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RESEARCH ARTICLE

Lycopene Attenuates Pyriproxyfen-Mediated Hepatic Dysfunction Through Antioxidant and Anti-Inflammatory Mechanisms in Japanese Quails

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ABSTRACT

Pyriproxyfen (PPF) is pesticide extensively used in agricultural field to control insects. High doses of PPF are associated with reproductive and developmental effects, such as decreased fertility, fetal abnormalities, and delayed puberty. PPF exposure is linked with hepatic and renal dysfunction due to oxidative stress in birds and rats. Lycopene (LYC) is a carotenoid extracted from tomatoes that have potential antioxidant properties. The recent experimental investigation was conducted to estimate the protective role of LYC against PPF-instigated liver damage in quails. Sixteen quails (Coturnix japonica) were categorized into four equal groups. Group-I was control group, Group II was treated with PPF (10 mg/kg B.W), Group III was co-treated with PPF (10 mg/kg B.W) and LYC (2 mg/kg B.W) and Group IV (positive group) was treated with LYC (2 mg/kg). After 30 days all birds were slaughtered. Blood plasma were collected in an EDTA tube. Hepatic damage markers, liver antioxidant enzymes, lipid profile, and histopathological parameters were evaluated. The exposure to PPF significant ($P<0.05$) increased in value of ALT, AST and ALP and activity of superoxide dismutase (SOD) and catalase (CAT) in PPF-treated group than control group. Moreover, the level of LDL, VLDL, cholesterol and triglyceride were significantly ($P<0.05$) increased than control group while the level of HDL significantly ($P<0.05$) decreased than control group. The body weight gained in PPF-treated group drop significantly ($P<0.05$) than control group. Furthermore, histopathological analysis showed that PPF considerably damaged the liver tissues. The current study revealed that PPF also induced behavioral abnormalities and non-significant difference in liver weight in quails. LYC reversed all the effects caused by PPF. The results of this study suggested that LYC can potentially alleviate PPF-induced liver damages due to its antioxidant and pro-inflammatory factor inhibitor as well as anti-apoptotic nature.

Key words: Pyriproxyfen (PPF), Antioxidant, Anti-Inflammatory.

INTRODUCTION

Various environmental pollutants have detrimental impacts on ecosystems such as alterations in biodiversity, disrupting the food chain, destroying habitats and disrupting the physiological system that leads to impaired reproductive efficiency (Wang et al., 2022). Chemical substances known as pesticides can either eliminate, stunt or inhibit the growth of any organisms that harms a crop. Pest management has allowed farmers to increase agricultural output which has significantly helped the world's expanding population during the past century (Carvalho, 2017). Brazil is one of leading agricultural manufacturer. So, it uses about 20 percent pesticides products being use worldwide (Lopes-Ferreira et al., 2022). However, extensive uses have a number of negative consequences (De Moraes et al., 2019).

Pyriproxyfen (PPF) (2- [1-methyl-2-(4-phenoxyphenoxy) ethoxy] pyridine) is used to control a variety of insects in residential, agricultural and

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horticultural areas including invasive red fire ants, mosquitoes, aphids, jassids, silver leaf, cutworms, bollworm, whiteflies and common houseflies throughout the world. PPF acts as a juvenile hormone when administered, interacting with receptors, interfering with the insect's morphogenesis and inhibiting insect development and transformation into adults. PPF is evidenced to disrupt the endocrine system (Liu et al., 2020; Maharajan et al., 2018). PPF is toxic to animals such as rat with different dose regime such as more than 1000 mg/kg through respiration, more than 2000 mg/kg for epidermis and more than 5000 mg/kg for oral intake (Truong et al., 2016; Lucas et al., 2008).

