RESEARCH ARTICLE

Acetamiprid Induced General and Reproductive Toxicity in Male Wistar Rats

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Abstract

Acetamiprid, a novel insecticide of neonicotinoid class, is used widely in agricultural practice for its significant insecticidal potential. Human and animal are directly or indirectly exposed to acetamiprid. The present study was planned to investigate the toxic potential of acetamiprid on male reproductive system of Wistar rats. Total 40 healthy male Wistar rats aged 6 to 8 weeks were randomly and equally divided in four groups (n=10) based on body weight. Group I served as control, while groups II, III and IV were dosed daily orally with acetamiprid @ 7.75, 15.5, and 31.0 mg/kg body weight, respectively, for 45 days. All the rats were examined daily for appearance of any abnormal clinical signs or mortality. Rats were weighed on weekly interval. There were no clinical signs or morality throughout the study. No significant alteration was found in haematological and serum biochemical analysis compared to control group, except slight increase in serum urea level in treatment group, yet statistically comparable with that of control group. The gross examination showed no detectable macroscopic lesions in any of the organs in any of the rats. There was no any deviation in sperm parameters as compared to control group. Microscopic examination of different organs revealed no any test article related abnormality, except few incidental changes with only minimal severity, such as vacuolation in hepatocytes and alveolar histiocytosis. In conclusion,oral acetamiprid did not produce adverse effect in male Wistar rats even at 31.0 mg/kg body weight.

Key words: Acetamiprid, Induced toxicity, Macro-microscopic alterations, Sperm parameters, Testes, Wistar rat

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Introduction

Higher production and lower loss through different organisms are todays need. The human population is increasing day by day occupying agricultural and forest land for their shelters. Thus, the land for food production is reducing and the demand of food is increasing. The main cause of low production is insect-pest. To control insects, farmers are using insecticides which may indirectly affect the human or animal health. Pesticides are utilized for the eradication of weeds, fungi, and insects as well as insect-transmitted diseases in agricultural, animal husbandry, and public health operations. The frequent and continuous use of pesticides has resulted in their widespread distribution in the environment (Mondal *et al*., 2014). Neonicotinoid is the newest class of insecticides, having outstanding potency and systemic action for crop protection against piercing-sucking pests, apart from highly effective in flea control on cats and dogs (Mondal *et al*., 2014). Male fertility has been steadily falling as social modernization levels have increased. Iammarrone *et al.* (2003) studied epidemiological data of infertility and experienced infertility in 13-18% of couples, mainly affecting male reproduction. Acetamiprid (E)-N [(6-chloro-3-pyridyl) methyl] is the newly developed neonicotinoid class of insecticides, which is frequently employed against a wide range of insect pests (Sanyal *et al.,* 2008). Tendency of acetamiprid to linger in the environment at small quantity made it dangerous for both the environment and human health as its metabolites may wind up in the water supply (Zhou *et al.,* 2006). However, systematic studies on its toxicity have not been reported in the literature, hence this study was planned on Wistar rats to evaluate whether the acetamiprid induced toxicity influences the general health and male reproduction.

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Materials and Methods

The acute study was carried out for LD50 of drug prior to reproductive analysis of acetamiprid in the Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Sardarkrushinagar, Gujarat (India) during January- February 2023. Both the protocols "acute oral toxicity study (up and down procedure) of acetamiprid" and "acetamiprid induced reproductive toxicity" in male Wistar rats were approved by in the Institutional Animal Ethics Committee (IAEC) of the College. According to approval total 55 male Wistar rats were procured from the Laboratory Animal Facility of Cadila Pharmaceuticals, Dholka, Ahmedabad, Gujarat, India. As per the standards set out by the CCSEA, New Delhi, India rats were housed with unlimited access to a regular palatable pellet feed and water during the experiment. Rats were identified by their tails marked with a permanent marker. Five rats were housed together in a cage.

