36 SD). However, a non-significant recovery was observed in plant-treated group E_5 (2.35 ± 0.30 SD). The group E_6 (1.82 ± 0.18 SD) was significantly recovered ($P < 0.05$) upon plant extract treatment.

3.3 Sperm Count and Motility

The reproduction rate depends on several factors including sperm quality and quantity. In the present study,

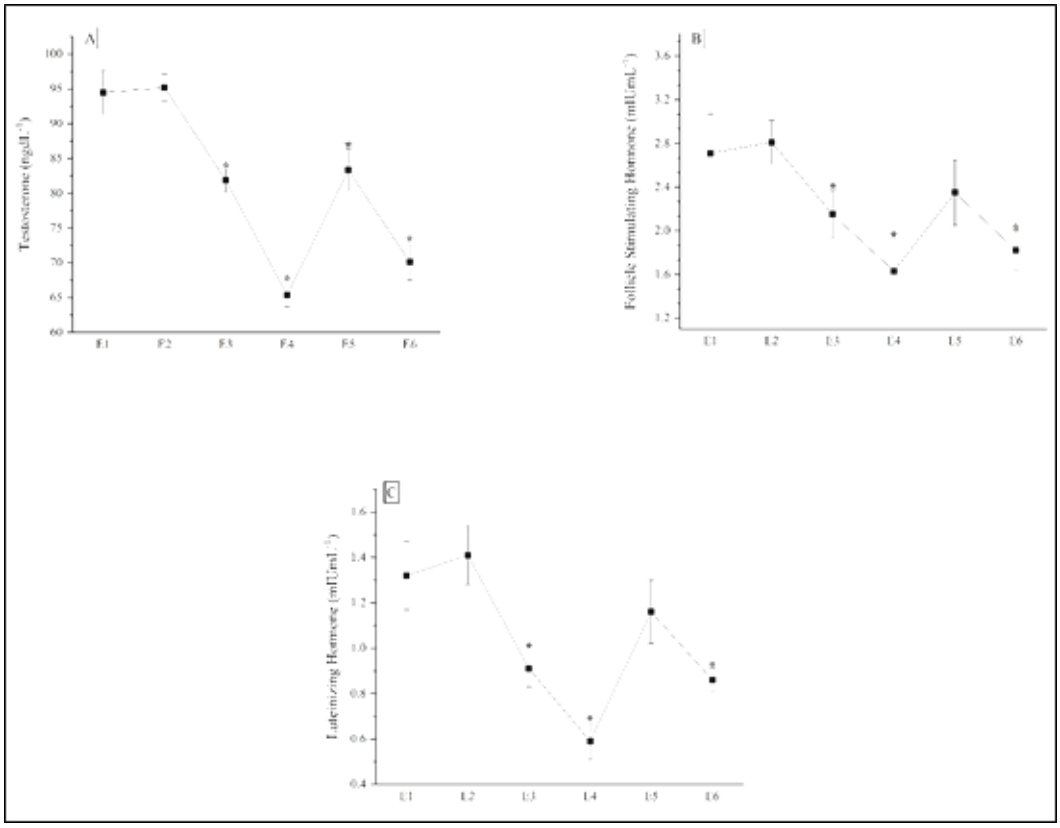


Figure 2. Scattered graphs showing **(A)** Testosterone, **(B)** Follicle Stimulating Hormone (FSH), and **(C)** Luteinizing hormone (LH) levels of experimental groups. The values are expressed as mean ± SD, n=06. Asterisk (*) indicates a significant difference at $p<0.05$ compared to the control group.

the sperm count was evaluated in all the experimental groups and presented in Figure 3. A significant decrease in sperm count was observed in low (8.21 ± 0.60 SD) and high dose (6.41 ± 0.41 SD) carbosulfan treated groups compared to the control group (10.63 ± 0.49 SD). However, co-treatment of plant extract manifested

