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# Fluopyram Induced Oxidative Stress, Biochemical and Histopathological Changes in Brain and Blood of Albino Rats

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***Abstract*:** Fluopyram, a widely used fungicide in agriculture, has raised concerns regarding its potential toxicity and adverse effects on non-target organisms. The present study was designed to investigate the adverse effect of two doses of fluopyram (0.5 and 2.5 mg/kg body weight/day) on antioxidant enzymes and histopathological changes in the brain of male and female albino rats for 28 days and its amelioration with ginger extract. Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were decreased and there was significant increase in lipid peroxidation (LPO) levels in the brain and blood plasma of male and female albino rats. Histopathological alterations in the brain revealed mononuclear cell infiltration, pyknotic neurons, degenerated neurons(DN), increased perinuclear space and hemorrhage in treated rats indicating fluopyram's neurotoxic potential. Rats treated with ginger extract and fluopyram showed restored antioxidant enzyme activities in blood and brain tissue and restored histopathological changes in brain. These pathophysiological alterations in blood and brain tissues could be due to potential toxic effects of fluopyram that is associated with generation of free radicals.

***Keywords***: Fluopyram, Albino rats, Ginger extract, Oxidative stress.

## 1. INTRODUCTION

Pesticides are chemical substances used to eradicate insect pests, weeds, fungus, and rodents. Plant growth regulators, fungicides, molluscicides, insecticides, herbicides, nematicides, fungicides and other substances are among them (Zhan et al., 2020; Bhatt et al., 2021a; Zhang et al., 2021). Fluopyram is a novel broad range fungicide created by Bayer Crop Science to manage plant pathogenic fungi like botrytis, black dot and white mold to fight fungal diseases in different fruits and vegetable crops (Tinwell et al., 2014). It prevents succinate dehydrogenase from accomplishing its function in the fungal mitochondrial respiratory chain and is classified as a succinate dehydrogenase inhibitor (SDHI) subclass of fungicides. Because of its widespread use, concerns regarding fluopyram's ecotoxicological effects have recently emerged (Matadha et al., 2019). From a human health perspective, it is crucial to recognize that numerous neurological disorders and various malignancies are associated with pesticide exposure (Yen et al., 2021). Fluopyram inhibits the mitochondrial complexes as it is an inhibitor of mitochondrial complex II, as these are the primary pathways in the brain tumors and diseases like parkinson's etc (Exner et al., 2012). In this context, assessing the toxicity of fluopyram on brain is very important.

Toxicological studies on fluopyram have highlighted its capacity to induce oxidative stress in various biological systems. Oxidative stress occurs when there is an imbalance between the production of reactive oxygen species (ROS) and the body's ability to neutralize these species with antioxidants (Sies, 2015). This imbalance can lead to cellular damage, inflammation and dysfunction, which are implicated in a variety of diseases and toxicological outcomes. In mammals, fluopyram exposure has been shown to lead to increased oxidative damage in multiple organs, including the brain and liver (Akinmoladun et al., 2020). The brain, being particularly vulnerable to oxidative stress due to its high metabolic activity and lipid-rich composition, is an important target for the toxic effects of environmental pollutants, including pesticides (Halliwell, 2006). Fluopyram-induced oxidative stress in the brain can result in neuro-degeneration, inflammation and impairment of cognitive functions (Kocaman et al., 2020). Similarly, fluopyram's effects on blood parameters have been investigated, with studies suggesting changes in hematological profiles, such as red and white blood cell counts and enzyme activities (Singh et al., 2019). Celik et al. (2012) conducted a study on rats by giving Luna Experience-

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SC 400 (fluopyram and tebuconazole) and observed cytotoxic and genotoxic potential in rat bone marrow. Anti-platelet, antioxidant, anti-tumor, anti-retroviral, anti-hepatotoxic and anti-arthritic properties are a few of the pharmacological properties that have been shown by ginger (Kamtchoving et al., 2002). Studies on the impact of fluopyram on the blood and brain tissue however, are lacking. Therefore, the purpose of this study was to assess how the fluopyram affects the antioxidant potential and histology of brain of male and female albino rats and its amelioration with ginger extract as ginger extract is well known for its antioxidant, anti-inflammatory and neuroprotective properties.

