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Effects of Fipronil Induced Testicular Dysfunction In Male Albino Rats

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ABSTRACT

Fipronil (FIP), a widely used phenylpyrazole insecticide, has raised significant concerns due to its environmental persistence, bioaccumulation, and potential adverse effect on non-target organisms, including humans. The study investigates the reproductive toxicity of Fipronil in male albino rats, focusing its impact on testicular structure and function. Male rats were administered varying doses of Fipronil to evaluate changes in body weight, testis weight, histopathological alterations, biochemical parameters and hormone levels. Result revealed dose-dependent reduction in body and testis weight, histological damage to seminiferous tubules, disrupted biochemical markers and significant alterations in serum testosterone, FSH and LH levels. These findings underscore Fipronil's potential to impair male reproductive health through mechanisms involving oxidative stress, hormonal disruption, and structural damage to testicular tissue.

INTRODUCTION

Fipronil is a widely used insecticide known for its effectiveness against a board spectrum of pests. This review examines its chemical properties, uses, and its significant impact on health and the environment (Kanojiya *et al.*,2025). In developed countries, pesticide are applied in agriculture to maximize crop yields (Mahmood *et al.*, 2016). However, these chemicals can have harmful effects on the environment, compromise food safety, and pose risk to both animal and human health (Kaur *et al.*, 2024). While highly effective, its persistence in ecosystems and potential toxicity to non- target species, including humans, raise important concerns (Chaware *et al.*,2025). Fipronil (FIP) is a board spectrum phenyl pyrazole insecticide developed by Rhone-Poulenc in 1987 and introduce commercially in 1933 (Ware *et al.*, 2000; Tingle *et al.*,2003). Registered in the United States in 1996, Fipronil has since been widely used in agriculture practices, veterinary medicine, residential pest control and turf management (Tomline *et al.*, 2000; Anadon and Gupta,2025). However, Fipronil's widespread use is accompanied by growing concerns over its environmental persistence and toxicity. A review on Fipronil, a widely used insecticide, has been linked to reproductive toxicity in various species (Malame *et al.*, 2025). The compound exhibits a long environmental half-life, allowing it to accumulate in soil and aquatic systems (Chagnon *et al.*,2015). Consequently, this persistence raises potential exposure risk for non-target organisms, including humans, through ingestion, inhalation, dermal contact (Chodorowski and Anand,2004; Mohamed *et al.*,2004). Both acute and chronic exposure have been linked to neurotoxicity, hepatotoxicity, immune suppression, reproductive toxicity and oxidative stress in animal models (Mahmoud *et al.*,2021; Kartheek *et al.*, 2018; Khan *et al.*, 2015; Bano *et al.*, 2020). Symptoms observed in humans and rodents include excitability, dizziness, seizures, nausea and behavioral alteration (Mohamed *et al.*, 2004; Tercariol andGodinho,2011). Furthermore, genotoxic and mutagenic effects have been documented in both mice and humans (De Oliveira *et al.*,2012; Celik *et al.*,2014). Research has also demonstrated the ability of Fipronil to interfere with GABAergic signaling in spermatozoa, leading to decrease sperm capacitation and fertility (Bae *et al.*,2020). The testis, comprising of seminiferous tubules and interstitial Leydig cells, is responsible for spermatogenesis and testosterone production, respectively (Russell *et al.*,1990; Foster, 2017). Disruption to any part of this system from hormone signaling pathways to sperm maturation processes in the epididymis can result in reduce fertility and altered reproductive capacity (Hall, 2015). It is imperative to look more closely at the mechanism of fipronil reproductive toxicity given its environmental durability, bioacumulation potential and known negative effects on the male reproductive system.

MATERIALS AND METHODS

Animal collection and Maintenance

Eighteen adult male albino rats (*Rattus norvegicus*), weighing 200-240 gm, were obtained from Global Bioresearch Solutions, Pune, Maharashtra, India. The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) and conducted in accordance with CCSEA guidelines (Reg.No.478/GO/Re/S/01/CCSEA). Rats were acclimatized for 7 days under standard Laboratory conditions (natural light, 26-28^0 C), housed in cages with stainless steel lids, with a commercial pellet diet and water.