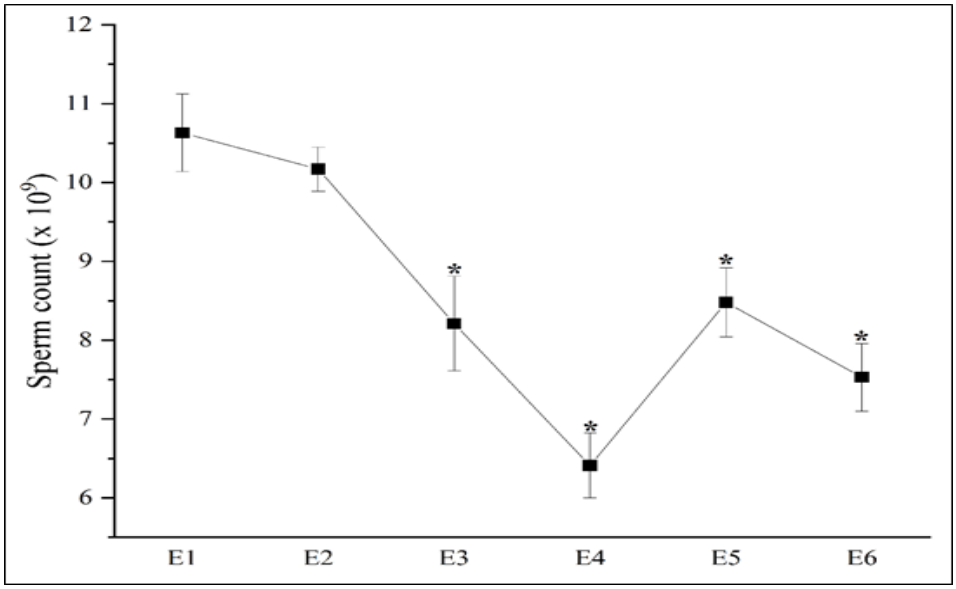


Figure 3. Scattered graphs representing the sperm count of experimental groups. The values are expressed as mean ± SD, n=06. Asterisk (*) indicates a significant difference at p<0.05 compared to the control group.

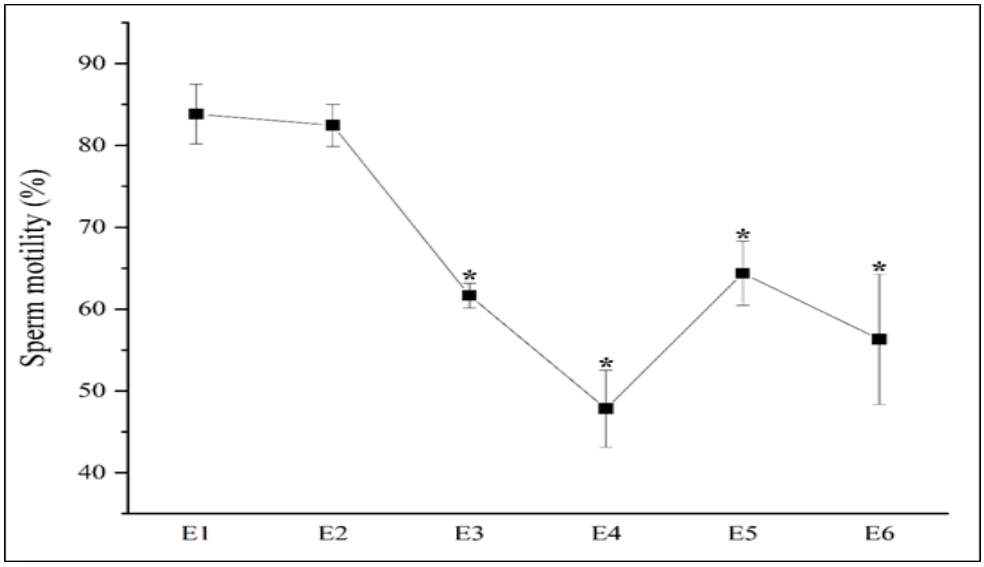


Figure 4. Scattered graph showing sperm motility of the experimental groups. The values are expressed as mean ± SD, n=06. Asterisk (*) indicates a significant difference at p<0.05 compared to the control group.

significant recovery in the sperm counts of low dose (8.48 ± 0.44 SD) and high dose (7.53 ± 0.43 SD) carbosulfan groups. Normal sperm motility was observed in E_1 (83.83 ± 3.65 SD) and E_2 (82.46 ± 2.58SD) groups (Figure 4). A significant decrease in sperm motility was observed in low (61.66 ± 1.50 SD) and high dose (47.83 ± 4.70 SD) carbosulfan treated groups compared to the control group. This decreased sperm motility was recovered significantly ($P < 0.05$) under the treatment of plant extract.