## 2. MATERIALS AND METHODS

### 2.1. Chemicals

Fluopyram(N-[2-[3-Chloro-5-(trifluoromethyl)-2-pyridinyl] ethyl]- 2(trifluoromethyl) benzamide; CAS: 658066-35-4; 34.48% W/W SC) was purchased from the local market of Ludhiana Punjab, India, manufactured by Bayer Crop Science Ltd. under the trade name Bayer Velum Prime.

### 2.2. Experimental Design

In this study, a total of 24 mature male and 24 female albino rats weighing 130 to 170 g were used. Rats were housed in polypropylene cages and were given water and food *ad libitum*. The rats were acclimatized for a period of 2 weeks before the start of the experiment and were randomly divided into four groups; 6 rats in each group. Group I served as control, Group II and III received 0.5 and 2.5 mg/kgbw/day of fluopyram through oral gavage and Group IV received 100 mg/kg aqueous extract of ginger and 2.5 mg/kgbw/day fluopyram Table 1.

The rhizomes of *Z. officinale* were shade dried and crushed to powder. 125 g of the powder was macerated in 1000 ml of distilled water for 12 h. at room temperature and was then filtered to obtain the final aqueous extract. In this study each animal was orally given 1 ml of the final aqueous extract (Kamtchoving et al., 2002). Two doses of fluopyram were dissolved in 0.1 ml DMSO and were given by oral gavage for 28 days. The experiment was performed after the approval of Institutional Animal Ethical Committee, Guru Angad Dev Veterinary and Animal Sciences University Ludhiana, India under protocol No. (GADVASU/2023/IAEC/69/04 -17/05/2023).

After the treatment animals were put to death by cervical dislocation. Blood was collected by cardiac puncture and then centrifuged to obtain plasma and brain tissue was removed and weighed after dissecting the animals. The tissues were sheared in 0.9% saline (chilled) and homogenization was done in 0.1 M phosphate buffer(pH 7.4). The homogenates were centrifuged for 30 min at 1000 rpm at $4^0$C to obtain supernatants. The plasma and tissue supernatants were used for the assay of different antioxidant enzymes.

### 2.3. Oxidative Stress Biomarkers in Blood and Brain

Superoxide dismutase(SOD) was determined according to method of Marklund and Marklund (1974), catalase (CAT) by Aebi (1983), glutathione peroxidase (GPx) by Hafeman et al. (1984) and lipid peroxidation(LPO) by Jollow et al. (1974) respectively. In the estimations of all the antioxidant enzymes the total soluble protein content was estimated by Lowry et al. (1951) taking BSA as standard.

**Statistical Analysis:** Software (SPSS) was used to analyze the data with post host Tukey's t-test was used for making comparisons between control and treated group of rats.

### 2.4. Histopathological Examinations

Brain tissue was cleared and fixed in 10% formalin for 24 hours. Then the tissues were dehydrated in different grades of ethanol, clearing was done in xylene and embedding was done in paraffin wax for the preparation of blocks. The 5-7µm thick sections were cut and stained in haematoxylin-eosin stain and mounted in DPX and slides were seen under

**Table 1.** Effect of fluopyram on net body weight and weight of brain of male and female rats

| Group | Treatment | Male rats | | | Female rats | | |
|---|---|---|---|---|---|---|---|
| | | Initial weight (g) | Final weight (g) | Brain weight (g/100g body weight) | Initial weight (g) | Final weight (g) | Brain weight (g/100g body weight) |
| I | Control | 151.66 | - | 4.28[a] | 130.00 | - | 4.32[a] |
| II | 0.5mg/kgbw/day | 153.00 | 156.66 | 4.22[a] | 135.00 | 136.66 | 4.28[a] |
| III | 2.5mg/kgbw/day | 153.00 | 156.66 | 3.90[a] | 140.00 | 146.00 | 4.24[a] |
| IV | Ginger + 2.5 mg/kgbw/day | 152.00 | 152.66 | 3.95[a] | 132.00 | 135.33 | 3.98[a] |

***Values are expressed as mean±SE***

***[a]represents no significant difference between treatments at p≤0.05 as compared to control***

microscope. The histological alterations in brain were scored as follows: normal appearance(-), mild(+), moderate(++), severe(+++).