It has the characteristics of lower solubility, more hydrophobicity, and partition coefficient (Truong 2016). It also has long persistence nature (more than six month) in their habitats (Mmbaga and Lwetoijera, 2023). It is stored in fat over the long term. WHO has set a daily tolerable PPF intake of hundred mg/kg of body weight. Their suggested concentration for tap water is 0.01 mg/l (Truong et al., 2016). Previously, it is non-genotoxic, have less carcinogenicity, less toxic to mammals and surrounding environment. Due to high safety impact and selective mode of action, it is widely recommended against insect control (De Oliveira et al., 2019; Mascari and Foil, 2010). However, effective dose use for mosquito control causes hazardous impact on environmentally friendly pest such as honey bees as well as small fish. Gradually, it accumulates in food chain due to stability and persistence nature and causes harmful impact on non-target organism (Lawler et al., 2017; Truong et al., 2016; Mehrnoush et al., 2013).

Report submitted by WHO indicated that PPF increased the weight and size of the liver, altered plasma lipid contents in rats, triggered the enzymes in the liver of dogs as well as disturbs the level of cholesterol. It occurs when application of PPF increase the tolerable concentration and PPF administered at higher doses (5000 mg/kg of body weight) (Organization 2008). The use of PPF is common in India and Pakistan for the control of Anopheles stephensi and Culex quinquefasciatus vectors (Maharajan et al., 2018). The presence of PPF residues in soil may result in contamination of food and can accumulate in a wide variety of animal organs, posing serious health risks (mutagenic effects) (Muñiz et al., 2017).

Additionally, PPF affect the reproductive system of honeybees (Chen et al., 2016), developmental neurotoxicity, reproduction toxicity in Daphnia magna, oxidative stress and DNA damage in Labeo rohita fish, disrupt the acetylcholinesterase activities in Hoplosternum littorale brain, mitochondrial dysfunction resulted apoptosis and teratogenic effect (Hafez and Mobarak, 2024).

Carotenoids are a group of tetraterpenoids which are the most common natural lipid-soluble pigments range from colourless to orange, yellow and red besides having a powerful antioxidant (Liang et al., 2023; Maoka et al., 2020). Lycopene (LYC) ($C_{40}H_{56}$) is naturally occurring lipid soluble pigment abundantly found in red vegetables and fruits such as tomatoes, watermelons, apricots, pink grapefruits, carrots, cranberries, papayas, pink guavas, and peaches (Khan et al., 2021; Carvalho et al., 2021). LYC are mostly accumulated in hepatic system (1.28–25.46 nmol/g) and fatty tissues (0.20–0.70 nmol/g) than another organ (Bohn et al., 2017). So, it is the most potent antioxidant in the carotenoid family. Oxidative damage occurs in cells and tissues can be reduced in vivo and in vitro due to its action against biologically reactive oxygen species (Boeira et al., 2014). Moreover, it also has anti-inflammatory, cardioprotective, anti-cancer properties, regulating body metabolism, immune system regulation and so on (Li et al., 2021; Khongthaw et al., 2022). Therefore, accordant with abovementioned pharmaceutical properties of LYC, the recent experimental investigation was conducted to estimate the palliative role of LYC against PPF-instigated liver damage in quails.

MATERIALS AND METHODS

Chemical Used

PPF (CAS No: 95737–68–1, Molecular Weight: 321.37) and LYC (CAS No: 502–65–8, Molecular Weight: 536.87) were purchased from Sigma Aldrich (Germany).

Animal

For this experiment, sixteen adult common quails (Coturnix japonica) having weight 120±20 g were employed. Scientific analysis of quails was carried out in the University of Agriculture Faisalabad (UAF) Animal House for thirty days. The quails were allowed to acclimate for 7 days prior to the experiment. Quails were maintained in rustless steel cages at a temperature of 20-22oC. The conventional food chaw and tap water were supplied to the quails with twelve-hour light and dark cycles. For the duration of the experiment, all of the rules about how to treat animals were followed in accordance with instructions issued by European Union of Animal Care and Experimentation (CEE Council 86/609) which were also authorized by the UAF's ethical committee.