3.4 Sperm Morphology

Sperm morphological defects and abnormality rates of all the experimental groups were investigated and

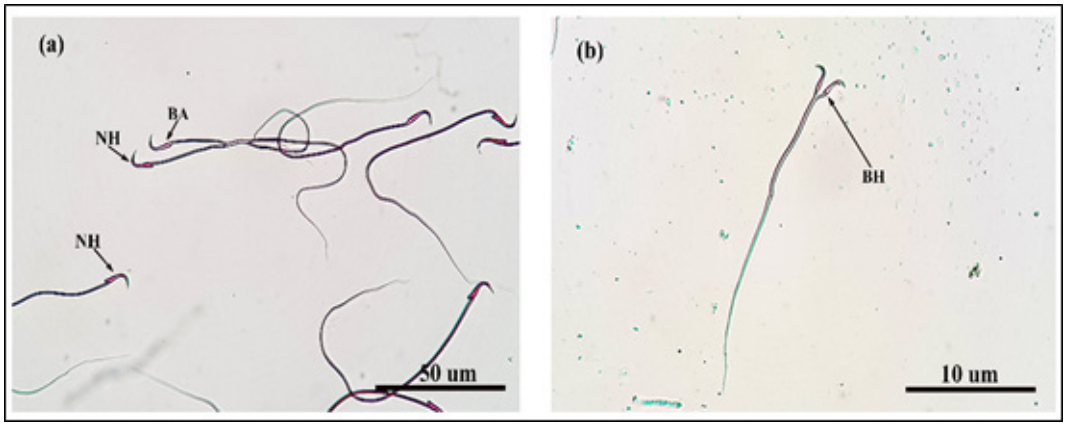


Figure 5. Photomicrographs of rat sperm morphology. **(a)** NH: Normal sperm head; (BA) Banana shape sperm head, and **(b)** BH: Bifid sperm head.

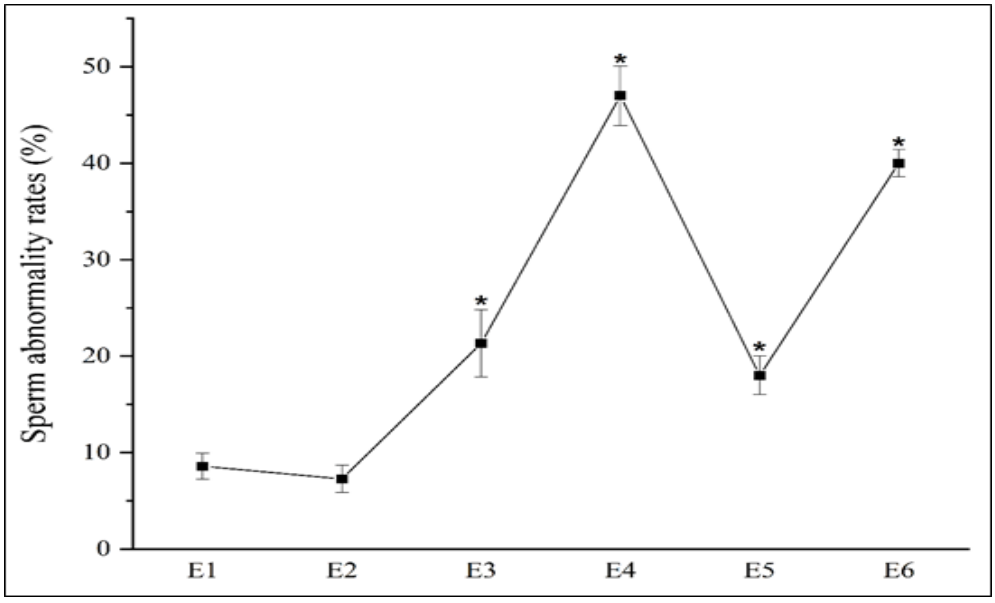


Figure 6. Sperm abnormality rates among experimental groups. The values are expressed as mean ± SD, n=06. Asterisk (*) indicates a significant difference at $p<0.05$ compared to the control group.

represented in the Figures 5 & 6. The E_1 & E_2 groups revealed usual abnormality rates of 8.58 ± 1.35 SD & 7.28 ± 1.40 SD respectively. The carbosulfan treated groups expressed significantly increased abnormality rates in E_3 (21.33 ± 3.50 SD) and E_4 (47 ± 3.09) groups. However, the co-treatment of plant extract significantly decreased the abnormality rates in E_5 (18 ± 2 SD) and E_6 (40 ± 1.41 SD) groups.