## 3. RESULTS AND DISCUSSION

No mortality was observed during the treatment period. Mean body weight of male and female albino rats did not vary significantly between rats of control and treated groups (0.5 and 2.5 mg/kgbw/day) (Table 1). Non-significant decrease in body weight was observed in male and female treated rats as compared to control rats. No significant difference was observed in the weights of brain of rats among all the treated and control groups. Different antioxidant enzymes were assessed in the plasma and brain of male and female rats exposed to fluopyram and the efficacy of ginger was evaluated at high dose post treatment of fluopyram. Rats of both sexes showed decreased catalase, SOD, GPx activity after being exposed to high dose of fluopyram, compared to the corresponding control groups in the plasma. The rats treated with ginger showed higher levels of catalase at high dose, i.e., 2.5 mg/kg, in both male and female animals in the plasma when compared with the untreated and fluopyram treated rats (Table 2, 3). Rats of both sexes showed increased LPO levels after treatment with high dose of fluopyram, compared to the corresponding control groups in the plasma. The rats treated with ginger had lower LPO at high dose, i.e., 2.5 mg/kg, in both male and female animals in the plasma (Table 2, 3) when compared with the untreated and fluopyram treated rats (low dose of fluopyram)

Male and female rats showed decreased catalase, SOD, GPx activity after being exposed to high dose of fluopyram, compared to the corresponding control groups in the brain tissue. The rats treated with ginger (ZO) had higher levels of catalase at high dose, i.e., 2.5 mg/kg, in both male and female animals in the plasma when compared with the untreated and fluopyram treated rats (Table 5). LPO levels were also increased after being treated with high dose of fluopyram, compared to control groups in the brain tissue. The rats treated with ginger (ZO) indicated lower LPO at high dose, i.e., 2.5 mg/kg, in both male and female animals in the

**Table 2.** Effect of fluopyram on antioxidant parameters of plasma in male rats

| Group | Treatment | Antioxidant parameters | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Superoxide dismutase (SOD) | Catalase (CAT) | Glutathione-S-transferase (GST) | Glutathione peroxidase (GPx) | Glutathione (GSH) | Glutathione reductase (GR) | Lipid peroxidation (LPO) |
| I | Control | 17.09$^{b}$ | 11.64$^{c}$ | 0.67$^{b}$ | 4.83$^{a}$ | 47.91 | 0.05$^{a}$ | 1.36$^{b}$ |
| II | 0.5mg/kgbw/day | 13.88$^{b}$ | 10.40$^{c}$ | 0.66$^{b}$ | 4.23$^{d}$ | 46.78$^{a}$ | 0.04$^{c}$ | 7.53$^{a}$ |
| III | 2.5mg/kgbw/day | 5.85$^{a}$ | 8.91$^{a}$ | 0.46$^{a}$ | 2.25$^{a}$ | 41.60$^{a}$ | 0.02$^{a}$ | 6.52$^{c}$ |
| IV | Ginger+2.5 mg/kgbw/day | 15.37$^{b}$ | 11.40$^{b}$ | 0.66$^{b}$ | 4.30$^{b}$ | 46.46$^{b}$ | 0.04$^{b}$ | 1.69$^{b}$ |

*Values are expressed as mean±SE of six animals in each group*

*$^{abc}$ represents significant difference between treatments at p≤0.05 as compared to control.*

*Units: SOD (U/mg protein), CAT (µmole of $H_2O_2$ decomposed/min/mg protein), GPx(U/mg protein), GST (µmoles of GSH-CDNB conjugate formed/min/mg protein), GSH (nmol/mg protein), GR (µmoles of NADPH oxidized/min/mg protein), Lipid peroxidation (nmol MDA/100 mg tissue)*

**Table 3.** Effect of fluopyram on antioxidant parameters of plasma in female rats

| Group | Treatment | Antioxidant parameters | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Superoxide dismutase (SOD) | Catalase (CAT) | Glutathione-S-transferase (GST) | Glutathione peroxidase (GPx) | Glutathione (GSH) | Glutathione reductase (GR) | Lipid peroxidation (LPO) |
| I | Control | 18.34$^{c}$ | 11.64$^{c}$ | 0.84$^{c}$ | 4.87$^{c}$ | 47.9 | 0.06$^{c}$ | 1.85$^{a}$ |
| II | 0.5mg/kgbw/day | 15.94$^{b}$ | 10.40$^{b}$ | 0.71$^{c}$ | 4.09$^{c}$ | 41.20$^{b}$ | 0.05$^{c}$ | 5.50$^{b}$ |
| III | 2.5mg/kgbw/day | 5.89$^{a}$ | 5.55$^{b}$ | 0.45$^{b}$ | 3.86$^{a}$ | 40.46$^{a}$ | 0.02$^{a}$ | 7.82$^{c}$ |
| IV | Ginger + 2.5 mg/kgbw/day | 17.95$^{b}$ | 12.85$^{c}$ | 0.80$^{b}$ | 4.83$^{c}$ | 46.46$^{c}$ | 0.04$^{b}$ | 1.64$^{a}$ |

