Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(1), 726-748 | ISSN:2251-6727

Assessment of the Protective Role of Natural Bee Honey Against Sub Chronic Chlorpyrifos Toxicity in Albino Mice: Focus on Brain, Intestines, and Spleen

[1]Muhammad Jawad, [2]Amber Maria, [3]Rasheeka Rasheed, [4]Dilshad Fatima, [5]Muhammad Zafran, [6]Aqsa Zaheer, [1,7]Babar Khan*

1 Department of Zoology, Government College University Faisalabad, Faisalabad 38000 Pakistan

2 Department of Biotechnology and Bioinformatics, Government college university Faisalabad, P/O box 38000 Faisalabad Pakistan

3 Department of Wildlife and Fisheries, University of Agriculture Faisalabad, P/O box 38000 Faisalabad Pakistan

4 Department of Zoology, Government College University Faisalabad, P/O box 38000 Faisalabad, Pakistan

5 Department of Zoology, Government College University Faisalabad, P/O box 38000 Faisalabad, Pakistan

6 Department of Biological Sciences, Quaid-I-Azam University, P/O box 45320 Islamabad,

***Corresponding author:**

College of Biology, School of Life Science, Nanjing Normal University, 1 Wenyuan Road, Nanjing 2130023, China.

(Received: 25 November 2025 Revised: 27 December 2025 Accepted: 11 January 2026)

KEYWORDS

Chlorpyrifos, Natural Honey, Hematology, Neurotoxicity, intestinal toxicity, Hepatotoxicity, Stress marker, Histopathology, Albino mice.

ABSTRACT:

Chlorpyrifos (CPF), a widely used pesticide in agriculture, has been linked to toxicity in the reproductive organs of both humans and animals. The study investigated the toxic effects of chlorpyrifos (CPF) on the brain, spleen, and intestines of albino mice while assessing natural honey's potential to mitigate these effects. Thirty-five adult mice were divided into seven groups to evaluate various doses of CPF and honey, with body weights tracked and organ assessments conducted post-treatment. Hematological analysis revealed significant abnormalities in CPF-exposed groups (G3, G4, and G5), including elevated white blood cell counts and reduced red blood cell counts, hemoglobin levels, and hematocrit values, indicating anemia. However, honey supplementation in Groups 6 and 7 improved these parameters, demonstrating its protective role against CPF-induced hematotoxicity. Oxidative stress markers in CPF-treated mice (Groups 3, 4, and 5) revealed increased malondialdehyde (MDA) levels and decreased antioxidant enzyme activity, highlighting oxidative damage. Histopathological examinations indicated CPF-induced structural damage across the brain (characterized by neuronal necrosis and degeneration), spleen (demonstrating lymphoid depletion), and intestines (showing villous atrophy). In contrast, the honey-treated groups (Groups 6 and 7) displayed improved tissue integrity and reduced inflammation, underscoring honey's protective effects against CPF toxicity. Overall CPF exposure adversely affected metabolic health, hematological function, and organ integrity in albino mice, while honey exhibited significant protective effects. Honey showed promise in countering CPF toxicity, requiring further research on its protective effects against pesticide health risks.

INTRODUCTION

Chlorpyrifos (CPF), chemically known as 0,0-diethyl 0-(3,5,6-trichloro-2-pyridinol) phosphorothionate, is a widely used broad-spectrum organophosphate insecticide. It has extensive applications in both agricultural and non-agricultural sectors globally. First developed by Dow Elanco and introduced to the American market in 1965, chlorpyrifos has played a significant role in crop protection against a wide array of pests. Despite concerns about its adverse health effects, including neurotoxicity, its usage continues worldwide due to its efficacy. The toxicity of chlorpyrifos arises mainly from its inhibition of acetylcholinesterase (AChE), an enzyme crucial for nerve function, leading to an accumulation of acetylcholine in synapses and overstimulation of the nervous system [1, 2].

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(1), 726-748 | ISSN:2251-6727

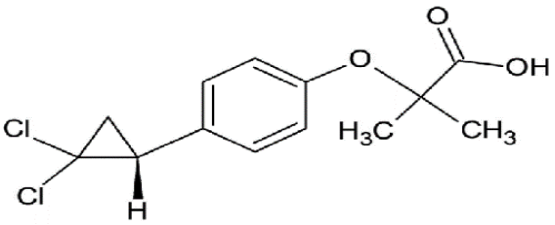

Figure 1. Structure of Chlorpyrifos

Acute human exposure manifests as symptoms such as headaches, dizziness, nausea, and respiratory distress, whereas chronic exposure has been linked to developmental disorders, neurobehavioral deficits, and increased cancer risks. Regulatory actions, including its ban by the USA Environmental Protection Agency in 2000, underscore growing safety concerns; nonetheless, chlorpyrifos persists in environments due to its continued global use [3, 4].

Environmental contamination from chlorpyrifos affects both terrestrial and aquatic ecosystems. It disrupts biogeochemical cycles and poses risks to non-target organisms, including beneficial insects, aquatic life, and birds. Its persistence in soil, water, and air leads to widespread exposure through food, water, dermal contact, and inhalation. Managing chlorpyrifos contamination is challenging, with current physicochemical and biological degradation methods often producing toxic byproducts and incomplete detoxification [5].Human exposure to chlorpyrifos is compounded by its systemic toxicity, which includes oxidative stress and inflammation in various organs such as the brain, intestines, and spleen. These effects compromise cellular integrity, leading to neurotoxicity, gastrointestinal dysfunction, and immunotoxicity [6].Natural bee honey has emerged as a promising protective agent against the toxic effects of chlorpyrifos. Renowned for its antioxidant, anti-inflammatory, and immune-modulating properties, honey contains bioactive compounds such as phenolics and flavonoids that neutralize free radicals, reduce oxidative stress, and promote tissue repair. Its prebiotic effects also support gastrointestinal health by enhancing beneficial microbiota and maintaining gut barrier function. Experimental studies have demonstrated honey's potential in alleviating toxicity induced by various chemicals and drugs, improving antioxidant enzyme activities, and mitigating histopathological damage [6, 7].