4. Discussion

Carbosulfan is a commonly used insecticide to control the pest population in agricultural fields. It causes occupational hazards affecting not only mammals but also fishes[6]. The farmers are highly vulnerable to insecticide exposure as they frequently spray it to the crops. It has previously been reported that carbosulfan was a germ cell mutagen that causes developmental disabilities in rats[8,30]. The rat possesses a spermatogenic cycle duration of 50 days. However, if immature spermatogonia are exposed to a toxicant, the effect can be seen after 7 weeks of exposure[31]. Our study lasted 8 weeks and revealed histological alterations such as displacement of spermatogonial cells, tubular degeneration, vacuolization, and sloughing of germ cells into the tubular lumen in the testes of experimental groups. Previous studies reported carbosulfan induced testicular toxicity via hypothalamo-hypophysial-testicular axis under sublethal exposure in mice[32]. Dose dependant histopathological alterations including degeneration, necrosis, oedema, and reduced number of spermatozoa in the lumen of seminiferous tubules were evident in rats treated with combined chlorpyriphos and cypermethrin[33]. In our study, the high dose carbosulfan treated group (E_4) was manifested highly affected testes histology while the low dose treated group (E_3) exhibited few pathological defects that indicate dose-dependent changes. However, histopathological changes were moderately alleviated in both plant extract treated groups (E_5& E_6). The ameliorative effect of *Cissus quadrangularis* might be owing to the antioxidative property of the plant extract. The previous study showed that the protective treatment of *Cissus quadrangularis* was effectively maintained the cholesterol level and restored the testicular oxidative damage and inhibition of steroidogenesis induced by quinalphos[23].

Vacuolisation of the germ cells might be an initial sign of toxicity, however, increased vacuolation could be attributed to metabolic disruption[34]. The male reproductive system is regulated by a negative feedback loop involving Hypothalamus, Anterior Pituitary (HPT & testicles) axis. Gonadotropin-Releasing Hormones (GnRH) including Follicle-Stimulating Hormone (FSH) and Luteinizing Hormone (LH) released and controlled via pituitary signals[35]. Alterations in the testicular histoarchitecture mainly depend on hormonal regulation, any variation in the gonadotropin hormones could cause cellular level damage. Endocrine-Disrupting Chemicals (EDCs) including insecticides affect GnRH and induces histological changes leading to infertility in male offspring[36]. In the present study histopathological alterations in both E_3 and E_4 groups might be owing to a significant decrease in Testosterone, LH, and FSH. Nonetheless, the hormonal levels were retrieved upon treatment of plant extract (Figure 2). Our results were in concordant with previous studies that reported similar alterations upon exposure to toxicants [23,34]. Recent studies support the plant extract-mediated alleviation of pesticide-induced toxicities. Aqueous extract of *Eruca sativa* ameliorated the histopathological and hormonal levels affected by abamectin exposure[37]. Considerable recovery was observed on co-administration of *Camellia sinensis* against clove or chlorpyrifos induced reproductive toxicity in male rats[38]. Fertility enhancer property of *Lannea acida* extract on insecticide-induced oxidative stress and the hormonal imbalance was recorded in rats, the plant extract has alleviated the damages caused by the insecticide[39].

The reduction in testosterone levels in E_3 & E_4 could be attributed to cellular damage in the Leydig cells of the testes. In this study, we have noticed vacuolisation of the interstitial cells and decreased Leydig cells. The groups treated with plant extract (E_5 & E_6) were also found with mild vacuolation in interstitial cells but recovered compare to carbosulfan treated groups. Considering the recovery in the cellular damage and hormonal variation in our study indicates the reversible cellular damage caused by the insecticide. However, some of the pathological observations including vacuolisation, lumen filled with germ cells, and tubular degenerations were moderately recoverable in high concentration treated animals (E_4)