Treatment

The administrated fipronil brought from Bharat. Agri. Pvt. Ltd. India, a powder was given orally mixed n distilled water in the

concentration of 30 mg/kg BW and 60 mg/kg BW daily in group B and C respectively. Group A used as control and feed with normal food pellet and distilled water. At the end of 21 days of treatment, animal weight was recorded, and testis was removed, clean and weighed ad processed for histology and estimation.

Body weight and Organ weight

The body weight of each animal was measured twice per week throughout the study period by using digital balance. The isolate Organs (testes) of all the control
and experimental animals were measured by using calibrated balance and data was maintained.

Histology

Testis of control and experimental animals were fixed in Bouin's fluid for 24hrs, then dehydrated by passing through graded series of ethyl alcohol, clear in xylene and after embedding in paraffin wax at 60-62^0 C, blocks were prepared. The photomicrograph were taken with the help of digital camera Nikon COOLPIX 8400attahed to the light microscope Nikon EclipseE200 and enlarged to required size.

Biochemical Analysis

The testis were dissected out and the surrounding fat was removed. The testis then homogenized for 5 minutes in different volumes of ice-cold distilled water. The total protein concentration in testis tissue was estimated using Lowry *et al.*, *(*1951) method. The intensity of the yellowish-brown color formed was measured at 490 nm using a spectrophotometer. A celebration curve was prepared. DNA concentration in testis tissue was estimated using the diphenyl anime method (Searchy and Maclnnis,1970a). RNA concentration in testis tissue was estimated using Dische-Orcinol technique (Searchy and Maclnnis,1970b).

Hormone analysis

Testosterone, Luitinizing hormone and follicle stimulating hormone was analyzed by using enzyme-linked immunoassay (ELISA) kit purchased from Himedia. All procedureadhere to the standard protocol supplied.

Statistical analysis

The data was statistically analyzed and expressed as mean ±SEM. Statistical analysis of the variance between control and experimental values was done using 't' test with the help of graph pad calculator (Graph pad. 2024).

RESULT

BODY WEIGHT

The control group exhibited a normal increase in body weight, rising from 216.67 ± 6.67g to 256 ±6.67g over the 21day period. In contrast to this, in the present study, the rats treated with low dose of fipronil (30 mg/kg body weight) showed a slight decrease in body weight, from 216.67±1.33g to 201.67±3.33g** while a more pronounced reduction was observed in the high-dose group (60 mg/kg body weight declined from 231.67±3.33g to 196.67±1.33g**.These result indicate a clear, dose-dependent decrease in body weight following sub-acute exposure to Fipronil for 21 days.

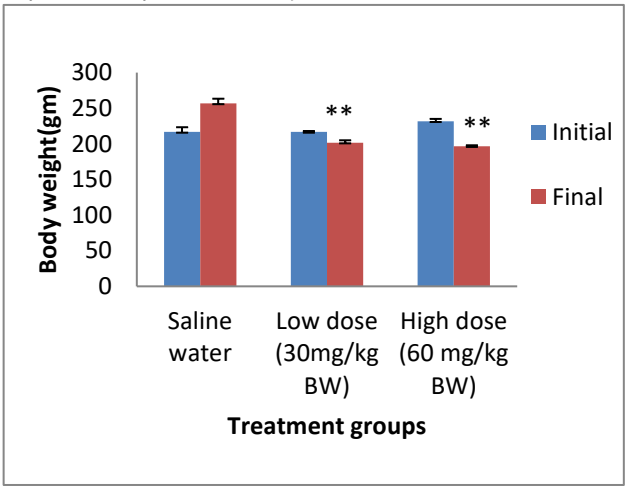


Fig 1: Showing the body weight of control and Fipronil treated rats for 21 days duration.

Values are expressed in mean ± standard Error Mean, N =6, P<0.5*, P< 0.01**, P< 0.001***.

TESTIS WEIGHT

The present investigation revealed that testis weight was found to decrease in rats treated with fipronil for 21 days. The control group, which received saline water, had a testis weight of 1.455±0.003. In the group treated with a low dose of Fipronil (30 mg/kg of body weight), decrease to 1.435 ±0.002** a more marked reduction was observed in the high-dose group (60mg/kg body weight), where the testis weight dropped to 1.384 ± 0.001***.