This study investigates chlorpyrifos-induced toxicity in albino mice, focusing on neurobehavioral, hematological, oxidative, and histopathological parameters. Additionally, it evaluates the protective role of natural bee honey in mitigating chlorpyrifos toxicity in the brain, spleen, and intestines. The findings aim to elucidate natural honey's therapeutic potential as a safe, accessible intervention against pesticide-induced health hazards.

MATERIAL AND METHOD

Animals

Thirty-five healthy adult albino mice were obtained. They were housed in plastic baskets with food and moved to the animal facility at the Pharmacy Department of the same university. The mice were kept in clean polypropylene cages with daily bedding changes to ensure a healthy environment. Throughout the study, they had unlimited access to commercial Kent rodent feed (16% protein) and distilled water. The animal room's temperature was kept at 24±2.50 degrees Celsius with a 12-hour light/dark cycle. All mice were allowed two weeks to adjust to these conditions.

Study design

Thirty-five healthy albino mice will be divided into 7 equal groups. Group 1 was treated with simple saline water and Group 2 was treated with natural honey (control group). Group 3: albino mice were orally administered CPF with a low dose. Group 4: albino mice received CPF with a medium dose. Group 5 was treated with a high dose of CPF. Groups 6 and 7 were treated with low and high doses of CPF with 25mg/kg of natural honey. The experiment was conducted for 28 consecutive days chlorpyrifos was given at an alternative day. Animals were euthanized by decapitation under anesthesia 24 hours following the last treatment, and blood was collected for further investigation. Biochemical tests and examinations were performed.

Ethical Statement

The use of animals was carried out using NIH Publication "Guide for the Care and Use of Laboratory Animals" (NRC 2004) and by the local bioethical committee of the University on animal experimentation [8]. The study was conducted in the research laboratory of the Department of Zoology, Government College University Faisalabad for sub chronic toxic effects of

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(1), 726-748 | ISSN:2251-6727

Chlorpyrifos orally administered in adult Albino Mice for 28 days. Body weight, hematology, oxidative stress markers and histology were used as parameters to investigate toxicity.

Physical Parameters

After 28 days of Chlorpyrifos exposure, the albino mice were fasted for 24 hours and then euthanized by placing them in a sealed jar containing a cotton piece soaked with chloroform. Blood samples were collected from each animal through the tail (caudal) vein for biochemical analysis. The brain, intestines, and spleen were carefully removed for the assessment of oxidative stress, histological examination, and other related analyses. Throughout the experimental period, the body weight of each albino mouse was recorded at both the beginning and the end of the trial using a digital balance, and their health condition was monitored daily. At the conclusion of the experiment, the mice were kept without the administration of Chlorpyrifos for an additional three days. Following this period, all mice were dissected after blood sampling, and the brain, intestines, and spleen were removed and preserved for further examination.

Figure 2. Albino mice were dissected after blood sampling and selected organ (Brain, Spleen and Intestines) were removed and preserved for analysis

Hematological Parameters

Blood was collected from each albino mouse through cardiac puncture using sterile syringes under anesthesia to ensure minimal distress. The collected blood samples were immediately transferred into EDTA-coated tubes to prevent coagulation and preserve sample integrity. These samples were then analyzed for a comprehensive set of routine hematological parameters, including red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin concentration, hematocrit, and platelet count. Differential blood cell counts were performed using prepared blood smears, which were stained and examined under a light microscope to assess the morphology and proportion of various blood cells. This detailed hematological evaluation provided insight into the physiological impact of Chlorpyrifos exposure and the potential protective effects of natural bee honey.

Biochemical Parameters

Measurement of Oxidative Stress Markers

Preparation of Tissue Homogenates

Freshly excised selected tissues were used for the preparation of tissue homogenates. Homogenization of brain, intestine, and spleen was done in 0.1 M Tris-HCL with pH-7.4 in bullet blender (Bullet blender 5 Eppendorf, Model-BBY5E-CE, USA) for 3-4 minutes. Homogenate was centrifuged at 10,000 rpm for 15 minutes at 4°C in refrigerated micro centrifuge (Sigma 1-14 refrigerated micro centrifuge, Germany). The supernatant from homogenates was poured carefully in 2 ml Eppendorf and stored at -80 °C for further analysis of stress biomarkers

Malondialdehyde (MDA)

To determine the extent of lipid peroxidation, levels of malondialdehyde (MDA), a byproduct of this process, were measured. The MDA concentration in tissue supernatant was determined following the method described by Ohkawa et al. (1979). Briefly, 0.2 ml of supernatant was mixed with 8.1% SDS, 1.5 ml of 20% acetic acid (pH 3.5), and 1.5 ml of 0.8% thiobarbituric acid, with distilled water added to bring the total volume to 4 ml. This mixture was then heated at 95 °C for 60 minutes in a water bath. After cooling to room temperature, n-butanol and pyridine (15:1 ratio) were added, and the mixture was vigorously shaken. Following centrifugation at 4000 rpm for 10 minutes, the upper organic layer was carefully extracted, and its absorbance was measured at 532 nm. Lipid peroxidation was expressed as µM of MDA per gram of tissue.

Glutathione (GSH)

The levels of glutathione (GSH) in the brain, intestine, and spleen after exposure to Chlorpyrifos (CPF) were quantified using the method of Sedlak and Lindsay (1968). A spectrophotometer (Model: U-2800 Hitachi,

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(1), 726-748 | ISSN:2251-6727

Germany) was used to measure GSH content at 412 nm, and the results were expressed as µM/g tissue. Tissue homogenates were precipitated with 50% trichloroacetic acid (TCA) and then centrifuged at 1000 rpm for 5 minutes. Next, 0.5 ml of the resulting supernatant was mixed with 2.0 ml of 0.2M Tris-EDTA buffer (pH 8.9) and 0.1 ml of 0.01M DTNB, and the mixture was allowed to react for 5 minutes at room temperature.