upon plant extract treatment. A previous study reported that fenitrothion-induced testicular damage including hormonal changes were recovered upon quercetin treatment, indicating retrievable toxicities caused by the pesticide[40]. Sperm count and motility is a crucial parameter to assess male fertility rates. In our study, epididymal sperm count and sperm motility was highly affected in E_3 & E_4 group and also elevation in sperm abnormality rate was observed compare to other groups. The decrease in sperm count might indicate the epididymal damage as the sperm was collected from epididymis for the sperm count assay. It could also be linked with epididymal tissue damage or contractility of the duct[41]. The increased sperm abnormality in E_3 & E_4 including head and tail damage in the present study could be either due to impaired spermatogenesis or damage of genetic material in the spermatogonia or spermatocytes[42,43]. In the present study, the increased abnormality rate was correlated to the altered testes histoarchitecture and hormonal imbalance. The sperm abnormalities including banana head and bifid head (Figure 5) could be a sign of germ cell toxicity of the insecticide, as previous study observed different concentrations of the fipronil caused dose-dependent reproductive damages via apoptosis of spermatozoa and oxidative stress[44].

Our results are in parallel with previous studies, where a similar decrease in sperm count, motility, and hormonal level with an increase in the insecticide dose was observed. However, the abnormalities were recovered upon plant extract treatment. The testes of male mice that were exposed to deltamethrin concentrations exhibited similar histopathological alterations indicating cellular level damage in the testes caused by the pesticide[45]. Significant recovery was observed in the testis of rats exposed to rotenone on co-treatment of olive leaves extract[46]. *Nigella sativa* oil mitigates efficiently the adverse effects induced by acetamiprid on reproductive parameters of male rats[47]. Green tea extract was efficiently protected the malathion-induced testicular oxidative damage and histopathological alterations in male rats[48]. The present investigation provides crucial evidence that the *Cissus quadrangularis* plant extract mitigates the reproductive toxicity caused by sublethal exposure to carbosulfan insecticide. These results could help in the future development of reproductive medicine and provide first aid methods in case of accidental occupational health hazards.

5. Conclusions

This study revealed that the extract of *Cissus quadrangularis* plant has mitigative properties on insecticide-induced toxicity and protects from most damages caused by the drug. The recovery in reproductive parameters i.e., sperm count and motility, sperm morphology, hormonal variation, and testes histology indicates the potentiality of the plant extract. Considering the obtained results, it is recommended that the plant extract might also be used as a first-aid medicine in case of accidental insecticide exposure or consumption.

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7. References

1. Karunarathne A, Gunnell D, Konradsen F, Eddleston M. How many premature deaths from pesticide suicide have occurred since the agricultural Green Revolution? Clin Toxicol. 2020; 58(4):227-232. https://doi.org/10.1080/15563650.2019.1662433. PMid:31500467.
2. Statistical Database, GOI (Internet). 2021. Cited 2021 Jun 3. Available from: http://ppqs.gov.in/statistical-database.
3. Guillet P, N'Guessan R, Darriet F, Traore-Lamizana M, Chandre F, Carnevale P. Combined pyrethroid and carbamate 'two-in-one' treated mosquito nets: field efficacy against pyrethroid-resistant *Anopheles gambiae* and *Culex quinquefasciatus*. Med Vet Entomol. 2001; 15(1):105-112. https://doi.org/10.1046/j.1365-2915.2001.00288.x. PMid:11297094.
4. Wei J, Furrer G, Schulin R. Kinetics of Carbosulfan Degradation in the Aqueous Phase in the Presence of a Cosolvent. J Environ Qual. 2000; 29(5):1481-1487. https://doi.org/10.2134/jeq2000.00472425002900050015x.
5. Campbell S, David MD, Woodward LA, Li QX. Persistence of carbofuran in marine sand and water. Chemosphere.