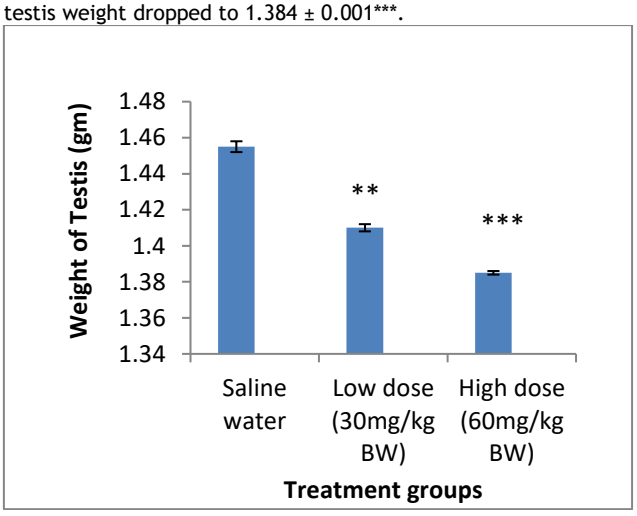
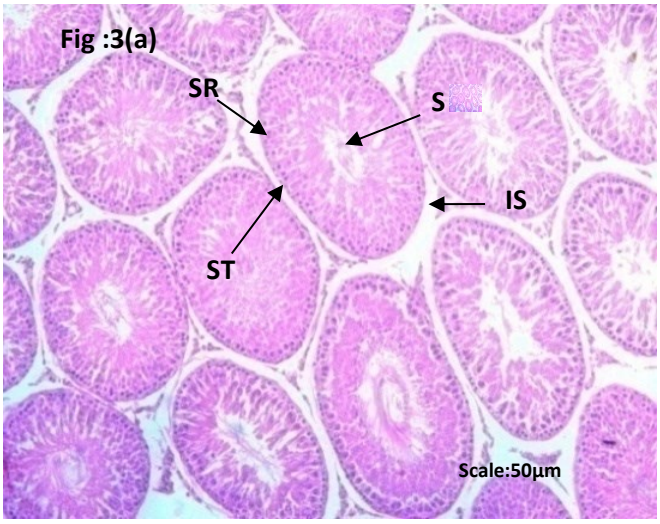


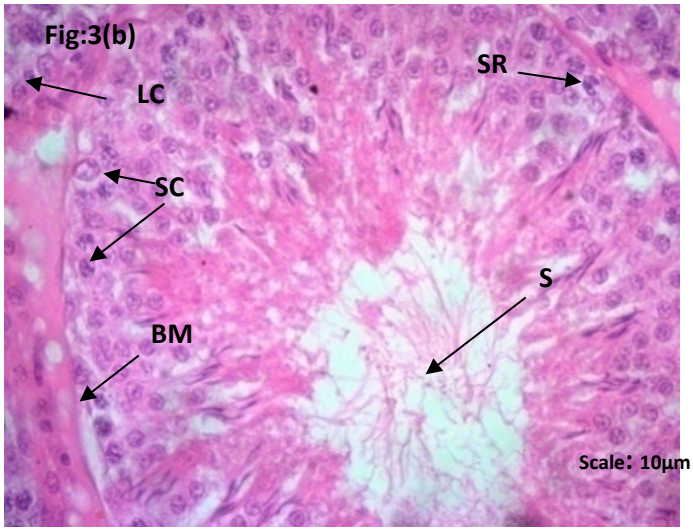
Fig 2: Showing the weight of testis of control and Fipronil treated rats for 21 days duration.

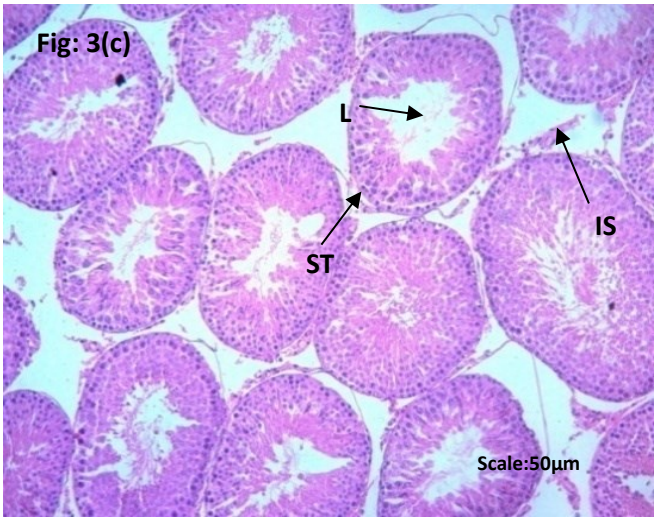
Value are expressed in mean ± standard Error Mean, N =6, P<0.5*, P< 0.01**, P< 0.001***.