Catalase (CAT)

Catalase activity in the brain, intestine, and spleen was determined using the method described by Aebi (1974). A reaction mixture was prepared containing 1.95 ml of phosphate buffer (50 mM, pH 7), 0.05 ml of supernatant, and 1 ml of hydrogen peroxide (30 mM). Absorbance was measured at 240 nm at 15-second intervals for 30 seconds. Catalase activity was expressed as Units per ml of tissue homogenate.

Histological Examination

Adult albino mice were subjected to detailed histological examinations of the brain, intestines, and spleen following oral exposure to Chlorpyrifos (CPF) at doses of 3 mg/kg, 5 mg/kg, and 8 mg/kg body weight.

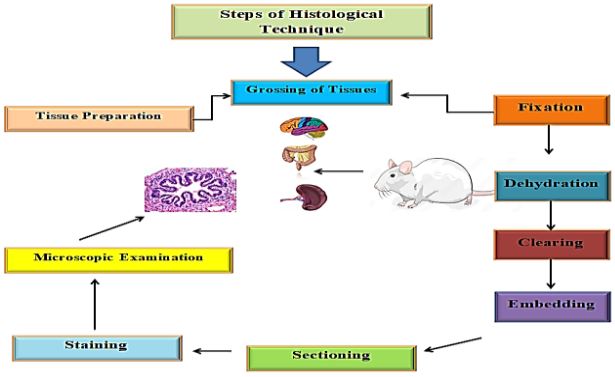

Figure 3. Histology was performed in stages using the above mentioned procedure.

These tissues were carefully extracted post-euthanasia to assess the structural and cellular alterations induced by sub-chronic toxicity. The histological evaluation was conducted in a series of well-defined stages to ensure the preservation and accurate analysis of tissue architecture. These stages included fixation, dehydration, clearing, infiltration, embedding, sectioning, staining, and microscopic examination. The protocol followed was based on established methods as described by Shahid et al. (2022), ensuring standardized processing and reliable interpretation of histopathological changes. This comprehensive histological assessment aimed to reveal the extent of tissue damage caused by CPF and evaluate the potential protective effects of natural bee honey.

Examination

Examination of prepared slides was done for alterations in tissues under light microscope and photographed under 40x and 100x objective lens (Nikon DS-Fi2).

Statistical analysis of Data

The data were statistically analyzed using Minitab17 software. One-way ANOVA was used to determine the treatment effects on various parameters. Tukey's test was then used to compare treatment means at $p<0.05$.

RESULTS

General observations

No signs of severe illness or mortality were observed during the experiment. Treated groups showed subtle behavioral changes, with slight reductions in activity in CPF-treated groups (3, 4, 5 and 7). Groups 1, 2, and 6 showed no differences in physical condition, behavior, or food and water consumption. Overall, slightly significant health issues were noted beyond expected effects of the treatments.

Body weight

Table 1 shows the weight of 35 of Albino Mice before and after the experiment. Body weight gain of mice across the groups showed no significant differences. All mice exhibited a normal increase in weight throughout the study period. Group 1 mice had an initial mean weight of 27.33±0.33g, which increased to 40.67±0.88g, resulting in a weight gain of 13.33±0.88g. Group 2 mice started with a mean weight of 27.67±0.33g and had a final weight of 35.00±0.58g, showing a weight gain of 7.33±0.67g. Group 3 (CPF) mice had an initial weight of 27.00±0.58g, which rose to 34.33±0.33g, with a weight gain of 7.33±0.33g. Group 4 (CPF) mice began with a weight of 27.67±0.67g, which increased to 31.00±0.58g, resulting in a weight gain of 8.33±0.58g. Group 5 (CPF) mice had an initial weight of 27.50±0.40g, which increased to 37.83±0.73g, showing a weight gain of 10.33±0.73g. Group 6 (CPF+Honey) mice started with 27.20±0.50g and ended at 32.50±0.90g, showing a weight gain of 5.30±0.90g. Finally, Group 7

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(1), 726-748 | ISSN:2251-6727

(CPF+Honey) mice had an initial weight of 27.45±0.35g, which increased to 39.52±0.80g, showing a weight gain of 12.07±0.80g.

Organs Weight

After the completion of 28 days' experiment, mice were sacrificed, dissected and their organs viz., brain, intestine, and spleen were removed, rinsed in phosphate buffer solution (PBS), dried and then weighed on digital electronic balance. There was no significant difference among the control, low, medium, and high dose chlorpyrifos treated mice for relative weight of the brain, and spleen while relative weights of the intestine were significantly increased in high dose treated groups ($p<0.05$).

Weight of Brain

Table 2 shows the control group receiving saline (G1) had an average brain weight of 348.75mg, while the honey group (G2) showed a slight increase to 361.15mg. The 3mg/kg BW treatment group (G3) had the highest brain weight at 461.40mg, suggesting a strong positive effect. However, the 5 mg/kg BW group (G4) showed a decrease to 423.60mg, indicating variability in response to this dose. The 8mg/kg BW group (G5) had a brain weight of 456.10mg, slightly lower than G3 but still higher than G1 and G4. The 3mg/kg BW + honey group (G6) had a lower brain weight of 398.25mg, suggesting that honey may have a complex effect. Meanwhile, the 8mg/kg BW + honey group (G7) recorded 452.80mg, indicating that honey influences brain weight, particularly at higher doses (Figure 4.3).

Weight of Intestine

The intestine weights of mice were recorded at the end of the experiment (Table 2). The control groups showed lower weights, with G1 (distilled water) at 2948.97mg and G2 (honey) slightly higher at 3185.42mg. Among the treatment groups, G3 (3mg/kg BW) increased to 3991.58mg, G4 (5 mg/kg BW) to 4512.32 mg, and G5 (8mg/kg BW) had the highest weight at 5083.14mg. The addition of honey influenced results differently, with G6 (3mg/kg BW + honey) at 4820.06mg and G7 (8mg/kg BW + honey) slightly lower at 4756.78mg. Overall, intestine weight increased dose-dependently, with 8mg/kg BW showing the highest values, while honey had varying effects.