*Values expressed as mean±SE*

*$^{abcd}$ represents significant difference between treatments at p≤0.05 as compared to control.*

*Units: SOD (U/mg protein), CAT (µmole of $H_2O_2$ decomposed/min/mg protein), GPx(U/mg protein), GST (µmoles of GSH-CDNB conjugate formed/min/mg protein), GR (µmoles of NADPH oxidized/min/mg protein), Lipid peroxidation (nmol MDA/100 mg tissue)*

plasma (Table 4 and 5) when compared with the untreated and fluopyram treated rats(low dose of fluopyram)

Shah et al. (2010) observed that tebuconazole was given in four different groups (0, 100, 400 and 1600 ppm) for 13 weeks during a subchronic study conducted in rats. The results showed that one male and one female rat died during the study, that the mean body weight decreased in the high-dose group despite eating more food, and that the cytochrome $P_{450}$ content and hepatic N-dimethylhylase activity increased. Gujral et al. (1978) observed- adult male and female rats showed reduced body weight, blood glucose, serum total cholesterol and serum alkaline phosphatase in response to ginger, demonstrating the hypocholesterolaemic effects of ginger.

Male and female rats have different responses to fluopyram due to hormonal differences, particularly the influence of sex hormones on oxidative stress and antioxidant enzymes. Estrogen showed neuroprotective effects and may help maintain antioxidant enzyme activity in females, while males may have a more pronounced reduction in SOD, CAT and GPx activity when exposed to fluopyram. The decrease in SOD, CAT and GPx activity was more in males as compared to females in plasma and brain of rats. However no significant difference was observed in between both the sexes.Due to factors like a high oxidative metabolic rate, a high ratio of membrane surface area to cytoplasmic volume, high levels of unsaturated lipids (mostly polyunsaturated fatty acids or PUFAs), high iron levels, increased free radical generation from dopamine metabolism and ineffective scavenging mechanisms, the central nervous system and specifically the spinal nerve, is particularly vulnerable to damage from free radicals (Abuja and Albertini, 2001).

The brain makes up only 2% of the body weight, but it has a high oxygen demand, around 20% of it. Higher concentrations of ROS are generated in the brain and glutathione-mediated detoxification is crucial (Abdollahi et al., 2004; Franco et al., 2010). The working of SOD and CAT

**Table 4.** Effect of fluopyram on brain antioxidant parameters of male rats

| Group | Treatment | Antioxidantparameters | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Superoxide dismutase (SOD) | Catalase (CAT) | Glutathione-S-transferase (GST) | Glutathione peroxidase (GPx) | Glutathione (GSH) | Glutathione reductase (GR) | Lipid peroxidation (LPO) |
| I | Control | $8.69^{c}$ | $12.98^{d}$ | $0.49^{c}$ | $0.69^{a}$ | $48.73^{c}$ | $0.058^{c}$ | $0.46^{a}$ |
| II | 0.5mg/kgbw/day | $7.07^{c}$ | $10.02^{c}$ | $0.43^{b}$ | $0.68^{a}$ | $41.32^{a}$ | $0.061^{c}$ | $0.52^{a}$ |
| III | 2.5mg/kgbw/day | $5.88^{a}$ | 9.82 | $0.41^{a}$ | $0.67^{a}$ | $40.86^{b}$ | $0.049^{b}$ | $0.75^{b}$ |
| IV | Ginger + 2.5 mg/kgbw/day | $8.79^{b}$ | $11.48^{d}$ | $0.48^{c}$ | $0.69^{a}$ | $46.77^{c}$ | $0.05^{b}$ | $0.47^{a}$ |