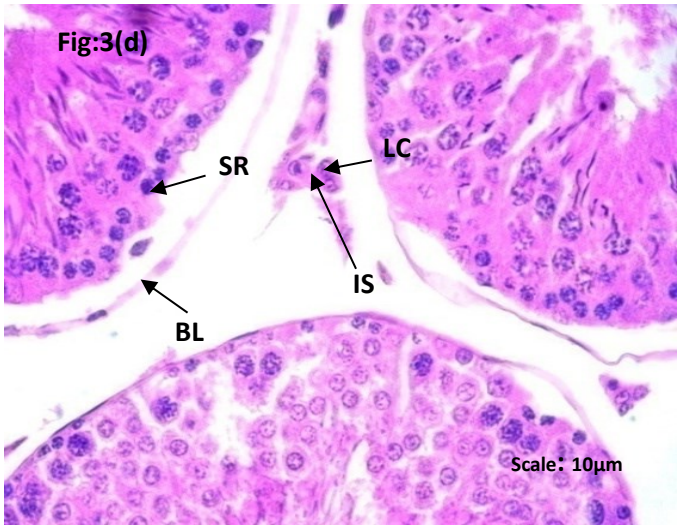
HISTOPATHOLOGICAL ASSESSMENT OF TESTICULAR TISSUE

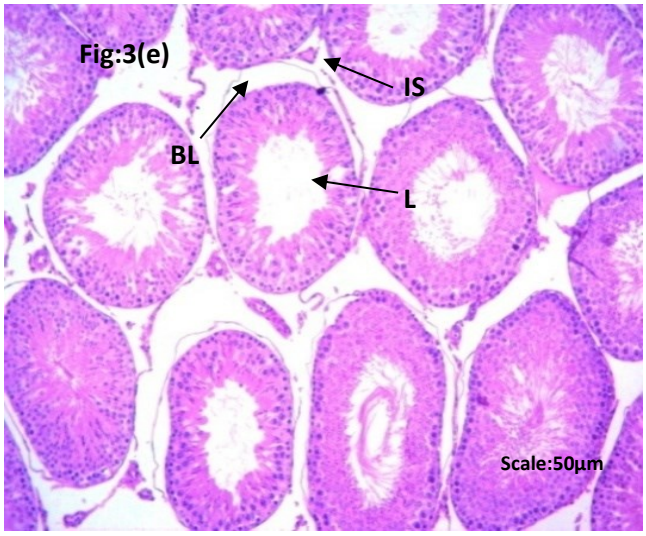
The transverse section of the testis in the control group in Fig **3 (a) and (b)** where seminiferous tubules (ST) displaying normal architecture. The germinal epithelium in intact with well- organized spermatogenic cells and abundant mature spermatozoa (S)in the Lumen (L), while Sertoli cells (SC) and Leydig cell (LC) appear normal. The rats treated with fipronil **Fig 3 (c) and (d),** (30 mg/kg BW) leads to severe degeneration of seminefeous tubules. The germinal epithelium is markedly thinned and disorganized, with extensive cell sloughing and detachment of spermatogonia (SR) from the basal lamina (BL). The lumen (L) lacks spermatozoa, and the interstitial space (IS) show a significant reduction in Leydig cells (LC), indicating serious impairment of testicular structure and function. This damage is more pronounced in Fig **3(e) and (f),** representing the group treated with Fipronil at 60 mg/kg BW for 21 days, reveal severe testicular damage. The germinal epithelium is partially disorganized, showing reduced and misaligned spermatogenic cells, with lumens containing exfoliated germ cells (EGC), debris and fewer spermatozoa. Signs of early apoptosis, such as mild vacuolization and nuclear condensation, are evident, and Sertoli cells appear loosely associated