Acetamiprid was purchased from Krishi Rasayan Exports Private Limited, Ahmedabad, Gujarat, India. The needed quantities of acetamiprid solutions were achieved by combining 0.5% carboxymethyl cellulose (CMC) with distilled water as a carrier, and the formulations were then kept at room temperature. The dose formulation was carried out on weekly basis. The acute toxicity testing of acetamiprid was undertaken in accordance with the OECD guidelines for Testing of Chemicals, Test No. 425 using 178 & 550 mg/kg single oral dose, and the main experiment was conducted in accordance with the OECD (2022) using graded dose of acetamiprid, *i.e.* 10% or below the acute toxicity dose LD50.

Acute Toxicity

The first animal received acetamiprid (175 mg/kg). The animal was still alive after 48 h, so the second animal was given the next dose of 550 mg/kg. Using the statistical software AOT425StatPgm, the estimated LD50 was determined. Individual animals were monitored for at least 30 min following single dosing @ 178 and 550 mg/kg orally (n=7 each), then every day for the subsequent 14 days. For the first 24 h, extra care was taken to monitor the animals closely. The concepts and guidelines of the OECD's humane endpoints guidance document were taken into consideration. Before administration of the test chemical, and at weekly thereafter the weight of each animal was recorded. Animals that were still alive by day 14 were weighed, euthanized and subjected to necropsy. Gross pathological alterations were seen and recorded.

Acetamiprid Induced General and Reproductive Toxicity in Male Wistar Rats

According to Section 4 of the OECD Guidelines for Testing of Chemicals, Test No.407 (OECD, 2008), the experiment was planned for acetamiprid. Every week, CMC (carboxymethyl cellulose) was suspended in distilled water as the vehicle for three dose formulations of acetamiprid @ 7.75, 15.50 and 31.00 mg/kg b.wt., which were then kept at room temperature. Each formulation was stirred with a magnetic stirrer until a homogenous suspension was obtained. During the 45-day study period, the test substance was given once daily by oral gavage. Weekly doses were prepared. 40 male Wistar rats were randomly and equally divided in four groups each of 10 animals. Group I served as the control group and received CMC alone orally for 45 days, which in treatment groups the acetamiprid LD50/40, LD50/20, and LD50/10 mg/kg body weight in CMC was administered to groups II, III, and IV, respectively.

Clinical Observations, Necropsy and Gross Pathology

All animals underwent observations twice daily for morbidity and mortality. Throughout the acclimatization phase, the clinical observations were recorded once daily. Clinical observations were noted at least twice (during and after the dose) on each dose day. The body weights of all rats were measured on the first day before dosing and then every week for the duration of the study.

All rats were given isoflurane anaesthesia on the 46th day and blood was collected from the retro-orbital plexus using a capillary tube and was collected into K_3EDTA tube for haematology and serum clot activator vial for serum biochemistry. Whole blood was used for haematology, while smears made were stained with Giemsa to check the cell morphology. Within 2 h of blood collection, serum was separated and stored at -20°C until analysed.

All the rats survived full experimental period, hence the final body weight of all rats was noted in the evening of day 45, and were fasted overnight before detailed necropsy. Next morning, animals were euthanized by over dose of Isoflurane, and all visceral organs were preserved in 10% neutral buffered formalin, including the liver, kidneys, lungs, heart, brain, spleen, adrenals, thymus, seminal vesicles, epididymides, and prostate. Testes and eyes were initially preserved in a modified Davidson solution for 48 h followed by being briefly rinsed and preserved in 10% neutral buffered formalin. Wet weight was recorded as soon as possible following dissection of each organ, and removal of any adhering tissue. Fixed and preserved tissues were used for histo-pathological examination under the microscope.