Experimental Design

Sixteen quail used in this experiment were purchased from the local bird market, Jhang bazaar, Faisalabad. The birds were separated into four equal groups (n=4/groups). Group-I was titled as control group. Group-II was titled treatment group acquired oral treatment of PPF (10 mg/kg B.W). Group-III as titled co-treatment group acquired oral treatment of PPF (10 mg/kg B.W) and LYC (2 mg/kg B.W). Group-IV was titled curative group acquired oral treatment of LYC (2 mg/kg B.W). The birds were sacrificed after 30 days of the experiment. Heparinized syringes were used to collect blood samples from the livers of all birds. Samples of blood plasma were obtained after centrifugation of blood for fifteen minutes at normal temperature.

Ethylenediaminetetraacetic acid (EDTA) tubes were used to collect and store blood plasma at -20°C for biochemical analysis. The livers were taken out and washed with ice-cold saline. The livers were cut in two equal parts. One sample of liver was used for biochemical analysis which was stored in –80°C temperature while other sample was used histopathological analysis after dipping and fixing in 10% formalin solution.

Biochemical Analysis of Hepatic Damage Markers

The level of hepatic function enzymes such as aspartate transaminase (AST), Alanine aminotransferase (ALT) and alkaline phosphatase (ALP) was analyzed by employing German commercial kits.

2.5. Evaluation of Lipid Profile. The cholesterol level was determined using the protocol of Allain et al., 1974). The low-density lipoproteins (LDL) and very- low density lipoproteins (VLDL) were ascertained following the procedure (Friedewald, 1972 and Warnick et al., 1990). respectively. The high-density lipoproteins (HDL) were assessed using the procedure of Albers et al., (1978).

Evaluation of Antioxidant Enzymes

The protocol of Kakkar et al, (1984) was employed to ascertain the Superoxide dismutase (SOD) activity. Catalase (CAT) activity was calculated with the help of protocol proposed by (Chance and Maehly, 1955).

Histopathological Analysis

Hepatic tissue histology was employed to ascertain the level of PPF noxiousness and the beneficial effects of LYC. Just after hepatic tissues had been removed, it was gently washed with cold saline solution and fixed using serum. After fixation process, dehydration was carried out at a constant temperature in the presence of increasing alcohol levels (80%, 90%, and 100%). Then it was fixed in paraffin wax blocks. A microtome was used to chop thin slices of paraffin-embedded tissues fixed on wooden planks at a size of 5 um (Thermo, Shandon finesse 325, UK) and stained with hematoxylin and eosin stain. Finally, slides were examined under a Light microscope (Nikon, 187842, Japan). German Leica LB microscope fitted with a Cannon digital camera was employed for microphotography (Japan). In the last step, slides were viewed under Leica microscope at 400X.

Statistical Analysis

After careful testation and investigation, all the derived findings were indicated as Mean±SEM and it was further analyzed by employing a basic statistical technique known as one-way analysis of variance (ANOVA) with Tukey's test. Comparing the experimental group with control group, graph pad prism 8 software was used. P<0.05 was kept as a significance level.

RESULTS AND DISCUSSION

Effect of PPF and LYC on Body Weight Gain

At the start of the experiment, the body weights of all birds were comparable. The body weight gain of the PPF-treated quails was considerably (P < 0.05) declined than control quails. However, no case of morbidity or mortality was observed in the treated quails. The body weight gains of co-treated (PPF + LYC) and positive group (LYC) were escalated remarkably than control group (Table 1). The obtained results are supported by the results given by Farag et al., (2023) and Allam et al., (2022). These findings were also similar to Shahid and Saher, 2020 who found that prenatal exposure of PPF revealed a notable (P<0.05, P<0.001) drop in body weight gains in treated pups than control pups. Fluctuations in the body weight of animals might indicate internal damage of body tissues caused by pesticides. Another possible reason for reduction in body weight gain in pesticide treatments might be the decreased muscle mass and cachexia due to oxidative stress induced by pesticides. No Literature cited on PPF and LYC plant extract regarding its combine effect on body weight. However, results of this study showed that LYC notably elevated the body weight gain of quails compare with PPF-treated group which is due to curative role of LYC.