2004; 54(8):1155-1161. https://doi.org/10.1016/j.chemosphere.2003.09.018. PMid:14664844.
6. David M, Lokeshkumar P. Studies on carbosulfan induced oxygen deprivation and behavioral anomalies in fresh water fish *Cyprinus carpio*. J Adv Sci Res. 2020; 11(4 Suppl 9):36-43.
7. Nwani CD, Agrawal ND, Raghuvanshi S, Jaswal A, Shrivastava S, Sinha N, Onyishi G, Shukla S. Toxicological effects of carbosulfan in rats: Antioxidant, enzymological, biochemical, and hematological responses. Toxicol Ind Health. 2016; 32(7):1335-1343. https://doi.org/10.1177/0748233714564243. PMid:25564597.
8. Giri S, Giri A, Sharma GD, Prasad SB. Mutagenic effects of carbosulfan, a carbamate pesticide. Mutat Res Genet Toxicol Environ Mutagen. 2002; 519(1-2):75-82. https://doi.org/10.1016/S1383-5718(02)00114-6.
9. Giri S, Giri A, Sharma GD, Prasad SB. Induction of sister chromatid exchanges by cypermethrin and carbosulfan in bone marrow cells of mice in vivo. Mutagenesis. 2003 Jan 1; 18(1):53-58. https://doi.org/10.1093/mutage/18.1.53. PMid:12473735.
10. Dhouib IE-B, Annabi A, Jrad A, El-Golli N, Gharbi N, Lasram MM, El-Fazaa. Carbosulfan-induced oxidative damage following subchronic exposure and the protective effects of N-acetylcysteine in rats. Gen Physiol Biophys. 2015; 34(03):249-261. https://doi.org/10.4149/gpb_2015005. PMid:25926552.
11. Dhouib, Ines El-Bini. Anti-inflammatory effects of n-acetylcysteine against carbosulfan-induced hepatic impairment in male rats. Recent Adv Biol Med. 2015; 01:29. https://doi.org/10.18639/RABM.2015.01.156935.
12. Kaliwal BB, Ksheerasagar RL. Histological and biochemical changes in the liver of albino mice on exposure to insecticide, carbosulfan. Casp J Environ Sci. 2006; 4(1):67-76.
13. Cui J, Wang F, Gao J, Zhai W, Zhou Z, Liu D, Wang P. Bioaccumulation and metabolism of carbosulfan in Zebrafish (*Danio rerio*) and the toxic effects of its metabolites. J Agric Food Chem. 2019; 67(45):12348-12356. https://doi.org/10.1021/acs.jafc.9b03674. PMid:31638788.
14. Nwani CD, Lakra WS, Nagpure NS, Kumar R, Kushwaha B, Srivastava SK. Mutagenic and genotoxic effects of carbosulfan in freshwater fish *Channa punctatus* (Bloch) using micronucleus assay and alkaline single-cell gel electrophoresis. Food Chem Toxicol. 2010; 48(1):202-208. https://doi.org/10.1016/j.fct.2009.09.041. PMid:19818828.
15. Abdelhamid FM, Mahgoub HA, Ateya AI. Ameliorative effect of curcumin against lead acetate-induced hematobiochemical alterations, hepatotoxicity, and testicular oxidative damage in rats. Environ Sci Pollut Res. 2020; 27(10):10950-10965. https://doi.org/10.1007/s11356-020-07718-3. PMid:31953765.
16. Farghali H, Canová NK, Zakhari S. Hepatoprotective properties of extensively studied medicinal plant active constituents: possible common mechanisms. Pharm Biol. 2015; 53(6):781-791. https://doi.org/10.1007/s11517-015-1286-7. PMid:25850981.
17. Jaiswal SK, Gupta VK, Siddiqi NJ, Pandey RS, Sharma B. Hepatoprotective effect of citrus limon fruit extract against carbofuran induced toxicity in Wistar rats. Chin J Biol. 2015; 2015:1-10. https://doi.org/10.1155/2015/686071.
18. Jainu M, Devi CSS. *In vitro* and *In vivo* evaluation of free-radical scavenging potential of *Cissus quadrangularis*. Pharm Biol. 2005; 43(9):773-779. https://doi.org/10.1080/13880200500406636.
19. Lee HJ, Le B, Lee D-R, Choi B-K, Yang SH. *Cissus quadrangularis* extract (CQR-300) inhibits lipid accumulation by down regulating adipogenesis and lipogenesis in 3T3-L1 cells. Toxicol Rep. 2018; 5:608-614. https://doi.org/10.1016/j.toxrep.2018.02.008. PMid:29854631 PMCid:PMC5977379.
20. Nash R, Azantsa B, Kuate D, Singh H, Oben J. The Use of a Stem and leaf aqueous extract of *Cissus quadrangularis* (CQR-300) to reduce body fat and other components of metabolic syndrome in overweight participants. J Altern Complement Med. 2018; 25(1):98-106. https://doi.org/10.1089/acm.2018.0016. PMid:29912570 PMCid:PMC6352552.
21. Sawangjit R, Puttarak P, Saokaew S, Chaiyakunapruk N. Efficacy and safety of *Cissus quadrangularis* L. in clinical use: A systematic review and meta-analysis of randomized controlled trials. Phytother Res. 2017; 31(4):555-567. https://doi.org/10.1002/ptr.5783. PMid:28165166.
22. Zenebe S, Feyera T, Assefa S. *In vitro* anthelmintic activity of crude extracts of aerial parts of *Cissus quadrangularis* L. and leaves of *Schinus molle* L. against *Haemonchus contortus*. Bio Med Research International. 2017; 2017:6. https://doi.org/10.1155/2017/1905987. PMid:29410960 PMCid:PMC5749317.
23. Kokilavani P, Suriyakalaa U, Elumalai P, Abirami B, Ramachandran R, Sankarganesh A, *et al*. Antioxidant mediated ameliorative steroidogenesis by *Commelina benghalensis* L. and *Cissus quadrangularis* L. against quinalphos induced male reproductive toxicity. Pestic Biochem Phys. 2014; 109:18-33. https://doi.org/10.1016/j.pestbp.2014.01.002. PMid:24581381.
24. Lekshmi RK, Sreekutty MS, Mini S. The regulatory effects of *Cissus quadrangularis* on some enzymes involved in carbohydrate metabolism in streptozotocin-induced diabetic rats. Pharm Biol. 2015; 53(8):1194-1200. https://doi.org/10.3109/13880209.2014.970286. PMid:25857823.