Clinical Pathology

Haematology was carried out by the Automated Haematology Analyzer (Exigo Haematology Analyzer, Boule Medical AB, Sweden) using the impedance method. Serum samples were examined for the biochemical parameters like, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline ahosphatase (ALP), urea, creatinine, total protein (TP), albumin, cholesterol, triglycerides, gamma glutamyl transferase, glucose, calcium, phosphorous, uric acid using a fully automated biochemistry analyser (RANDOX-RX Monaco, United Kingdom) and commercially available reagent kits (Randox).

Tissue Processing and Staining

Tissues were subjected initially to trimming, labelling, and washing followed by tissue processing. With the use of increasing percentages of isopropyl alcohol (30%, 70%, 90%, and absolute alcohol), dehydration was achieved.

The Leica EG1160 paraffin and Leica TP1020, an automated tissue processor, were used for the entire tissue processing. The 4 to 5 μm thin sections were cut using a semi-automated rotary microtome. The tissue sections were taken on slides coated with 2% 3-Aminopropyltriethoxysilane (APES), deparaffinized with xylene and then rehydrated with descending grades of isopropyl alcohol and water. The hydrated tissue sections were stained with haematoxylin & eosin and mounted with DPX (Suvarna *et al.,* 2018).

Sperm Evaluation

The sperm evaluation was performed according to the method of Hood (2012). For sample preparation a small portion of cauda from the epididymis was collected in 5 mL of 0.9% w/v normal saline in a petri dish. The dish was kept at room temperature for approximately 5 min to allow the sperm to ooze out. The homogenate was then prepared by swirling the contents of the dish with a pipette tip to get maximum free sperm.

To check sperm viability 5 μL of the sperm sample was collected using a micropipette on a glass slide labelled with the animal ID. The sample was mixed with 5 μL of 0.5% eosin by swirling action using a micropipette tip. A coverslip was then applied to the slide, and the sample was observed under the microscope at 40X objective for counting viable and non-viable sperm. Viable sperm remained colourless, while non-viable sperm took a pink/faint red stain. A total of 100 sperm (viable and non-viable) were counted, and the viability percentage was recorded.

To observe sperm morphology 100 μL of the sample was diluted with 1 mL of 10% Neutral buffered formalin in a microcentrifuge tube and mixed gently. 2-3 drops of 1% eosin yellow were added and mixed by agitation. The sample was then incubated at room temperature (15°C to 30°C) for 45-60 min to allow the sperm to stain. 1-2 drops of the stained sample from the tube were placed on a grease-free glass slide, followed by mounting the slide using DPX mountant. A total of 100 sperm were counted, and the abnormality percentage was recorded.

The total sperm were counted using WBC counting method of charged Neubaurg chamber with diluted semen through WBC pipette under the 10X objective and recorded as millions/mL and per gram cauda epididymis as follows:

Total sperm count (million/mL) = (N X 20 X 1000 X Dilution Volume)/ 0.4

Where, N=Number of sperm in 4 WBC counting squares, 20= Dilution factor of pipette, 0.4= Number of squares counted X Volume of 1 square.

Sperm/gram cauda epididymis (million/mL) =Total sperm count (10^6/mL) / Weight ofcauda (g)

Statistical Analysis

A two-way analysis of variance was used for the statistical analysis of the data obtained on various parameters. Using Duncan's test, pairwise comparisons with controls were done for each group separately (Snedecor and Cochran, 1994).

Results and Discussion

The assessment of acute oral toxicity of acetamiprid with help of AOT425StatPgm, suggested the median lethal dose (LD50) as 310 mg/kg once, hence for subsequent trails the dose requirements were met with accordingly.

Clinical Observations

During 45-days oral exposure of acetamiprid the male rats did not show any abnormal behaviour or clinical signs. There was no mortality in any of the four groups of rats throughout the study. There was no alteration in mean weekly body weight of any of the treatment groups of rats (Table 1). These findings regarding absence of clinical signs and mortality were in accordance with previous investigators. Chakroun *et al.* (2016), also did not find any abnormal physical or mental behaviours in rats with zero mortality. The current mortality findings also concurred with Arican *et al.* (2020) and El-Hak *et al.* (2022). Apart from clinical signs, mortality and body weight findings were contradicted with Brahmbhatt *et al.* (2016) as they found weakness, lethargy, paleness of mucous membrane and rough hair coat in mice treated at 10 mg/kg/day (mid dose) and 20 mg/kg/day (high dose) and decreased body weight in high dose group.

