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AMELIORATIVE POTENTIAL OF GINGER FOR MITIGATING LIVER DAMAGE CAUSED BY FLUOPYRAM IN MALE ALBINO RATS

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ABSTRACT

Fluopyram is a widely used new generation broad spectrum fungicide and a variety of biochemical and histopathological alterations in the livers of albino rats. These include inflammatory cells, dilated sinusoids, haemorrhage, dilation of central veins, congestion and presence of erythrocytes. Significant increase in blood alanine aminotransferase (ALT), aspartate amino transferase (AST), acid phosphatise (ACP) and decrease in alkaline phosphatise (AKP) enzyme levels were seen in high dose of fluopyram treated rats as compared to control. The oxidative stress and antioxidant enzymes showed a significant reduction in superoxide dismutase (SOD), catalase (CAT) and increase in malondialdehyde (MDA) level. Rats treated with fluopyram and ginger showed improved histopathological changes in liver. Ginger extract also showed decreased serum levels of malondialdehyde and raised serum levels of antioxidant enzymes. According to the current study, strong antioxidant activity of ginger mediates its protective effect against fluopyram induced liver damage.

Key words: Fluopyram, albino rats, histopathology, dose, ginger extract, liver, oxidative stress, histopathology, protein, liver marker enzymes, haemorrhage

Pesticides are recognised as hazardous materials because of their acute and long-term toxicity, but their usage is practically inevitable (Wei et al*.,* 2016). A broad-spectrum fungicide called fluopyram (FL) (N-(2-[3-chloro-5-(trifluoromethyl)-2-pyridinyl] ethyl)-2-(trifluoromethyl) benzamide) produced by Bayer Crop Science to fight fungal diseases in more than 70 crops including fruits (Li et al*.,* 2020; Tzatzarakis et al*.,* 2020). Excessive use of fluopyram might cause instability in the associated agroecosystem, thereby affecting the non-targetted organisms. Acute fluopyram exposure results in non-specific, temporary functional effects on the neurological system as well as thyroid tumours in mice and liver tumours in rats (Dong and Hu, 2016). At lower dose weight of liver was found to be increased and hepatocellular hypertrophy was also observed in rats. Fluopyram's is commonly sprayed at fruiting stage of vegetables (chilli, tomato, cucumber) and fruits (banana, grape and citrus) and waiting period of 28 days is suggested for safe consumption of vegetables applied with this fungicide as the initial deposits of fluopyram were 5.59 and 3.54 mg/ kg at 0th and 3rd day at recommended dose but in later days residues will reach lower level. So hence it is essential to evaluate its toxic effect on non-target species. Also wide range of natural botanical like ginger (*Zingiber officinale*) is a valuable anti-platelet, antioxidant, anti-tumor, anti-retroviral, anti-hepatotoxic and anti-arthritic (Kamtchoving et al., 2002). When the rats were exposed to ginger, it extract reduced body weight, blood glucose, serum total cholesterol, and serum alkaline phosphatase and also had hypocholesterolaemic effects in male rats (Gujral et al., 1978). So, this study will illustrate the potential risk of fluopyram as a funguicide and also in combination with ginger extract in humans intake through consumption of vegetables and fruits based on its mechanisms of toxicity in albino rats for treated a period 28 days.

MATERIALS AND METHODS

Fluopyram (purity: 94.7% w/ w) was purchased from the local market of Ludhiana, Punjab, India. This study used 24 mature male albino rats weighing 130 to 170 g, which were randomly divided into four groups and were given water and food ad libitum and were acclimatized for two weeks. Group I served as control, Group II was treated orally with 0.5 mg/ kgbw/ day dose (low dose) of fluopyram, Group III was treated with 2.5 mg/ kgbw/ day dose (high dose) and Group IV was treated with 100 mg/ kg aqueous extract of ginger and 2.5 mg/ kgbw/ day fluopyram. The rhizomes of *Z. officinale* were shade dried at room temperature and were crushed to powder. 125 g of the powder was macerated in 1000 ml of distilled water for 12 h. at room temperature and were then filtered to obtain the final aqueous extract. The concentration of the extract is 24 mg/ ml equal to 100 mg/ kg. In this study each

animal was orally given 1 ml of the final aqueous extract (Kamtchoving et al., 2002). Doses 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).