Weight of Spleen

Table 2 shows the spleen weights of mice in both the control and treatment groups were measured at the end of the experiment, as shown in Table 4.3. After 28 days, the control group treated with distilled water (G1) had a spleen weight of 268.00±4.50 mg, while the control group receiving honey (G2) showed a slightly higher weight of 283.75±5.60 mg. Among the treatment groups, G3 (3mg/kg BW) had a spleen weight of 293.60±6.10 mg, followed by G4 (5mg/kg BW) with a weight of 308.20±5.30mg. The highest spleen weight was observed in G5 (8mg/kg BW) at 313.90±6.70mg. G6 (3mg/kg BW + honey) showed an increased spleen weight of 321.50±5.75mg, the highest among all the treatment groups. Lastly, G7 (8mg/kg BW + honey) had a spleen weight of 319.00± 6.40 mg, which was slightly lower than G6. Overall, the results indicated a dose-dependent increase in spleen weight, with the addition of honey enhancing the effect of the treatment (Figure 4.4).

Oxidative Stress Markers in Brain (Catalase, GSH, MDA)

Table 3 present the oxidative stress markers varied significantly ($P<0.05$) among treated groups compared to the control. CAT levels declined with increasing doses, except in honey-treated groups, where improvement was observed. The lowest CAT level (0.210±0.009 U/ml) was in G2 (3mg/kg BW), while G5 (3mg/kg BW + Honey) showed the highest (0.260±0.006U/ml), suggesting a protective effect of honey. GSH levels were lowest in G4 (0.070±0.003µM/g), indicating heightened oxidative stress. However, honey supplementation improved GSH, with G5 (0.185±0.002µM/g) and G6 (0.172±0.004µM/g) showing recovery. MDA levels, an indicator of lipid peroxidation, peaked in G3 (0.072±0.005µM/g), suggesting increased oxidative damage at this dose. Honey supplementation in G5 (0.035±0.002µM/g) and G6 (0.043±0.001µM/g) reduced MDA, indicating its protective role. Overall, higher doses of the compound increased oxidative stress, while honey supplementation mitigated damage by enhancing antioxidant defense.

Oxidative Stress Markers of Intestine (Catalase, GSH, MDA)

Table 4 shows the significant difference ($P<0.05$) in oxidative stress markers among all treated groups compared to the control. The CAT (Catalase) levels were

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(1), 726-748 | ISSN:2251-6727

highest in Group 5 (CPF) (0.201±0.030U/ml), suggesting an improved antioxidant response, while the lowest CAT level was observed in Group 2 (0.095±0.011U/ml), indicating increased oxidative stress. Honey supplementation in Groups 6 and 7 resulted in moderate CAT levels, reflecting a potential protective effect. Similarly, the GSH (Glutathione) levels were lowest in Group 2 (0.185±0.015µM/g), suggesting reduced antioxidant capacity and greater oxidative stress, whereas the highest GSH level was found in Group 6 (CPF+Honey) (0.320±0.004µM/g), reinforcing honey's beneficial role in restoring antioxidant balance. Conversely, MDA (Malondialdehyde) levels, a marker of lipid peroxidation, were highest in Group 2 (0.670±0.013µM/g), indicating severe oxidative damage. However, honey-treated groups (G6 and G7) exhibited lower MDA levels compared to their non-honey counterparts, demonstrating honey's potential in mitigating oxidative damage. Overall, these findings suggest that higher doses of the tested compound significantly increase oxidative stress, as reflected by decreased CAT and GSH levels and elevated MDA levels. Group 2 (20mg/kg BW) experienced the highest toxicity, evidenced by the lowest CAT and GSH levels and the highest MDA levels. In contrast, honey supplementation (Groups 6 and 7) helped counteract oxidative stress by enhancing antioxidant defenses and reducing lipid peroxidation, highlighting its protective effect against oxidative damage.

Oxidative Stress Markers of Spleen (Catalase, GSH, MDA)

Table 5 shows the impact of varying doses of a compound and honey supplementation on oxidative stress markers in mice. CAT (Catalase), an antioxidant enzyme, shows a significant increase in activity with higher doses, peaking in Group 7 (CPF+Honey) (8 mg/kg + Honey), where it reaches 0.700±0.00400 U/ml, suggesting a strong antioxidant response, especially when honey is included. The control group had a baseline level of 0.340±0.00280 U/ml, and non-honey treated groups exhibited progressively higher CAT levels with increasing doses, reaching 0.630±0.00350U/ml in Group 5 (CPF) (8mg/kg). In contrast, GSH (Glutathione) levels, another important antioxidant marker, decreased with increasing doses of the compound, with Group 5 (CPF) (8mg/kg) showing the lowest level of 1.100±0.02100µM/g, indicating a decrease in antioxidant defense at higher doses. However, Groups 6 and 7, which received honey supplementation, exhibited improved GSH levels (2.650±0.03700 µM/g and 2.300± 0.02800µM/g, respectively), demonstrating that honey supplementation helps mitigate oxidative stress. Lastly, MDA (Malondialdehyde), a marker of oxidative damage, was highest in Group 5 (CPF) (8mg/kg) at 0.720±0.00410µM/g, indicating significant lipid peroxidation and oxidative stress. The lowest MDA levels were found in the control group (0.410±0.00280µM/g) and in honey-treated groups (0.310±0.00260µM/g for Group 6 (CPF+Honey) and 0.440±0.00300µM/g for Group 7 (CPF)), suggesting that honey supplementation effectively reduces lipid peroxidation and oxidative damage.

Hematological Analysis for 28 Days Trial

Table 6 shows the significant differences in the values of hematological parameters ($p < 0.05$) In this study significant increase in the value of white blood cells (WBCs), mean corpuscular hemoglobin (MCH) and platelets (PLT) while significant decrease in red blood cells (RBCs), hemoglobin (HB), mean corpuscular hemoglobin concentration (MCHC), and Hematocrit (HCT) was observed in treated groups than control and placebo groups.