Effect of PPF and LYC on Liver Weight

A non-substantial (P>0.05) escalate in liver weight was observed in PPF-treated groups than control quails. Furthermore, non-substantial (P>0.05) expansion in the liver weight was also evaluated in co-treated quails as compared to the PPF-treated quails. Organ weight of LYC positive group was very close to the control group (Table 1 & Figure 1). The result of Farag et al., (2023) was different who demonstrated no change occur in liver after pesticide exposure in chickens. Anyhow, these findings were similar to Shahid and Saher who found that relative liver weight of prenatally exposed pups in relation to their body weights were noteworthy (P<0.001) elevate in PPF exposed group than control group (Shahid and Saher, 2020). The non-substantial (p<0.05) rise in liver weight and non-substantial (P>0.05) fall in liver weight in experimental groups could be due to the binary mixture and anti-inflammatory effect of LYC in this current research. No Literature cited on PPF and LYC plant extract regarding its combine effect on liver weight. Results of this study showed the ameliorative effect of LYC against liver weight.

Table 1: Effect of PPF and LYC on Body Weight Gains and Liver Weight

Parameters	Control	PPF	PPF + LYC	LYC
Body weight gains (g)	25.3 ± 6.11^{a}	19.5 ± 2.73^{b}	46.25 ± 1.94^{c}	46.5 ± 1.27^{c}
Liver Weight (g)	3.5275 ± 0.394^{a}	4.0025 ± 0.633^{a}	4.1925 ± 0.943^{a}	3.715 ± 0.782^{a}

Results are represented as Mean±SEM. The mean sharing different letters (a - c) are statistically significant at p<0.05 (ANOVA).

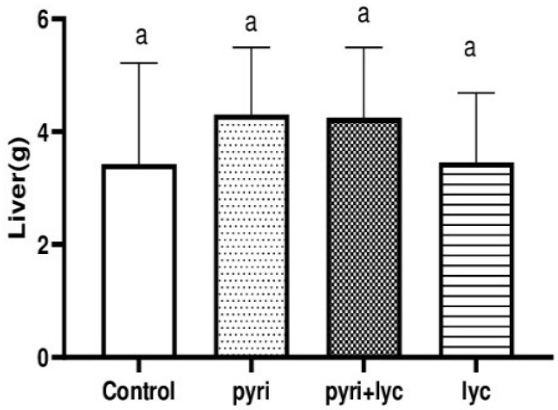

Fig. 1: Effect of PPF and LYC on liver weight in quails. Results are represented as Mean±SEM. The mean not sharing different letters are not statistically significant at P<0.05 (ANOVA).

Effect of PPF and LYC on Hepatic Enzymes in Liver of Quails

A noteworthy (P<0.05) rise in enzymatic activity of ALP, ALT and AST were seen in PPF-treated group than control group. Nevertheless, PPF + LYC co-treatment showed substantial decline in ALP, ALT and AST levels in contrast to PPF-treated quails. Furthermore, in LYC positive group, these levels were decline than PPF-treated quails and control quails (Table 2; Figure 2). Results of this research is aligned with the finding of Osman et al., (2018) and Sharma et al., (2023) in which ALT, AST and ALP were increase in birds. Detoxification of xenobiotics and metabolism occur in liver. The elevation of hepatic serum biomarker is mainly due to hepatocytes injury caused by PPF. This injury disturbs the morphology and function of liver such as anabolism of macromolecules. The liver damage also disturbs the detoxification process of xenobiotics. The liver dysfunction alters the permeability of the cell membrane, which causes the leakage of these liver transaminases into the bloodstream and caused hepatocyte necrosis (Badraoui et al., 2020; Wang et al., 2019; Ali et al., 2016). However, current research revealed that LYC restored these levels which may be the attenuative property of LYC.