The liver was removed and weighed after dissecting the animals under experiment. The tissues were sheared in 0.9% saline (chilled) and homogenized in 0.1 M phosphate buffer (pH 7.4). The homogenates were centrifuged for 30 min at 1000 rpm at 4^0C to obtain supernatants which were then used for the assay of different antioxidant enzymes like superoxide dismutase (SOD), glutathione-S-transferase (GST), catalase (CAT), glutathione (GSH), glutathione peroxidase (GPx), glutathione reductase (GR) and lipid peroxidation (LPO) following the standard methodology given for estimation of these enzymes given by Marklund and Marklund, 1974; Habig et al., 1974; Hafeman et al., 1984, Carlberg and Mannervik, 1985; Aebi, 1983; Stocks and Dormandy, 1971; and Jollow et al., 1974, and liver marker enzymes by colorimetric method of Reitman and Frankel as described by Bergmeyer (1974). 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 was done using Statistical package for Social Sciences (SPSS). One way analysis of Variance (ANOVA) with post host Tukey's t-test was used for making comparisons between control and treated group of rats. A value of $P<0.05$ indicated significant differences.

RESULTS AND DISCUSSION

Fluopyram is a broad-spectrum fungicide that inhibits the activity of succinate dehydrogenase enzyme. The liver was found to be the primary target organ during the general toxicity evaluation of fluopyram in all rodent trials, regardless of the species, sex and mode of administration and the duration of the investigation. Though there was no mortality in any of the treated groups, minor clinical symptoms like lethargy and change in daily activity as compared to control rats was observed. Fluopyram treatment resulted in slight decrease in body weight and increase in the relative weights of the kidney and liver particularly at high dose (2.5 mg/ kgbw/ day) as compared to control rats as shown in Table 1. Oxidative stress may be the reason for the reduction in body weight of treated rats. Increased hepatocellular proliferation, increased liver weight and lobular hypertrophy were among the additional liver alterations seen after both short-term and subchronic/ chronic exposure to fluopyram in albino rats (Tinwell et al*.,* 2014). Heise et al. (2018) also reported alterations in liver weight of albino rats when treated with three azole fungicides to study its hepatotoxicity.

Table 1 demonstrates that rats treated with high dose of fluopyram (2.5 mg/ kgbw/ day) had significantly lower levels of superoxide dismutase (SOD), glutathione transferase (GST) and glutathione peroxidase (GPx) as compared to control rats while low dose of fluopyram (0.5 mg/ kgbw/ day) showed insignificant lower levels of superoxide dismutase (SOD), glutathione transferase (GST) and glutathione peroxidase (GPx) as compared to controls. However, animals treated with fluopyram plus ginger had significantly higher serum levels of SOD during the whole treatment period. There was decrease in level of catalase (CAT) and glutathione reductase (GR) at high and low dose level and the decrease was significant at high dose as compared to low dose level. When comparing the serum levels of malondialdehyde (MDA) in rats treated with fluopyram to the control group, all treatment durations showed a substantial increase ($p<0.05$). On the other hand, rats given fluopyram and ginger had lower serum MDA levels comparable to that of control.

Compared to physiological indications, biochemical markers are more sensitive to changes (Aquilano et al., 2014). Sharma et al. (2023) also found significant reduction in antioxidant enzymes after oral administration of dimethoate and fluoride to Wistar rats. Aprioku et al. (2023) also reported reduced activities of SOD, catalase, glutathione peroxidase and reduced glutathione after administration of mancozeb to mature wistar rats for 10 days. Measurements of reactive oxygen species (ROS) generation were used to assess the oxidative stress in order to ascertain the mechanisms underlying fluopyram toxicity in albino rats. Numerous processes can produce ROS and when ROS levels are not maintained at the proper levels, oxidative stress and cellular damage occurs (Goswamy and Irazoqui, 2021). Lipid peroxidation (LPO) is linked to the pathophysiology of certain liver and kidney ailments and is known to disrupt the integrity of cellular membranes. As a result, it has been proposed as one of the molecular pathways underlying pesticide-induced toxicity and utilised as a biomarker of oxidative stress caused by pesticides (El-Sayed et al., 2018).