Table 1: Mean (±SE) weekly body weights (gm) of Wistar rats under acetamiprid oral toxicity study (n=10)

Day	Group I	Group II	Group III	Group IV
0	313.90 ± 9.83	315.20 ± 15.09	309.20 ± 11.97	318.80 ± 15.60
7	337.20 ± 11.61	345.90 ± 16.37	336.30 ± 23.28	339.30 ± 16.62
14	352.90 ± 13.32	357.40 ± 22.02	351.00 ± 23.23	357.20 ± 16.30
21	360.50 ± 14.00	361.60 ± 17.78	356.40 ± 24.91	367.30 ± 17.46
28	378.10 ± 14.28	375.20 ± 19.18	371.60 ± 24.86	386.40 ± 19.08
35	383.30 ± 13.20	381.40 ± 18.64	375.60 ± 25.26	379.00 ± 23.13
42	387.10 ± 15.51	384.50 ± 18.24	376.10 ± 28.13	396.40 ± 25.71
45	377.70 ± 12.57	372.00 ± 20.16	367.20 ± 27.21	385.20 ± 23.33

Haematological Findings

Haematological data of all treated groups showed non-significant decrease in platelet count, and the other parameters were statistically similar to control (Table 2). These observations were in agreement with Kanungo and Solecki (2012), who mentioned that the administration of acetamiprid in rats orally for 13 weeks did not cause any differences in haematological parameters as compared to the control group. However, the observations in our experiment were in contrast with reports of Singh (2012), Brahmbhatt *et al.* (2016) and Chakroun *et al.* (2016), who recorded significant alterations in various haematological parameters such as total WBC, total platelet count, MCV, MCHC, RBC count, haemoglobin, and HCT inacetamiprid administered rats.

Biochemical Alterations

There was non-significant increase in urea, triglyceride, ALP in treatment groups, while other biochemical parameters were comparable to control animals (Table 3). In contrast to the present serum biochemical findings, Brahmbhatt *et al.* (2016) Chakroun *et al.* (2016), Singh (2012) and Zhang *et al.* (2011) noted significant alterations in various biochemical parameters like AST, ALT, and ALP. Similarly, Arican *et al.* (2020) noted a dose-dependent decrease in cholesterol levels in the test group compared to the control group. El-Hak *et al.* (2022) found decreased serum Ca and TP levels at high doses.

Organ Weight

There were no any gross abnormalities in any of the organs of rats of any of the groups. Also, there was no statistical alteration in absolute and relative organ weight in the treatment groups from that of control group (Table 4). With respect to gross organ weights, the findings concurred well with Kanungo and Solecki (2012), and Brahmbhatt *et al.* (2016), except for thyroid gland and liver weight in later study. However, Zhang *et al.* (2011) and Mosbah *et al.* (2018) reported a decrease in the absolute weight of the prostate gland, seminal vesicles, testes, and epididymides of the treatment group as compared to the control group.