Effect of PPF and LYC on Lipid Profile in Liver of Quails

3.4.1. Effect of PPF and LYC on Cholesterol and Triglyceride Levels in Liver of Quails. PPF intoxication prompted a noteworthy (P<0.05) escalation in cholesterol and triglyceride content than control animals. However, PPF + LYC co-treatment prompted a substantial decline in cholesterol and triglyceride level than PPF-treated group. Moreover, LYC-only treatment further decreases these levels than PPF-treated quails (Table 2). The results of Saoudi et al., (2023) give the similar result who explained the elevated level of cholesterol and triglyceride in rats after chlorpyrifos pesticide exposure. Lipid especially unsaturated fatty acids are vital part of plasma membrane. It is noted that oxidative stress exerts harmful effect on cholesterol and triglyceride due to high sensitivity. The reason for this increase is PPF treatment which causes hepatic injury. This injury also alters the permeability of plasma membrane (El-Demerdash and Nasr 2014). It also causes hypothyroidism and cholestasis (Öner et al., 2008). The increase in level of serum triglycerides is due to PPF administration which reduced the removal of triglycerides from cell membrane and increase mobilization of triglycerides from liver (Mahmoud et al., 2012). However, present study described that LYC decrease cholesterol and serum triglycerides which is due to protection against PPF hepatotoxicity.

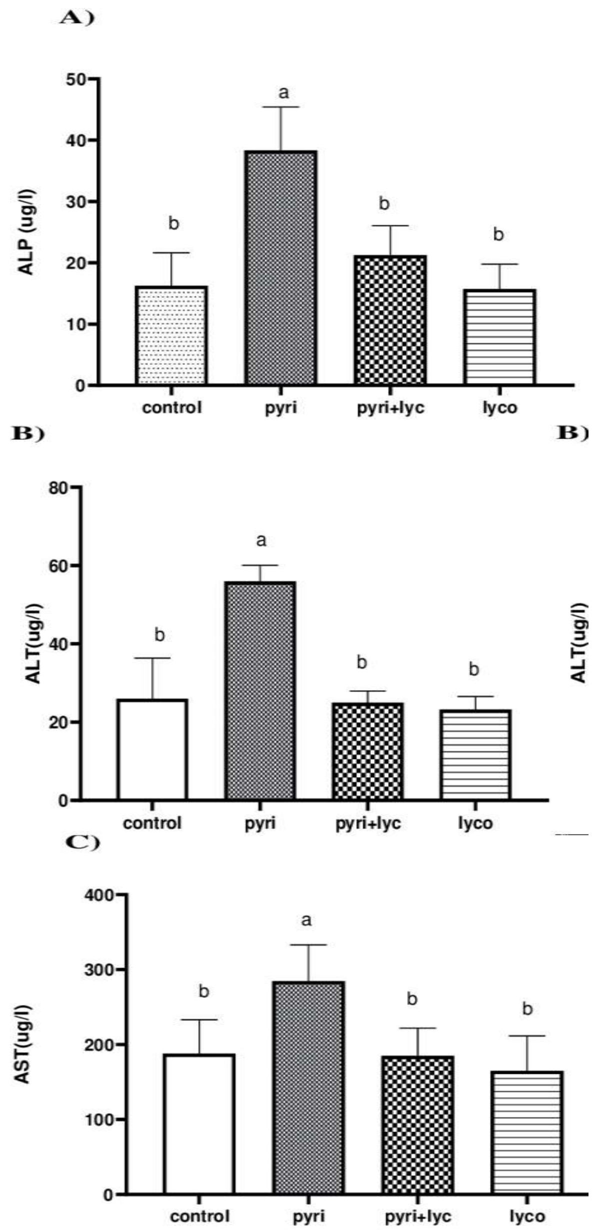

Fig. 2: Effect of PPF and LYC on (A) ALP (B) ALT (C) AST in quails. Results are represented as Mean±SEM. The mean sharing different letters are statistically significant at p < 0.05 (ANOVA).