25. Lekshmi RK, Rajesh R, Mini S. Ethyl acetate fraction of *Cissus quadrangularis* stem ameliorates hyperglycaemia-mediated oxidative stress and suppresses inflammatory response in nicotinamide/streptozotocin induced type 2 diabetic rats. Phytomedicine. 2015; 22(10):952-960. https://doi.org/10.1016/j.phymed.2015.06.014. PMid:26321745.
26. Test No. 425: Acute Oral Toxicity: Up-and-Down Procedure (En) OECD (Internet). 2021. Cited 2021 May 15. Available from: https://www.oecd.org/env/test-no-425-acute-oral-toxicity-up-and-down-procedure-9789264071049-en.htm.
27. Bancroft JD. Theory and Practice of Histological Techniques. Elsevier Health Sciences; 2008.
28. Hood RD, Hood RD. Developmental and Reproductive Toxicology: A Practical Approach. CRC Press; 2005. https://doi.org/10.1201/9781420040548.
29. World Health Organization, Editor. WHO Laboratory Manual for the Examination and Processing of Human Semen. 5th Ed. Geneva: World Health Organization; 2010.
30. Banji D, Banji OJF, Ragini M, Annamalai AR. Carbosulfan exposure during embryonic period can cause developmental disability in rats. Environ Toxicol and Pharmacol. 2014; 38(1):230-238. https://doi.org/10.1016/j.etap.2014.05.009. PMid:24973665.
31. Dirican EK, Kalender Y. Dichlorvos-induced testicular toxicity in male rats and the protective role of vitamins C and E. Exp Toxicol Pathol. 2012; 64(7):821-830. https://doi.org/10.1016/j.etp.2011.03.002. PMid:21458248.
32. Ksheerasagar RL, Kaliwal BB. Temporal effects of carbosulfan on testicular biochemical parameters and enzyme activities in albino mice. Int J Pharm Bio Sci (B). 2013; 4:173-181.
33. Alaa-Eldin EA, El-Shafei DA, Abouhashem NS. Individual and combined effect of chlorpyrifos and cypermethrin on reproductive system of adult male albino rats. Environ Sci Pollut Res. 2017; 24(2):1532-1543. https://doi.org/10.1007/s11356-016-7912-6. PMid:27785720.
34. Abdellatief RB, Elgamal DA, Mohamed EEM. Effects of chronic tramadol administration on testicular tissue in rats: an experimental study. Andrologia. 2015; 47(6):674-679. https://doi.org/10.1111/and.12316. PMid:25228095.
35. Hayes FJ, DeCruz S, Seminara SB, Boepple PA, Crowley Jr WF. Differential regulation of gonadotropin secretion by testosterone in the human male: Absence of a negative feedback effect of testosterone on follicle-stimulating hormone secretion. J Clin Endocrinol Metab. 2001; 86(1):53-58. https://doi.org/10.1210/jcem.86.1.7101. PMid:11231978.
36. Patrick SM, Bornman MS, Joubert AM, Pitts N, Naidoo V, de Jager C. Effects of environmental endocrine disruptors, including insecticides used for malaria vector control on reproductive parameters of male rats. Reprod Toxicol. 2016; 61:19-27. https://doi.org/10.1016/j.reprotox.2016.02.015. PMid:26928317.