Table 1. Effect on body weight parameters (Grams) of male SD mice exposed to CPF orally for 28 days

Group	Treatment	Initial Weight (g)	Final Weight (g)	Weight Gain (g)
G1	**Control (S.W)**	27.33±0.33^{B}	40.67±0.88^{D}	13.33±0.88^{B}
G2	**Control (Honey)**	27.67±0.33^{A}	35.00±0.58^{B}	7.33±0.67^{B}
G3	**3mg/kg (CPF)**	27.00±0.58^{A}	34.33±0.33^{A}	7.33±0.33^{A}
G4	**5mg/kg (CPF)**	27.67±0.67^{A}	31.00±0.58^{A}	8.33±0.58^{A}
G5	**8mg/kg (CPF)**	27.50±0.40^{A}	37.83±0.73^{E}	10.33±0.73^{A}
G6	**3mg/kg (CPF+Honey)**	27.20±0.50^{C}	32.50±0.90^{C}	5.30±0.90^{C}

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(1), 726-748 | ISSN:2251-6727

G7	**8mg/kg (CPF+Honey)**	27.45±0.35^{C}	39.52±0.80^{C}	12.07±0.80^{C}

Means sharing similar letters are statistically non-significant (P > 0.05) Means sharing. Different letters are statistically significant (P < 0.05).

Table 2. Mean (SD) weights of brain, intestine and spleen in control, low, medium, and high dose chlorpyrifos treated groups exposed orally for 28 days.

Group	Treatment	Brain Weight (mg)	Intestine Weight (mg)	Spleen Weight (mg)
G1	**Control(S.W)**	348.75± 5.20^{A}	2948.97± 22.15^{a}	268.00± 4.50a
G2	**Control (Honey)**	361.15± 4.80^{A}	3185.42± 18.67^{a}	283.75± 5.60a
G3	**3mg/kg (CPF)**	461.40± 5.65^{C}	3991.58± 26.83^{b}	293.60± 6.10b
G4	**5mg/kg (CPF)**	423.60± 5.90^{B}	4512.32± 29.11^{c}	308.20± 5.30c
G5	**8mg/kg (CPF)**	456.10± 6.00^{D}	5083.14± 32.54^{d}	313.90± 6.70c
G6	**3mg/kg (CPF+Honey)**	398.25± 6.10^{B}	4820.06± 34.78^{d}	321.50± 5.75^{d}
G7	**8mg/kg (CPF+Honey)**	452.80± 6.50^{E}	4756.78± 25.64^{d}	319.00± 6.40^{d}

Means sharing similar letters are statistically non-significant (P > 0.05) Means sharing. Different letters are statistically significant (P < 0.05)

Table 3 Oxidative stress markers (Mean±SD) in brain of mice in control, low, medium, and high dose chlorpyrifos treated groups exposed orally for 28 days.

Factors	G1 Saline Water	G2 (Honey)	G3 (3mg/kg BW)	G4 (5mg/kg BW)	G5 (8mg/kg BW)	G6 (3mg/kg BW + Ho	G7 (08 mg/kg BW + Honey)
CAT (U/ml)	0.245 ± 0.005^{a}	0.210± 0.009^{c}	0.215± 0.011^{bc}	0.230± 0.013^{b}	0.260± 0.006^{a}	0.250± 0.007^{a}	0.220± 0.008^{bc}
GSH (µM/g)	0.162 ± 0.002^{b}	0.180± 0.005^{a}	0.145± 0.004^{c}	0.070± 0.003^{d}	0.185± 0.002^{a}	0.172± 0.004^{b}	0.158± 0.003^{bc}
MDA (µM/g)	0.024 ± 0.006^{c}	0.019± 0.003^{c}	0.072± 0.005^{a}	0.054± 0.004^{b}	0.035± 0.002^{c}	0.043± 0.001^{b}	0.065± 0.004^{a}

Different letters indicate statistically significant differences (P<0.05) within each parameter. Values sharing the same letter are not significantly different from each other. The presence of honey in G5 and G6 appears to enhance antioxidant defense by increasing CAT and GSH while reducing MDA levels.

Table 4 Oxidative stress markers (Mean±SD) in intestine of mice in control, low, medium, and high dose chlorpyrifos treated groups exposed orally for 28 days.

Factors	Group 1 (Saline Water)	Group 2 (Honey)	Group 3 (3mg/kg BW CPF)	Group 4 (5mg/kg BW CPF)	Group 5 (8mg/kg BW CPF)	Group 6 (3mg/kg BW + Honey)	Group 7 (8mg/kg BW + Honey)
CAT (U/ml)	**0.105± 0.012^{a}**	**0.095± 0.011^{a}**	**0.158 ± 0.020^{b}**	**0.175 ± 0.022^{b}**	**0.201 ± 0.030^{c}**	**0.135± 0.009^{ab}**	**0.180± 0.014^{b}**
GSH	**0.230±**	**0.185±**	**0.312**	**0.260**	**0.275**	**0.320±**	**0.295±**

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(1), 726-748 | ISSN:2251-6727

(µM/g)	**0.007[a]**	**0.015[b]**	**± 0.005[c]**	**± 0.010[d]**	**± 0.012[d]**	**0.004[c]**	**0.006[cd]**
MDA (µM/g)	**0.190± 0.005[a]**	**0.670± 0.013[e]**	**0.450 ± 0.015[d]**	**0.500 ± 0.020[cd]**	**0.590 ± 0.028[c]**	**0.410± 0.009[ab]**	**0.525± 0.018[c]**

Means sharing similar letters are statistically non-significant (P > 0.05), Means sharing different letters are statistically significant (P < 0.05).

Table 5 Oxidative stress markers (Mean±SD) in intestine of mice in control, low, medium, and high dose chlorpyrifos treated groups exposed orally for 28 days.