Table 2: Effects of PPF and LYC on Liver Function Markers

Parameters	Control	PPF	PPF + LYC	LYC
ALP (U/L)	16.25±2.69^{b}	38.35±3.53^{a}	21.25±2.39^{b}	15.75±2.02^{b}
ALT (U/L)	26±5.19^{b}	56±2.04^{a}	25±1.47^{b}	23.25±1.65^{b}
AST (U/L)	188±19.58^{b}	284.75±20.91^{a}	185±6.01^{b}	165±20.16^{b}
HDL (U/L)	112±8.22^{a}	50±5.40^{b}	100±5.49^{a}	101.25±4.27^{a}
LDL (U/L)	53.75±7.76^{b}	96.25±5.54^{a}	48.5±3.62^{b}	46.25±8.01^{b}
VLDL (U/L)	35.25±7.08^{b}	99.75±4.75^{a}	43.5±1.85^{b}	43±6.44^{b}
Cholesterol (mmol/l)	147.5±20.11^{b}	227.5±9.24^{a}	132.5±19.31^{b}	110±9.128^{b}
Triglyceride (mmol/l)	128.75 ±7.46^{b}	192.25±15.34^{a}	115±5.40^{b}	113±6.25^{b}

Effect of PPF and LYC on HDL, LDL and VLDL in Liver of Quails

A substantial ($P<0.05$) drop in the level of HDL and escalation in LDL and VLDL was estimated in PPF-treated group than control group. Nevertheless, in PPF + LYC co-treated group, HDL was notably increased and level of LDL and VLDL was notably decreased compared to the PPF-treated group. However, values of HDL in LYC positive group were more than co-treated quails and less than control quails. But level of LDL and VLDL was notably decreased in LYC positive group than PPF-treated quails (Table 2). The previous study of Mahmoud et al. is consistence with results of this study in which decrease in levels of HDL and induction in LDL and VLDL in quails occur in malathion-treated quails than the control quails (Mahmoud et al., 2012). This elevation in LDL and VLDL could be due to blockage occurred in bile duct of liver which causes drop in its secretion. This occurs due to PPF which increases the hyper adrenal activity. The decrease in HDL might be due to increase in serum enzyme activity occurred by PPF treatment. However, the results of the study showed that LYC decrease the level of LDL, VLDL and increase levels of HDL which is due to pharmaceutical activity of LYC against PPF hepatotoxicity.

Effect of PPF and LYC on Antioxidant Enzymes Activity in Liver of Quails

PPF intoxication prompted a remarkable ($P<0.05$) increase in antioxidant enzymes activity (CAT and SOD) than control quails. Although, PPF + LYC co-treatment prompted a notable decline in antioxidant enzymes activity (CAT and SOD) than PPF treated group. Moreover, in LYC positive group, these antioxidant enzymes activity (CAT and SOD) were near to the control quails (Table 3). The results of the study were corroborated with Maharajan et al, (2018), who demonstrated that enzymes such as CAT and SOD were increases in zebrafish embryos (Danio rerio). But, notable rise in CAT and SOD was seemed only at higher doses of PPF treatment (1.66 µg/mL). Anyhow, given result of Mohammed et al. was inverse in which CAT and SOD was decreased after thiamethoxam pesticide exposure in chickens (Mohammed et al., 2024). Oxidative stress is a complex mechanism against environmental stresses which occur due to imbalance between reactive oxygen species (ROS) and antioxidant molecules. The free radicals production resulted PPF intoxication causes harm to cellular organelles and increased lipid peroxidation levels (Ahmed et al., 2017). On the other hand, antioxidant enzymes SOD and CAT play a crucial role in first line defensive system to keep the cells safe from scavenging activities of free radicals (Blahová et al., 2013). SOD plays a crucial role in catalyzing superoxide radicals to H_2O_2 and O_2. Detoxification of deleterious hydrogen peroxide is further proceeded by CAT enzyme which degraded H_2O_2 and O_2 (Adeyemi et al., 2015). Of note, remarkable escalation in SOD and CAT enzymes might be due to protection of quails by scavenging activities against oxidative stress produced by ROS (Saoudi et al., 2017). The insufficient antioxidant activities could be due to liver damage and death of hepatocytes induced by PPF (Amri et al., 2017). However, Results of this study showed that LYC restored the SOD and CAT activity which is due to pharmaceutical property of LYC.