37. Hassan HF, Meligi NM. Effects of sublethal Abamectin exposure on some hormonal profiles and testicular histopathology in male albino rats and the possible ameliorative role of *Eruca sativa*. Environ Sci Pollut Res. 2017; 24(31):24690-24697. https://doi.org/10.1007/s11356-017-0112-1. PMid:28913779.
38. Akinwande DV, Adeyemi JA, Olawuyi ST, Akinola BK, Adedire CO. Protective effects of *Camellia sinensis* on *Syzygium aromaticum* or chlorpyrifos-induced reproductive toxicity in male Wistar rats. J Basic Appl Zool. 2019; 80(1):48. https://doi.org/10.1186/s41936-019-0122-2.
39. Tetsatsi ACM, Nkeng-Effouet PA, Alumeti DM, Bonsou GRF, Kamanyi A, Watcho P. Colibri® insecticide induces male reproductive toxicity: Alleviating effects of *Lannea acida* (Anacardiaceae) in rats. Basic Clin Androl. 2019; 29(1):16. https://doi.org/10.1186/s12610-019-0096-4. PMid:31890217 PMCid:PMC6924042.
40. Saber TM, Abd El-Aziz RM, Ali HA. Quercetin mitigates fenitrothion-induced testicular toxicity in rats. Andrologia. 2016; 48(5):491-500. https://doi.org/10.1111/and.12467. PMid:26264430.
41. Costa NO, Vieira ML, Sgarioni V, Pereira MRF, Montagnini BG, Mesquita S de FP, Gerardin DC. Evaluation of the reproductive toxicity of fungicide propiconazole in male rats. Toxicology. 2015; 335:55-61. https://doi.org/10.1016/j.tox.2015.06.011. PMid:26169826.
42. Taşdemir I, Taşdemir M, Tavukçuoglu S, Kahraman S, Biberoğlu K. Effect of abnormal sperm head morphology on the outcome of intracytoplasmic sperm injection in humans. Hum Reprod. 1997; 12(6):1214-1217. https://doi.org/10.1093/humrep/12.6.1214. PMid:9222003.
43. Wyrobek AJ, Bruce WR. Chemical induction of sperm abnormalities in mice. Proc Nat Acad Sci. 1975; 72(11):4425-4429. https://doi.org/10.1073/pnas.72.11.4425. PMid:1060122 PMCid:PMC388734.
44. Khan S, Jan MH, Kumar D, Telang AG. Firpronil induced spermotoxicity is associated with oxidative stress, DNA damage and apoptosis in male rats. Pestic Biochem Phys. 2015; 124:8-14. https://doi.org/10.1016/j.pestbp.2015.03.010. PMid:26453224.
45. Ben Slima A, Chtourou Y, Barkallah M, Fetoui H, Boudawara T, Gdoura R. Endocrine disrupting potential and reproductive dysfunction in male mice exposed to deltamethrin. Hum Exp Toxicol. 2017; 36(3):218-226. https://doi.org/10.1177/0960327116646617. PMid:27164926.

46. Sarbishegi M, Alhagh Charkhat Gorgich E, Khajavi O. Olive leaves extract improved sperm quality and anti-oxidant status in the testis of rat exposed to rotenone. NephroUrol Mon. 2017; 9(3):1-7. https://doi.org/10.5812/numonthly.47127.
47. Mosbah R, Djerrou Z, Mantovani A. Protective effect of *Nigella sativa* oil against acetamiprid induced reproductive toxicity in male rats. Drug Chem Toxicol. 2018; 41(2):206-212. https://doi.org/10.1080/01480545.2017.1337127. PMid:28669218.
48. Nahid Z, Tavakol HS, Abolfazl GK, Leila M, Negar M, Hamed F, *et al*. Protective role of green tea on malathion-induced testicular oxidative damage in rats. Asian Pac J Reprod. 2016; 5(1):42-45. https://doi.org/10.1016/j.apjr.2015.12.007.