Factors	G1 Saline Water	G2 (Honey)	G3 (3mg/kg BW)	G4 (5mg/kg BW)	G5 (8mg/kg BW)	G6 (3mg/kg BW + Honey)	G7 (08 mg/kg BW + Honey)
CAT (U/ml)	0.340 ± 0.0028[a]	0.350± 0.0023[a]	0.460± 0.0028[c]	0.510± 0.0023[cd]	0.630± 0.0035[e]	0.590± 0.0031[de]	0.700± 0.0040[f]
GSH (µM/g)	2.750 ± 0.048[a]	2.450± 0.042[b]	1.750± 0.030[d]	1.400± 0.028[e]	1.100± 0.021[f]	2.650± 0.037[ab]	2.300± 0.028[cd]
MDA (µM/g)	0.410 ± 0.0028[a]	0.380± 0.0029[a]	0.500± 0.0029[c]	0.610± 0.0034[d]	0.720± 0.0041[e]	0.310± 0.0026[b]	0.440± 0.0030[c]

Means sharing similar letters are statistically non-significant (P > 0.05) ,Means sharing different letters are statistically significant (P < 0.05)

Table 6 Mean (SD) hematological parameters of mice in control, low, medium, and high dose chlorpyrifos treated groups exposed orally for 28 days.

Parameters	Units	Normal Control	Animals Treated with CPF	Animals Treated with CPF and Honey
Hemoglobin	(g/dL)	13.78±1.145	10.50±1.103	12.30±1.073
WBC	(10^9/L)	9.7±1.12	32.7±3.72	18.5±1.61
Total RBC	(10^12/L)	5.98±1.3	5.94±0.76	6.10±0.68
HCT	(%)	47.5±8.67	35.0±4.75	40.0±3.58
MCV	(fL)	63.67±5.96	58.0±6.04	65.00±5.20
MCH	(pg)	24.87±3.21	17.6±2.55	20.00±2.81
MCHC	(%)	30.7±3.92	30.4±5.12	31.0±4.55
Platelets	(10^9/L)	410±6.78	1768±208.01	884±104.01
PCT	(%)	4.8±1.2	1.39±0.19	0.65±0.10
PDW	(FL)	2.12±1.54	27.7±1.78	13.3±0.84
GRAN	(%)	1.3±1.04	81.5±5.25	42.0±2.62
GRAN#	(10^9/L)	34.7±31.5	10.6±1.35	5.3±0.68

Means sharing similar letters are statistically non-significant (P > 0.05) Means sharing. Different letters are statistically significant (P < 0.05)

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(1), 726-748 | ISSN:2251-6727

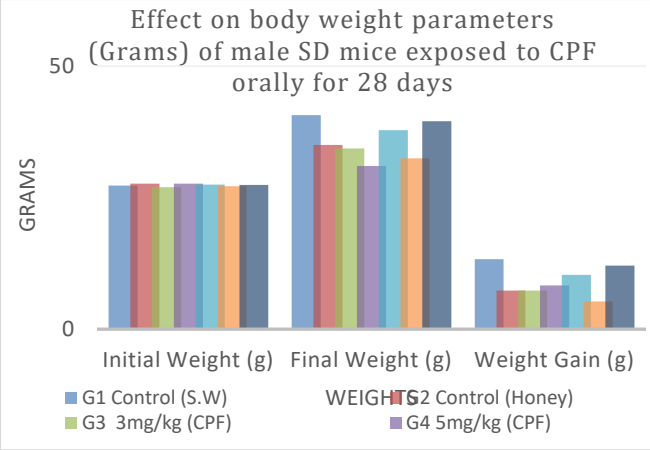

Figure 4. Shows the weight of 35 of Albino Mice before and after the experiment.

Histological Studies

A comprehensive 28-day histological investigation on Albino mice assessed the toxicological effects of chlorpyrifos at various dosage levels on critical organs, including the brain, intestine, and spleen. The control group displayed intact and normal tissue architecture, serving as a benchmark for comparison.

In the brain, exposure to higher doses of chlorpyrifos resulted in mild histopathological changes such as axonal dystrophy and vacuolation. In contrast, moderate doses induced more significant alterations, manifesting as neurophagia and cerebral edema. Intriguingly, even low doses produced severe necrosis and distortion of pyramidal nuclei, revealing that lower exposure levels could paradoxically have profound effects on brain integrity.

Within the intestine, the impact of chlorpyrifos was similarly concerning. High doses led to notable villous atrophy and inflammatory changes in the submucosa, indicating an acute inflammatory response. Moderate doses caused degeneration in the cryptic areas, and low doses resulted in severe necrosis and deformation of the villi, suggesting that even minimal exposure can trigger critical tissue damage.

The spleen also displayed significant adverse effects due to chlorpyrifos. At high doses, the organ exhibited lymphoid depletion and vascular congestion. Moderate levels caused disorganization of the white pulp and mild hemosiderosis, indicative of iron accumulation and tissue stress. Alarmingly, low doses elicited pronounced histopathological changes, including marked lymphocytic depletion, an increased area of red pulp, and splenic tissue necrosis, underscoring a shift towards heightened toxicity.

This study illuminates the substantial histopathological damage caused by chlorpyrifos across all examined organs. Notably, even lower doses can lead to severe tissue alterations, raising important concerns about the neurotoxic and immunotoxin potential of this pesticide. These findings warrant further investigation into the safety and regulatory standards surrounding chlorpyrifos exposure. Honey played a significant role in the recovery of tissues affected by histopathological damage, particularly following exposure to chlorpyrifos. Its rich antioxidant content helped neutralize free radicals generated by oxidative stress, thereby reducing oxidative damage to cells and promoting healing. The anti-inflammatory properties of honey contributed to minimizing inflammation in the brain, intestine, and spleen, facilitating the restoration of normal tissue architecture and function.

Brain Histology

Effects of Chlorpyrifos on the structural changes in the brain of Albino mice orally exposed for 28 days.

Group 1 Simple Saline Water (Control group)

Effects of chlorpyrifos on the histopathological changes in the brain of Albino mice were studied after 28 days. The H&E-stained slide sections of mice from the control group which treated with only Saline Water exhibited normal characteristics of the brain structure i.e., normal Glial Cell (GC), Neuronal Cells (NC), Oligodendroglia (OD) and Normal Arrangement of neurons (NA) in Albino mice (Figure 5).