***Values expressed as mean±SE***

***$^{abcd}$ represents significant difference between treatments at p≤0.05 as compared to control.***

***Units: SOD (U/mg protein), CAT (µmole of $H_2O_2$ decomposed/min/mg protein), GPx(U/mg protein), GST (µmoles of GSH-CDNB conjugate formed/min/mg protein), GR (µmoles of NADPH oxidized/min/mg protein), Lipid peroxidation (nmol MDA/100 mg tissue)***

**Table 5.** Effect of fluopyram on brain antioxidant parameters of female rats

| Group | Treatment | Antioxidantparameters | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Superoxide dismutase (SOD) | Catalase (CAT) | Glutathione-S-transferase (GST) | Glutathione peroxidase (GPx) | Glutathione (GSH) | Glutathione reductase (GR) | Lipid peroxidation (LPO) |
| I | Control | $10.86^{d}$ | $11.70^{c}$ | $0.49^{d}$ | $0.71^{a}$ | $48.59^{d}$ | $0.06^{b}$ | $0.39^{a}$ |
| II | 0.5mg/kgbw/day | $8.69^{a}$ | $8.52^{b}$ | $0.43^{c}$ | $0.69^{a}$ | $41.32^{b}$ | $0.049^{b}$ | $0.75^{b}$ |
| III | 2.5mg/kgbw/day | $6.07^{a}$ | $8.79^{b}$ | $0.41^{a}$ | $0.67^{a}$ | $40.77^{b}$ | $0.03^{a}$ | $0.93^{d}$ |
| IV | Ginger+2.5 mg/kgbw/day | $9.82^{d}$ | $11.48^{c}$ | $0.48^{d}$ | $0.70^{a}$ | $46.77^{d}$ | $0.06^{b}$ | $0.38^{a}$ |

***Values expressed as mean±SE***

***$^{abcd}$ represents significant difference between treatments at p≤0.05 as compared to control.***

***Units: SOD (U/mg protein), CAT (µmole of $H_2O_2$ decomposed/min/mg protein), GPx(U/mg protein), GST (µmoles of GSH-CDNB conjugate formed/min/mg protein), GR (µmoles of NADPH oxidized/min/mg protein), Lipid peroxidation (nmol MDA/100 mg tissue)***

is interrelated as superoxide molecule which is dismuted to hydrogen peroxide by the enzyme SOD followed by removal of hydrogen peroxide molecule by GSH-Px and CAT (found in peroxisome). The functions of GPx are performed in cytosol which limits its role in comparison to other antioxidant enzymes. Superoxide dismutases (SOD) helps in protecting the cells from molecular oxygen and also removes superoxide radicals and decreased SOD level in liver may lead to free radical damage at large scale. Decreased level of SOD will decrease the production of hydrogen peroxide and it also reduces the protection against free radicals. Decrease in the concentration of CAT will decrease the $H_2O_2$ concentration which in turn will decrease the free radicals. Glutathione peroxidase is known for the reduction of various hydro peroxides to $H_2O$ through oxidation of reduced GSH into glutathione disulphide (GSSH). Keshav et al. (2024) reported the neurotoxic effect of dichlorvos in male and female Wistar rats and attenuation of toxicity was observed with ginger extract. Post treatment with ginger resulted in increased levels of SOD, CAT, GSH, GPx and GR and declined levels of LPO were reported. Increased lipid peroxidation seems to be indicator of several disorders in cells, tissues or organs and it might be involved in different diseased state of cell such as aging, nervous disorders etc. (Meng et al., 2002 a b). Sahoo et al. (2000) also reported neurotoxicity in rats following lindane exposure. The work by Dwivedi et al. (2010), showed a decrease in GSH level in the brain following exposure to monocrotophos and dichlorvos, is consistent with the lower GSH level seen in experimental animals.

Histologically, brain tissue section of control rats and fluopyram treated rats (0.5 and 2.5 mg/kgbw/day) showed mononuclear cell infiltration, pyknotic neurons, degenerated neurons(DN), increased perinuclear space,