As shown in Table 1, rats treated with 0.5 mg/ kg of fluopyram showed an insignificant increase in aspartate

Table 1. Effect of fluopyram on male albino rats

Liver weight (g/ 100g b.w.) and body weight				
Initial weight	118.33± 7.26	111.66± 9.27	105.66± 3.48	109.33± 1.76
Final weight	110.33± 6.38	106.66± 9.27	100.00± 3.78	102.33± 1.45
Liver weight	0.018± 0.0005 [a]	0.016± 0.001[a]	0.011± 0.001[a]	0.017± 0.0005[a]
Antioxidant parameters of liver				
Enzymes	Control rats	Fluopyram (mg/ kgbw/ day)		
		0.5 mg	2.5 mg	2.5 mg+Ginger
Superoxide dismutase (μ/ mg)	12.57± 0.15[c]	13.26± 0.15[c]	7.20± 0.47[a]	11.68± 0.05[a]
Catalase (μ mole)	11.46± 0.03[d]	12.90± 0.03[d]	9.81± 0.38[c]	10.86± 0.02[b]
Glutathione-S-transferase (μ/ mg)	0.43± 0.006[b]	0.49± 0.001[c]	0.53± 0.012[b]	0.41± 0.002[a]
Glutathione peroxidise (μ moles)	0.91± 0.008[b]	0.93± 0.01[b]	0.96± 0.01[b]	0.91± 0.008[b]
Glutathione reductase (μ moles)	0.06± 0.05[a]	0.05± 0.0009[b]	0.03± 0.003[a]	0.05± 0.0009[a]
Lipid peroxidation (μ mol/ 100 mg tissue)	0.30± 0.01[a]	0.39± 0.01[a]	0.83± 0.01[b]	0.46± 0.02[a]
On liver marker enzymes of male rats				
ALT	133.2±5.1[c]	135.2± 0.15[c]	140.2± 0.47[a]	134.6± 0.05[a]
AST	59.6± 0.03[d]	61.5± 0.03[d]	65.7± 0.38[c]	60.6± 0.02[b]
ALP	0.43± 0.006[b]	0.49± 0.001[c]	0.43± 0.012[b]	0.41± 0.002[a]
AKP	0.91± 0.008[b]	0.93± 0.01[b]	0.86± 0.01[b]	0.91± 0.008[b]

Values expressed as mean ± SE; [abcd]represents significant difference between treatments at p≤0.05 as compared to control.

aminotransferase (AST) and alanine aminotransferase (ALT), acid phosphatase (ACP) and decrease in alkaline phosphatase (AKP) in their plasma, but in 2.5 mg/ kg dose treated rats, there was a substantial significant increase (P<0.05) in AST, ALT, ACP and decrease in AKP enzyme. However, as compared to the control group, the animals given fluopyram and ginger showed a negligible rise in these parameters. The levels of ALT, AST, AKP and ACP in the sera of the ginger-treated animals and the control group do not differ significantly. Liver biomarkers like AST, ALT and ALP are mainly used to evaluate the damage in liver caused by various pesticides. Transaminases (AST and ALT) play an important role in amino acids catabolism and biosynthesis. They are responsible for the metabolism, biosynthesis and detoxification processes of energetic macromolecules for various vital functions and they are employed as particular markers for liver damage (Rjeibia et al., 2016). The elevation in these enzymes may be related to liver dysfunction and disturbance in the production of these enzymes with modification in the permeability of liver takes place (Nahas et al., 2018). The liver structure of the control animals, which were fed water or ginger orally, was normal. Examining liver sections taken from rats given low dose of fluopyram treatment for 30 days revealed that the typical cord-like arrangement of the normal liver cells had disappeared, and the hepatic lobules' usual structural organization had been compromised. There was congestion in the portal and central veins (Fig. 1). Liver sections of rats taken from high dose treated fluopyram showed that many hepatic cells were damaged and lost their distinctive look, while others displayed extensive cytoplasmic vacuolization in some cells to the point where only a small remnant of the cytoplasmic mass cells were seen, often forming a narrow peripheral rim. Similar histopathological findings were also observed by other scientists while reporting the toxicity of fungicides in albino rats (Heise et al., 2015, Schmidt et al., 2016). High dose of fluopyram (2.5 mg/ kgbw/ day) had detrimental physiological effects on albino rats' growth and liver. These effects were accompanied by an increase in ROS generation, increased liver biomarker and histopathological changes in liver. Present findings illustrates the possible toxic effects of fluopyram to non target animals/ mammals and the environment while also offering crucial information about the processes behind fluopyram's toxicity to albino rats. At a dose of 2.5 mg/ kg, fluopyram was clearly toxic to the liver, as evidenced by decrease in liver weight, as well as an increase in the enzymatic activities and administration of fluopyram and ginger to rats ameliorated the histopathological and enzymatic alterations caused by fluopyram.