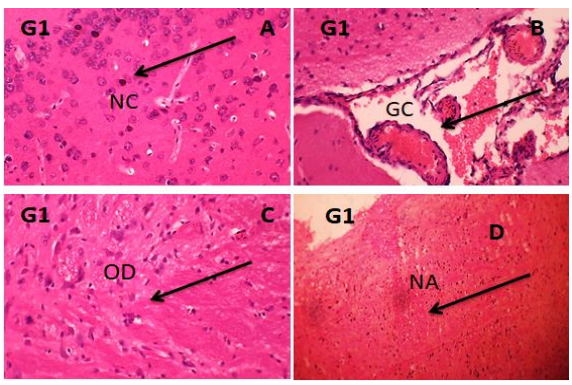

Figure 5 Photomicrograph (H&E; X40 & X100) of brain tissues in Group 1 (control) which has been treated with

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(1), 726-748 | ISSN:2251-6727

distilled water exhibited normal characteristics of the brain structure i.e., normal Glial Cell (GC), Neuronal Cells (NC), Oligodendroglia (OD) and Normal Arrangement of neurons (NA) in Albino mice.

Group 2 Treated with Natural Honey Bee Only (Control Group)

Histology of Group 2 mice revealed well-developed neural tissues with distinct cortical layers (CL) and mature neurons cells (NC). The interstitial spaces (IS) contained prominent glial cells (GC), contributing to the supportive structural framework of the brain. Additionally, treatment with honey demonstrated neuroprotective effects, enhancing neuronal density and promoting the maturation of synaptic connections. The presence of honey was associated with reduced oxidative stress markers and improved overall brain morphology, suggesting potential benefits for cognitive function.

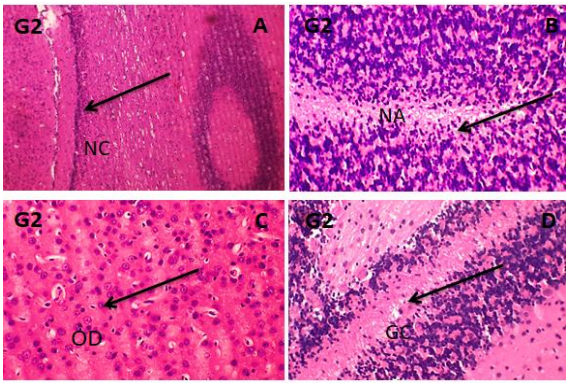

Figure 6 Photomicrograph (H&E; X40 & X100) of brain tissues in Group 2 (control) treated with natural honey exhibits typical characteristics of healthy brain structure. The images display normal Glial Cells (GC), Neuronal Cells (NC), and Oligodendroglia (OD), along with a well-organized arrangement of neurons (NA) in albino mice. These findings suggest that natural honey may support the maintenance of neural integrity and overall brain health.

Group 3(CPF) (Chlorpyrifos Low Dose)

An effect of chlorpyrifos on the histopathological changes in the brain of Albino mice were studied after 28 days. The H&E-stained slide sections of the brain tissues of Mice in this group were administered low-dose chlorpyrifos (3 mg/kg BWT) orally. Mild histopathological changes were noted, including neuronal shrinkage (NS), gliosis (GL), and mild perineuronal edema (PE), suggesting early oxidative stress and neurotoxicity. The brain still maintained some degree of structural integrity, but subtle alterations were evident compared to the control (Figure 3).

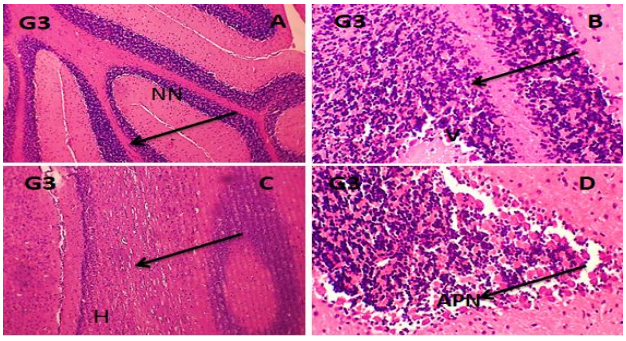

Figure 7 Photomicrographs (H&E; X40 & X100) of the brain tissues of Albino mice treated with a low dose (3mg/kg BWT) of chlorpyrifos (Group 3), showing mild histological alterations such as Neuronal Shrinkage (NS), Gliosis (GL), and Perineuronal Edema (PE). The brain structure remains largely intact, but signs of oxidative stress are evident.

Group 4 (CPF) (Chlorpyrifos Medium Dose)

Effects of chlorpyrifos on the histopathological changes in the brain of Albino mice were studied after 28 days. The H&E-stained slide sections of brain tissues of Albino mice. This group received a moderate dose of chlorpyrifos (5mg/kg BWT), leading to more pronounced neurotoxic effects. Moderate alterations included Neurophagia (NP), Vacuolation (VQ), and Axonal Dystrophy (AD), indicating neuronal stress and damage. The affected neurons exhibited pyknotic nuclei, and inflammatory cell infiltration was also noted, reflecting an immune response to cellular injury (Figure 8).

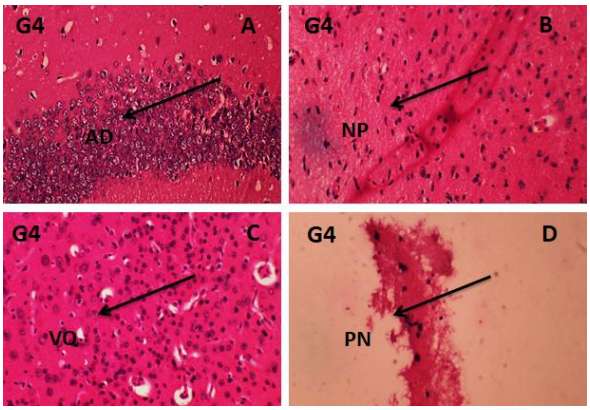

Figure 8 Photomicrographs (H&E; X40 & X100) of the brain tissues of Albino mice treated with a moderate dose

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(1), 726-748 | ISSN:2251-6727

(5 mg/kg BWT) of chlorpyrifos (Group 4), displaying histological abnormalities such as Neurophagia(NP),Axonal Dystrophy(AD), Vacuolation (VQ), and Pyknotic Nuclei (PN). The brain tissue shows moderate structural disruption with inflammation and neuronal damage.