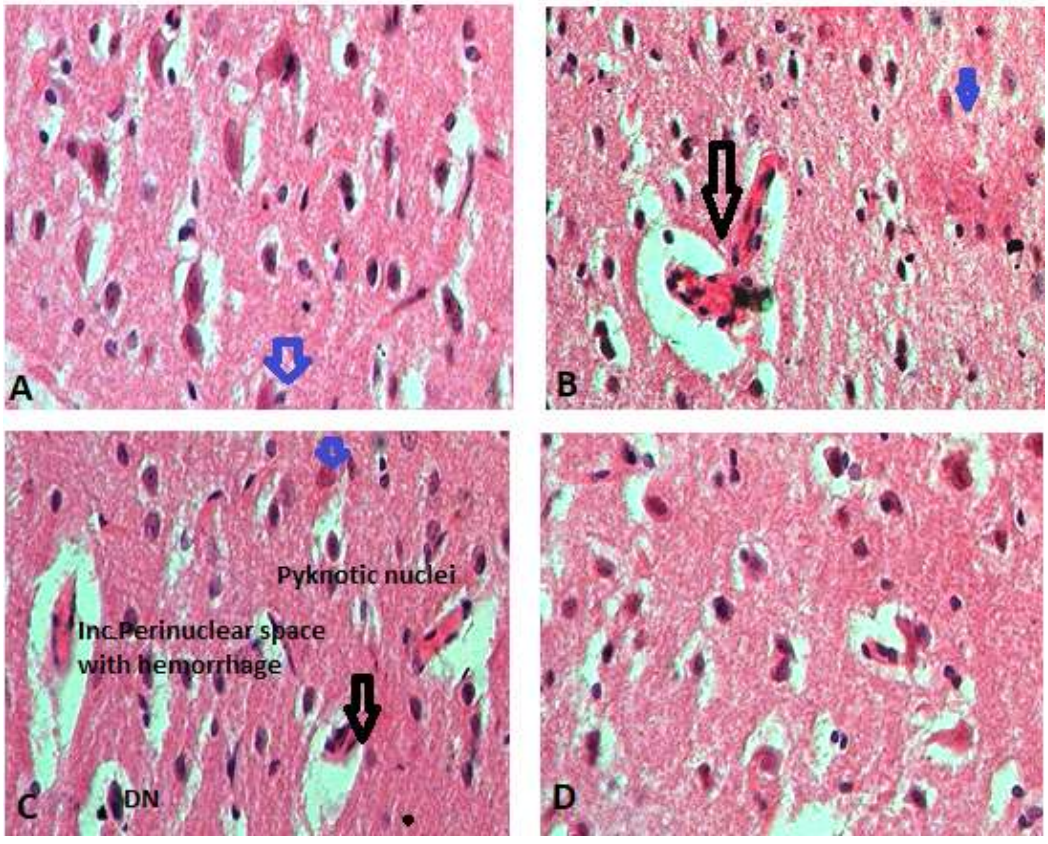


**Figure 1.** Brain Tissue Section from Control rats showed normal nerve or glial cells(A) In 0.5 mg/kgbw/day treated group brain showed mononuclear cell infiltration (black arrows), hemorrhage (blue arrows) (Fig 1 B) 2.5 mg/kgbw/day treated group showed pyknotic neurons, degenerated neurons(DN), increased perinuclear space and hemorrhage(blue arrows) (Fig 1 C) tissue section from rats treated with ginger extract and 2.5 mg/kgbw/day showed restored histology almost similar as that of control group observed by light microscopy with H&E (X400 magnification)

hemorrhage and tissue section from rats treated with 2.5 mg/kgbw/day and ginger extract showed restored histology almost similar as that of control group observed by light microscopy (Figure 1).

## 4. CONCLUSION

Decreased antioxidant enzymes influence the brain to increase free radical damage which in turn affects the defense system of body. The widespread application of fluopyram heightens the probability of environmental dispersal, resulting in the pollution of soil, water, and food products. Consequently, non-target organisms, such as animals, aquatic species, beneficial insects and soil microbes may experience direct or indirect exposure. It can be concluded from this study that ginger (post treatment) ameliorates the toxicity caused by fluopyram in brain of rats of both the sexes. This protection was provided by ginger as it improves the antioxidant defense system of rats. Therefore, daily dietary ginger intake can help mitigate the pesticide-induced toxicity. Further work is needed to understand the toxicological role of fluopyram on multiple organs of mammals.

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**Authors' Contributions**

PS planned the conceptualization and methodology of study. PS, NV and NK help in data curation, analysis and writing of manuscript. All the authors read and approved the manuscript.

**Declaration of Generative AI and AI-assisted technologies in the writing process**

The authors declare that generative AI and AI-assisted technologies were not used in the writing or preparation of this manuscript.

**Conflicts of Interest**

All authors declare there is no conflict of interest.

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