Table 2: Mean (±SE) haematology data of Wistar rats under acetamiprid oral toxicity (n=10)

Parameter	Unit	Group I	Group II	Group III	Group IV
Total leukocyte count	10^3/µL	12.8 ± 2.64	13.05 ± 1.93	14.27 ± 2.69	13.19 ± 3.13
Neutrophils	10^3/µL	3.02 ± 0.61	3.00 ± 0.71	3.81 ± 0.94	3.65 ± 1.40
Lymphocytes	10^3/µL	9.20 ± 2.00	9.44 ± 1.29	9.78 ± 2.04	8.92 ± 1.81
Monocytes	10^3/µL	0.28 ± 0.15	0.38 ± 0.18	0.34 ± 0.15	0.46 ± 0.26
Eosinophils	10^3/µL	0.28 ± 0.20	0.32 ± 0.96	0.34 ± 0.17	0.35 ± 0.12
Basophils	10^3/µL	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Total erythrocyte count	10^6/µL	8.18 ± 0.46	8.00 ± 0.26	7.77 ± 0.31	8.00 ± 0.34
Haemoglobin	g/dL	16.18 ± 0.76	16.27 ± 0.63	15.98 ± 0.66	15.83 ± 0.68
Haematocrit	%	41.40 ± 2.19	41.59 ± 1.04	41.01 ± 1.76	40.57 ± 1.77
Mean Corpuscular Volume	fL	50.42 ± 1.52	51.84 ± 1.24	52.29 ± 1.21	50.70 ± 0.92
MCH	Pg	19.79 ± 0.28	20.35 ± 0.29	20.58 ± 0.57	19.80 ± 0.31
MCHC	g/dL	39.27 ± 1.08	39.27 ± 1.12	39.36 ± 0.94	39.06 ± 0.47
Red cell distribution width	%	18.66 ± 0.26	18.17 ± 0.25	18.17 ± 0.20	18.63 ± 0.61
Platelets	10^3/µL	1075.1 ± 83.9	966.3 ± 145.1	987.0 ± 118.6	996.8 ± 113.0
Mean platelet volume	fL	5.32 ± 0.21	5.07 ± 0.15	5.10 ± 0.14	5.17 ± 0.32

Table 3: Mean (±SE) serum chemistry of Wistar rats under acetamiprid oral toxicity (n= 10)

Parameter	Unit	Group I	Group II	Group III	Group IV
Blood urea	mg/dL	47.71 ± 6.11	55.47 ± 5.06	60.55 ± 9.51	60.75 ± 3.64
Triglyceride	mg/dL	68.41 ± 18.34	86.68 ± 10.36	82.68 ± 13.81	86.40 ± 23.37
Alanine transaminase	U/L	39.10 ± 13.27	44.44 ± 9.82	36.63 ± 8.01	39.55 ± 5.67
Aspartate transaminase	U/L	134.25 ± 46.51	172.11 ± 68.50	129.08 ± 42.39	122.82 ± 35.27
Alkaline phosphatase	U/L	209.20 ± 28.72	245.40 ± 50.52	236.00 ± 27.25	221.10 ± 27.95
Gamma glutamyl transferase	U/L	3.20 ± 2.04	2.40 ± 1.35	2.90 ± 2.025	4.00 ± 2.45
Total protein	g/dl	6.21 ± 0.19	6.89 ± 0.75	6.45 ± 0.68	6.06 ± 0.35
Albumin	g/dl	3.25 ± 0.09	3.28 ± 0.18	3.24 ± 0.23	3.14 ± 0.14
Creatinine	mg/dL	0.50 ± 0.04	0.62 ± 0.08	0.55 ± 0.08	0.57 ± 16.27
Uric Acid	mg/dL	1.77 ± 0.45	1.39 ± 0.80	1.073 ± 21.14	1.57 ± 0.30
Cholesterol	mg/dL	49.53 ± 5.34	44.18 ± 7.38	48.17 ± 9.72	56.18 ± 21.35
Glucose	mg/dL	80.80 ± 13.55	73.10 ± 20.87	82.90 ± 26.72	74.00 ±17.11
Calcium	mg/dL	8.82 ± 0.29	9.02 ± 1.36	8.71 ± 1.40	8.77 ± 0.36

Table 4: Mean (±SE) values of absolute and relative organ weight of rats (n=10)