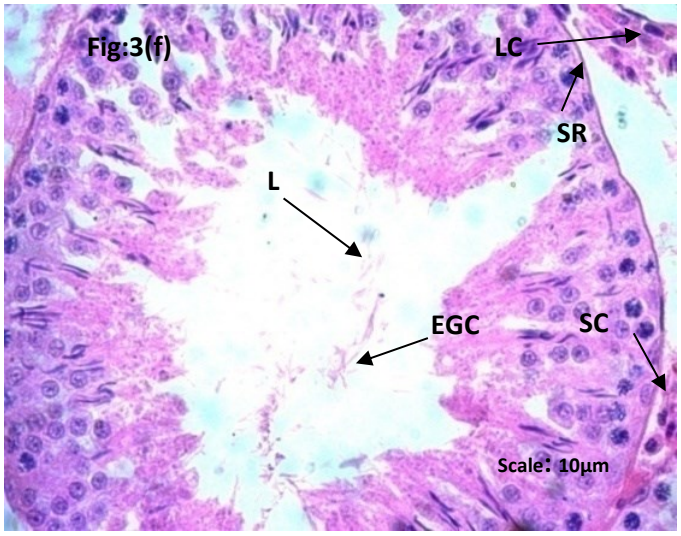


Fig.3 **a, b),** T.s of testis of male albino rats in control group show seminiferous tubules **(ST)** with normal architecture. Fig. 3(**c, d**) T.s of testis o albino rats treated with Fipronil (30 mg/kg BW) for 21 days show severe degeneration. Seminiferous tubules are disorganized with a thinned germinal epithelium, extensive cell sloughing, and spermatogonia (**SR)** detached from the basal Lamina (**BL**). Interstitial space (IS) show reduced Leydig cells (LC), In Fig 3(**e, f),** T.s of testis of albino rats treated with Fipronil (60 mg/kg BW) for 21 days show severe damage. Lumen contain exfoliated germ cells (EGC), debris, and fewer spermatozoa.

BIOCHEMICAL ANALYSIS

Fipronil exposure for 21 days lead to a significant reduction in testicular protein level in male rats. The control group showed a protein concentration of 0.350±0.0018 mg/100mg tissue. This decreased to 0.252±0.0015*** mg in low dose-group (30 mg/kg) and

further decline to 0.175±0.0013***mg/100mg in high dose group (60 mg/kg), indicating a dose- dependent effect. A similar pattern was observed for DNA levels. The control group recorded a concentration of 0.183±0.0026 mg/100mg tissue. Fipronil exposure reduced this to 0.143±0.0015***mg/100mg in the low-dose group to 0.130 ± 0.0026*** mg/100mg in the high-dose group, showing a marked and statistically significant decline. RNA also showed the significant decline after Fipronil treatment. The control group had an RNA level of 0.272±0.001mg/100mg tissue. In the low-dose, this dropped to 0.264±0.002**mg/100mg, and in the high- dose group, it further decrease to 0.247± 0.002** mg/100mg tissue.

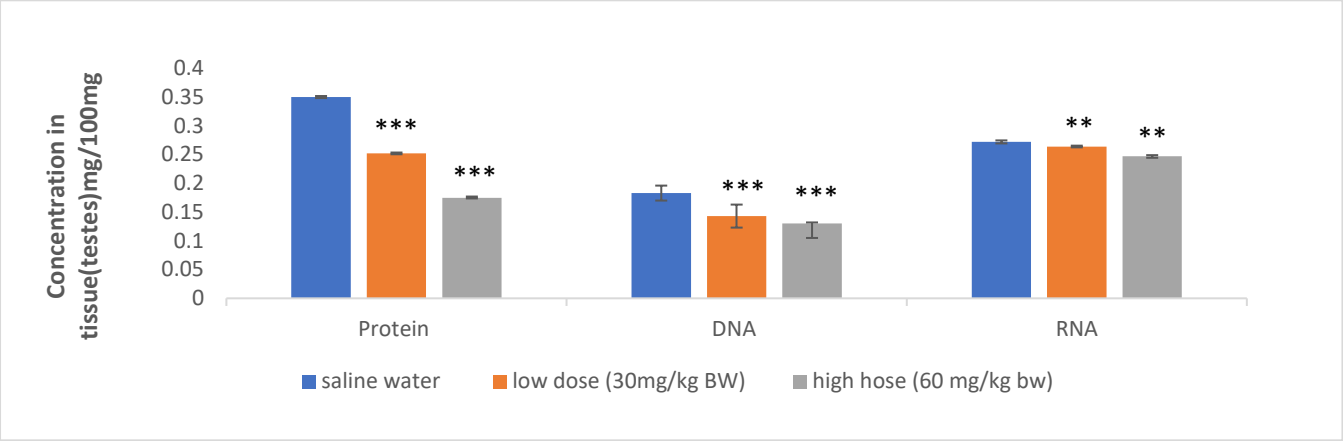


Fig 4: Showing the protein, DNA and RNA concentration in control and fipronil treated rats for 21 days duration,