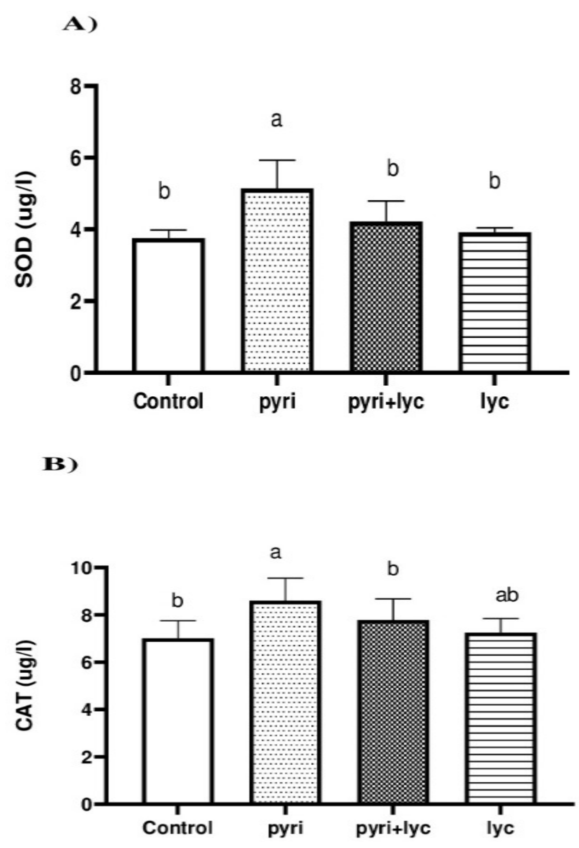

Fig. 3: Effect of PPF and LYC on (A) SOD (B) CAT in quails. Results are represented as Mean±SEM. The mean sharing different letters are statistically significant at $P<0.05$ (ANOVA).

Table 3: Effect of PPF and LYC on Biochemical Parameters

Parameters	Control	PPF	PPF+ LYC	LYC
CAT (U/mg protein)	7.015±0.37^{b}	8.603±0.74^{a}	7.0375±0.38^{b}	7.2525±0.^{29ab}
SOD (U/mg protein)	3.758±0.11^{b}	5.145±0.39^{a}	4.220±0.28^{b}	3.918±0.06^{b}

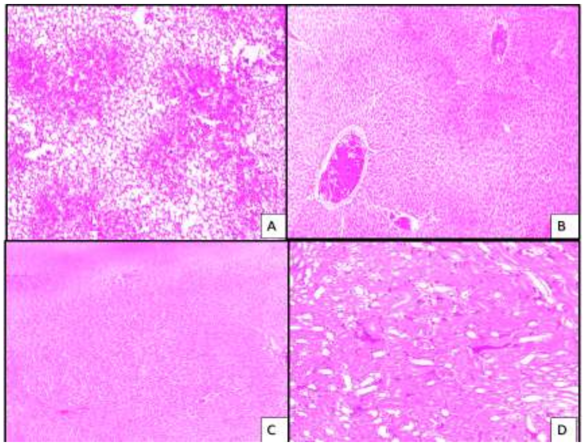

Fig. 4: Photomicrographs of hematoxylin and eosin-stained quail's hepatic sections from different treatments (A) Control with normal histology (B) PPF treatment induced significant damage in liver (C) PPF + LYC treatment represent restored damages (D) LYC treatment showed histology similar to the control animals.