Weight	Organ	Group I	Group II	Group III	Group IV
Absolute organ weight (gm)	Liver (gm)	10.31 ± 1.26	9.98 ± 1.26	10.35 ± 1.18	12.15 ± 2.09
	Kidneys (gm)	2.33 ± 0.20	2.20 ± 0.22	2.19 ± 0.15	2.37 ± 0.23
	Brain (gm)	2.05 ± 0.08	1.97 ± 0.12	2.02 ± 0.12	2.02 ± 0.16
	Epididymides (gm)	1.28 ± 0.15	1.14 ± 0.07	1.24 ± 0.11	1.21 ± 0.16
	Spleen (gm)	0.72 ± 0.07	0.65 ± 0.12	0.68 ± 0.12	0.74 ± 0.07
	Testes (gm)	2.96 ± 0.34	2.94 ± 0.29	3.11 ± 0.21	3.11 ± 0.21
	Adrenals (gm)	0.06 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.06 ± 0.01
	Thymus (gm)	0.52 ± 0.08	0.41 ± 0.09	0.51 ± 0.8	0.56 ± 0.11
Relative organ weight (%)	Liver (%)	2.73 ± 0.30	2.68 ± 0.30	2.82 ± 0.20	3.15 ± 0.44
	Kidneys (%)	0.62 ± 0.05	0.59 ± 0.04	0.60 ± 0.04	0.62 ± 0.07
	Brain (%)	0.54 ± 0.02	0.53 ± 0.03	0.55 ± 0.05	0.53 ± 0.06
	Epididymides (%)	0.34 ± 0.05	0.29 ± 0.05	0.34 ± 0.03	0.32 ± 0.05
	Spleen (%)	0.19 ± 0.02	0.17 ± 0.03	0.19 ± 0.03	0.19 ± 0.02
	Testes (%)	0.79 ± 0.10	0.79 ± 0.06	0.85 ± 0.05	0.81 ± 0.05
	Adrenals (%)	0.02 ± 0.01	0.01 ± 0.003	0.01 ± 0.003	0.01 ± 0.02
	Thymus (%)	0.14 ± 0.02	0.11 ± 0.02	0.14 ± 0.03	0.14 ± 0.03

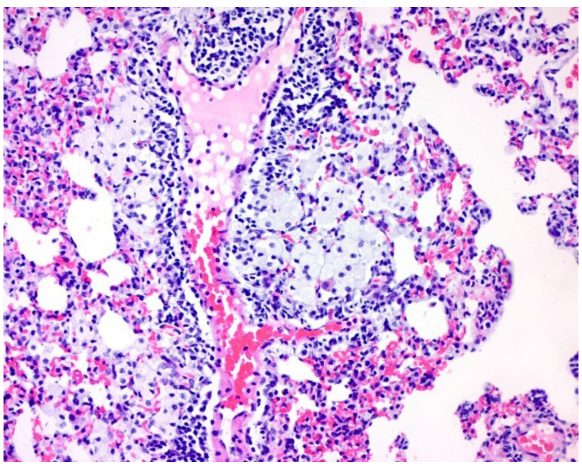

Fig. 1: Group IV: Lung alveoli showing histiocytosis (H&E, 200X)

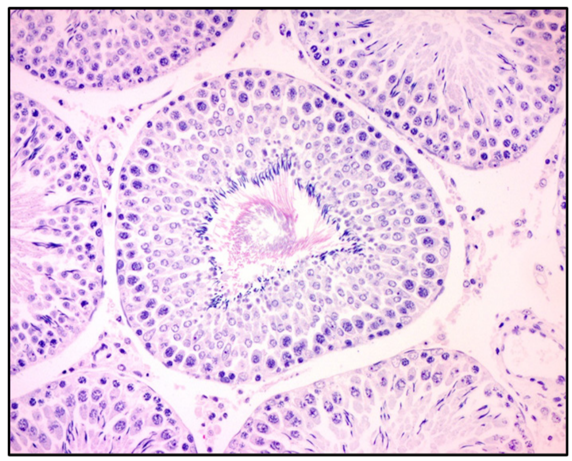

Fig. 2: Group IV: Seminiferous tubules showing normal histological architecture (H&E, 200X)