Values are expressed in mean ± standard Error Mean, N =6, P<0.5*, P< 0.01**, P< 0.001***.

HORMONE ANALYSIS

Twenty-one days of Fipronil exposure in male rats resulted in significant, dose dependent decline in reproductive hormones. In the control group, testosterone levels 4.11±0.013 ng/ml. In rats treated with a low dose of Fipronil (30 mg/kg body weight), testosterone decreased to 3.61 ± 0.026***ng/ml, and further dropped to 3.19 ± 0.029*** ng/ml in the high-dose group (60 mg/kg body weight). Luitinizing hormone (LH) levels also declined from 0.519±0.003 ng/ml in control to 0.417±0.001***ng/ml (30 mg/kg) and 0.353 ±0.001***ng/ml (60 mg/kg body weight). Similarly, follicle stimulating hormone (FSH) levels were dropped from 3.596 ± 0.051 mlU/ml in controls to 2.862 ±0.029*** mlU/ml and 2.320±0.046*** mlU/ml low and high doses, respectively. These results demonstrate that Fipronil exposure at both 30 and 60 mg/kg body weight significantly disrupts hormonal regulation essential for male reproductive system

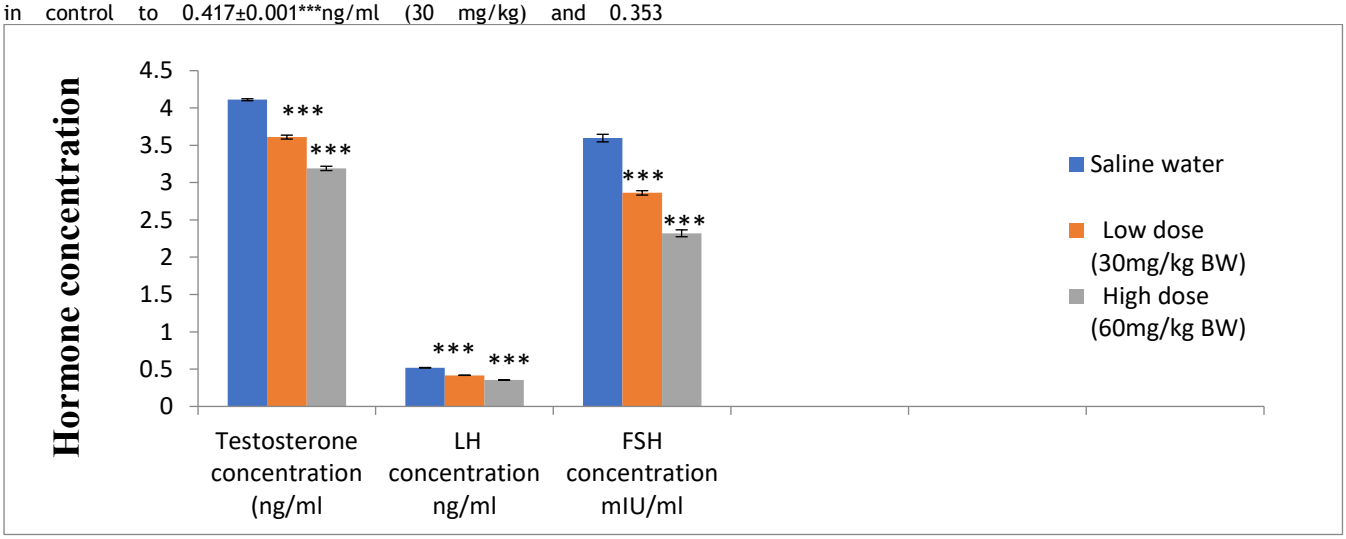


Fig 5: Showing the hormone concentration in control and fipronil treated rats for 21 days duration

Values are expressed in mean ± standard Error Mean, N=6, P<0.5*, P< 0.01**, P< 0.001***.