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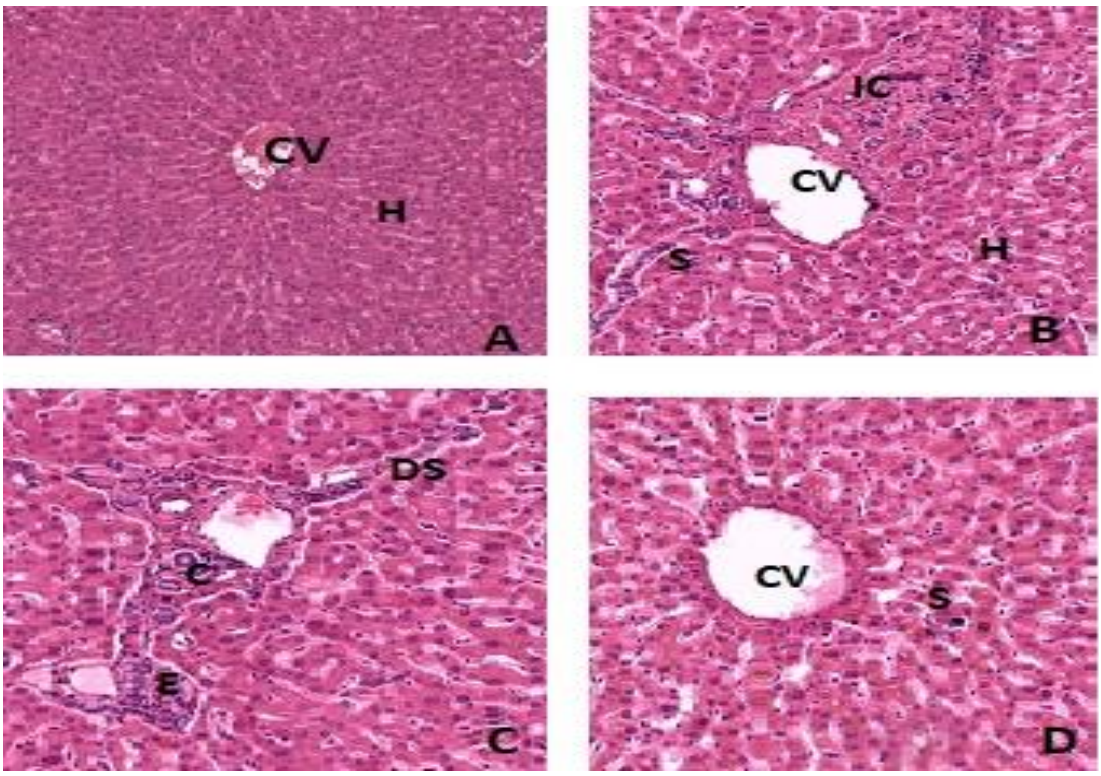

Fig. 1. Liver tissue section of control and fluopyram treated rats Fig. 1 (A) showed normal central vein (CV), hepatic cord (H) and sinusoids(S) whereas 0.5 and 2.5 mg/ kgbw/ day (B, C) treated groups showed inflammatory cells(IC), dilated sinusoids (DS), haemorrhagic spots or haemorrhage on the surface of liver, dilation of central veins, congestion(C) and presence of erythrocytes (E), Section treated (D) with 2.5 mg/ kgbw/ day dose and ginger group showed normalization of hepatic cords, central vein and sinusoids observed by light microscopy with X400 magnification

AUTHOR CONTRIBUTION STATEMENT

PS and NS designed research. PS conducted experiments and wrote the manuscript. NS and PS read and approved the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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