Gross and Histopathological Alterations

Gross evaluation of organs of test animals was similar to control animals. The acetamiprid did not produce any considerable histological alterations in any of the organs examined, and those were comparable to control except some spontaneous histological changes were noted in a few animals in either group, irrespective of test material. The microscopic examination of the lungs revealed alveolar histiocytosis of minimal severity (Fig. 1) observed in three animals of the control group and in only one animal of group IV. but these changes were noted only in a few animals, with some changes in both groups of animals. A minimal degree of perivascular cuffing was noted in two animals in group I but not in any animals in the higher-dose group. Liver of one control rat showed mild hepatocellular vacuolation and no such changes were evident in any of the dose group, hence being considered an incidental finding. One high dosed rat had basophilic tubules of minimal severity, whereas the kidneys of all other male rats in group IV did not show any change related to acetamiprid treatment and were comparable to the control group animals. Besides the lungs, liver, and kidneys histopathological examination of other reproductive organs (Fig. 2) were comparable with control animals.

Similar to the microscopic findings of the present study, Devan *et al*. (2015) and Kanungo and Solecki (2012) also noted no test substance related histological changes in treated groups as compared to control animals. In contrast, Zhang *et al.* (2011) and Brahmbhatt *et al*. (2016) and in mice, Kong *et al*. (2017) and Arican *et al*. (2020) in rats reported test compound related histopathological changes in testes,

Table 6: Mean (±SE) sperm parameters of Wistar rats under acetamiprid oral toxicity (n= 10)

Sperm parameters	Group-I	Group-II	Group-III	Group-IV
Total sperm count (million/mL)	24.53 ± 2.72	24.15 ± 3.41	24.45 ± 3.65	23.53 ± 2.04
Live sperm (%)	86.90 ± 3.25	86.30 ± 3.13	85.90 ± 2.47	85.20 ± 2.04
Abnormal sperm count (%)	7.10 ± 3.93	4.20 ± 1.99	5.70 ± 2.54	3.80 ± 1.48

which were collectively characterized as vacuolization in the germinal epithelium, decreased spermatids, Leydig cell population and vacuolar degeneration of tubular epithelium and focal necrosis of tubules, depletion of lymphocytes in thymus and splenic follicles. Whereas Terayama *et al*. (2018) noted distorted or disrupted epithelium of seminiferous tubules in mice. The absence of typical microscopic changes related to the test material in any of the tissues, including reproductive organs in the current study, might be attributed to the administration of acetamiprid at a lower dose rate than that could potentially result in damage to various tissues and organs in the treated animals.

Sperm Evaluation

The sperm evaluation was performed on all the samples collected from the epididymis heads of all the groups, including the control and treatment groups. Data obtained from statistical analysis of all groups of animals are presented in Table 5. The sperm parameters investigated in all rats represent no test item related abnormality, including total sperm count, sperm viability, and sperm morphology were comparable to the control. In a challenge to the present study, Zhang *et al*. (2011) Mosbah *et al*. (2018) Arican *et al*. (2020), and El-Hak *et al*. (2022) observed that low the number of spermatids and decreased epididymal sperm count in the treatment group as compared to the control group.

Conclusions

The present investigation observed that oral administration of acetamiprid up to the dose rate of 31 mg/kg b.wt. for 45 consecutive days being safe did not induce a significant effect on body weight, any abnormal clinical signs or mortality, organ weight, or haematological parameters. The increase in serum urea level was observed in a dose dependent manner, but no associated gross or microscopic changes were noted in the liver or kidney; hence, this increase in urea level was not considered acetamiprid related. The assessment of reproductive toxicity also showed no test substance related abnormalities in sperm count, sperm morphology, and sperm viability at this dose rate.

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