Histopathology of Liver

Photomicrograph of control group show the normal appearance of hepatocytes and endothelial lining of the central vain and sinusoidal spaces (H & E stained 400X). The administration of PPF instigated various hepatic damages such as severe hyperemia, sinusoidal accumulation of erythrocytes and fatty acids, hepatocyte necrosis and vacuolar degeneration of hepatocytes, hydropic degeneration in large parts of the tissue and cell fats were more severe and remnants of the hepatocyte nuclei were present. Infiltration of mononuclear inflammatory cells and central vein degeneration was also evident. However, the administration of PPF + LYC notably reduced aforementioned hepatic damages in quail such as degeneration of fewer hepatocytes and mild infiltration of mononuclear inflammatory fibrosis in portal spaces. The administration of LYC reduced hydropic degeneration, multiloculated mononuclear inflammatory cells and central vein degeneration comparable to the control quails (Table 4; Figure 4). The present findings are in agreement with the results of previous investigations after exposure to different types of pesticides in birds (Mohammed et al., 2024; Farag et al., 2023; Emam et al., 2018). Results of this study are also corroborated by Akter et al., (2020), who recorded alteration in the hepatic tissues in Heteropneustes fossilis fish exposed to the toxicant. These damages could be attributed to oxidative stress and inflammatory responses in the liver tissues which causes harm to cellular organelles, hepatocytes as well as necrosis (Abou-Zeid et al., 2021; Ahmed et al., 2017). However, there were fewer degenerations of hepatocytes and mild infiltrations of mononuclear inflammatory fibrosis in portal spaces in co-treated group (PPF + LYC) compared to the PPF treated group. Moreover, LYC treatment recovered all histopathological alteration induced by PPF which is due to antioxidant and anti-inflammatory nature.

Table 4: Showing Damages in the Liver of Quails due to PPF and LYC

Damages	Control	PPF	PPF + LYC	LYC
Increased sinusoidal space	¥	¥¥¥	¥¥	¥
Tissue damages and congestion	¥	¥¥¥	¥¥	¥
Necrosis of hepatocyte	¥	¥¥¥	¥	¥
Vacuolar deterioration	¥	¥¥¥	¥	¥
Karyolitic	¥	¥¥¥	¥¥¥	¥

Normal (¥), normal change (¥¥), sever change (¥¥¥)

Conclusions

PPF treatment remarkably escalated hepatic serum enzymes, altered lipid profile and harmed histopathology in Quails. Moreover, PPF treatment escalates the activities of antioxidant enzymes (CAT and SOD) by impairing antioxidant defence capacity and exacerbating the ROS production. But, administration of LYC potentially improved these damages. Therefore, it is concluded that LYC can potentially alleviate PPF-induced liver damage due to its antioxidant nature.

DECLARATIONS

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Conflict of Interest: The authors have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability: The data will be available upon request to the corresponding authors.

Ethics Statement: This study was conducted in strict accordance with the National Institutes of Health (NIH) guidelines for the care and use of laboratory animals (NIH Publication No. 8023, revised 1978). Ethical approval was obtained from the Institutional Biosafety and Bioethical Committee (IBC) of University of

Agriculture Faisalabad, Faisalabad, Pakistan (Ref. No. DGS No.). All efforts were made to minimize animal suffering and to reduce the number of animals used.

Authors' Contribution: Muhammad Sajid; Data Curation, Methodology, Original draft Write up, Yasir Jamal; Formal Data Analysis, Writing, Arooj Fatima; Review and Editing, Data Analysis, Muhammad Zaid; Review and Editing, Data Analysis; Minahil Shuakat, Review and Editing/Writing.

Generative AI Statement: The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

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