DISCUSSION

The present study investigated the sub-acute toxic effects of Fipronil on physiological, biochemical and histological parameters in male albino rats. Result demonstrated that Fipronil exposure significantly affected body weight, reproductive organ weight, testicular biochemistry and tissue architecture, indicating its potential to disrupt male reproductive health. Body weight significantly declined in fipronil treated groups, supporting Verma (2022), who suggested that Fipronil may cause appetite loss, impaired nutrient absorption, and oxidative stress-induced metabolic disturbances. Similarly, testicular weight was significantly reduced, likely due to the disrupted endocrine function and impaired spermatogenesis, aligning with Tohamya *et al.*, (2021), who reported reproductive organ atrophy linked to decreased testosterone and sperm production. Histopathological evaluation provided further evidence of reproductive toxicity. The control group showed normal testicular architecture. In contrast, testis sections from rats treated with 30 mg/kg BW fipronil for 21 days showed moderate Separation of spermatogonia from basal lamina and thinning of germinal epithelium reflect advanced disorganization of semineferous tubules. Cellular sloughing, germ cell degeneration and an almost complete loss of mature spermatozoa in the lumen confirm impaired spermatogenesis. This aligns with the findings of Tohamy *et al.*, (2021), Suliman (2020)

and Nakul (2024) who reported germ cell loss and structural deterioration of seminiferous tubules due to hormonal imbalance and direct cellular toxicity. A marked reduction in leydig cells in the interstitial spaces was more prominent, suggesting impaired testosterone synthesis. This finding supports the work of (Abdel-Mobdy *et al*., 2024). At a higher dose of fipronil 60 mg/kg BW for 21 days testicular damage was significantly more severe structural disorganization. Partial disintegration of the germinal epithelium and reduced number and misalignment of spermatogenic ells indicated impaired spermatogenesis. Exfoliated germ cells and cellular debris observed within the lumen, with mild vacuolization and nuclear condensation suggesting early apoptotic activity. These findings are consistent with Khan *et al., (*2015) and Badawy *et al.,* (2018), both of them documented oxidative-stress testicular damage, including apoptosis and architecture disruption. Biochemical estimation further confirm the toxic potential of fipronil on testicular tissue. Protein levels in the testis were also notably reduced following fipronil exposure. This may be due to disrupted protein synthesis and increase catabolism, or the excretion oh high molecular weight proteins, as suggested in studies on Fenitrothion treated rats by El-Demerdash *et al*., 2013.Protein depletion in testicular cells impairs the structure and function o germ cells, further contributing to subfertifity. There was a significant decline in nucleic acid content (DNA and RNA) indicating reduced cell proliferation and impaired transcriptional activity. The study has been in agreement with the findings of Badaway *et al.,* (2018), who reported that subacute exposure to a combination of imidacloprid and fipronil resulted in decreased testicular nucleic acid content due to oxidative damage. Such damage compromises gene expression essential for normal spermatogenesis. There was a significant decline in Testosterone, LH and FSH levels reinforcing the evidence that fipronil exerts dose-dependent, longterm effect on male reproductive function, likely through interference with the hypothalamic Pituitary –Gonadal axis, as also suggested by De Barros *et al.,* (2016) and Zohree *et al.,* (2018) and Nakul *et al*., (2024). A significant reduction in FSH levels was also observed, indicating potential disruption of the hypothalamic-pituitary –gonadal axis. This, findings are consistent with wit those of Nithya and Elango (2015), and parallel to the effect of lindane, highlighting is anti-androgenic potential

CONCLUSION

Exposure to fipronil exerts reproductive toxicity in experimental models, dose –dependent reduction in body and testis weight, along with histological damage to the testicular tissue including disrupted seminiferous tubules and decrease in testicular protein, DNA and RNA levels indicate impaired cellular and metabolic activity. The hormonal analysis further supports this, showing suppressed levels of testosterone, luitinizing hormone and follicle stimulating hormone (FSH).

ETHICAL STATEMENT

All the animal experiment protocols were done under the guidelines and rules of the Department of Zoology RTM Nagpur University Institutional Animal Ethics Committee.

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