Group 5(CPF) (Chlorpyrifos High Dose)

Mice in this group were subjected to high-dose chlorpyrifos exposure (8 mg/kg BWT), resulting in severe histological damage.Severe changes included widespread neuronal necrosis (NN), pyknotic nuclei (PN), disorganization of neuronal layers (DN), and severe axonal degeneration (SAD).The neurons showed marked vacuolation and gliosis, suggesting significant oxidative stress and neuroinflammation (Figure 9).

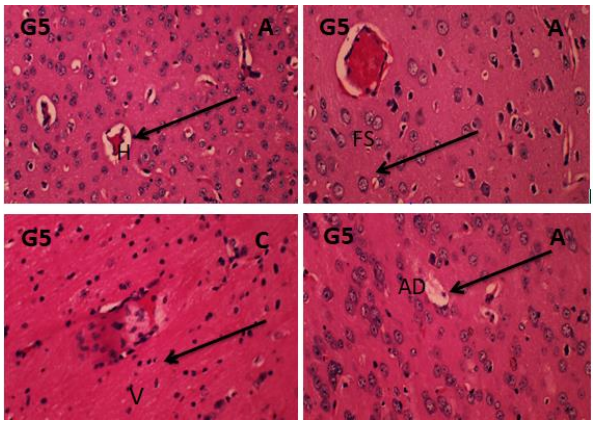

Figure 9 Photomicrographs (H&E; X40 & X100) of the brain tissues of Albino mice treated with a high dose (8mg/kg BWT) of chlorpyrifos (Group 5), revealing severe histopathological changes including Widespread Neuronal Necrosis (NN), Severe Axonal Degeneration (SAD), Disorganization of Neuronal Layers (DN), and Vacuolation (V). Hemorrhage (H) is also visible, indicating extensive neurotoxicity and cellular damage.

Group 6 (CPF+Honey) (Chlorpyrifos Low Dose+ Honey)

This group received low-dose chlorpyrifos (3 mg/kg BWT) along with natural honey, to assess the protective effects of honey against CPF-induced neurotoxicity. Compared to Group 3, histological damage was significantly reduced. The brain tissue displayed mild vacuolation (MV) and reduced neuronal degeneration (RND).Glial cells (GC) and neuronal cells (NC) remained largely intact, suggesting honey mitigated CPF-induced damage and helped preserve neuronal function (Figure 10).

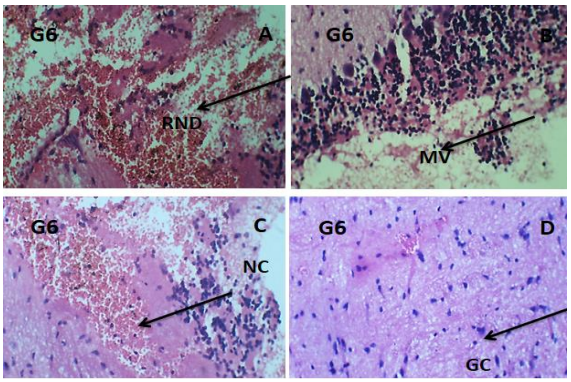

Figure 10 Photomicrographs (H&E; X40 & X100) of the brain tissues of Albino mice treated with a low dose (3 mg/kg BWT) of chlorpyrifos along with honey supplementation (Group 6), showing a significant reduction in neurotoxic damage. Mild Vacuolation (MV) and Reduced Neuronal Degeneration (RND) are observed, with relatively preserved brain architecture. Glial Cells (GC) and Neuronal Cells (NC) appear intact, indicating a protective effect of honey.

Group 7(CPF) (Chlorpyrifos High Dose+Honey Bee)

This group received high-dose chlorpyrifos (8 mg/kg BWT) combined with honey, to evaluate whether honey could counteract the severe neurotoxic effects observed in Group 5.Although some degenerative changes persisted, honey supplementation significantly reduced neuronal necrosis (RNN), limited perineuronal edema (LPE), and improved neuronal arrangement (INA).The overall damage was less compared to Group 5, indicating that honey partially counteracted oxidative stress and neuronal injury at higher CPF doses (Figure 11).

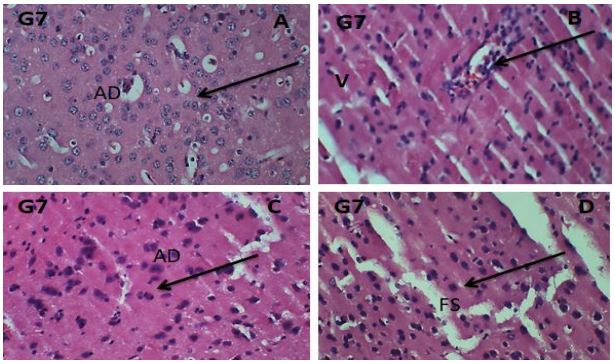

Figure 11 Photomicrographs (H&E; X40 & X100) of the brain tissues of Albino mice treated with a high dose (8 mg/kg BWT) of chlorpyrifos along with honey

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(1), 726-748 | ISSN:2251-6727

supplementation (Group 7), demonstrating reduced histopathological damage compared to Group 5. Although some Neuronal Necrosis (NN) and Mild Vacuolation (MV) persist, Perineuronal Edema (LPE) and Axonal Degeneration (AD) are significantly reduced. Improved Neuronal Arrangement (INA) suggests that honey mitigates CPF-induced neurotoxicity.

Intestine Histology

The histopathological effects of chlorpyrifos (CPF) exposure on the small intestine of Albino mice were evaluated over a 28-day period. Hematoxylin and Eosin (H&E)-stained sections of the intestinal tissue revealed significant alterations depending on the administered dose and the presence of honey as a protective agent.

Group 1 (Control group)

The control group (G1) exhibited normal intestinal histology, including well-organized villi (V), intact goblet cells (GC), a structurally normal submucosa (S), and properly arranged crypts of Lieberkühn (CL) (Figure 4.21). There were no signs of inflammation, necrosis, or villous deformation.

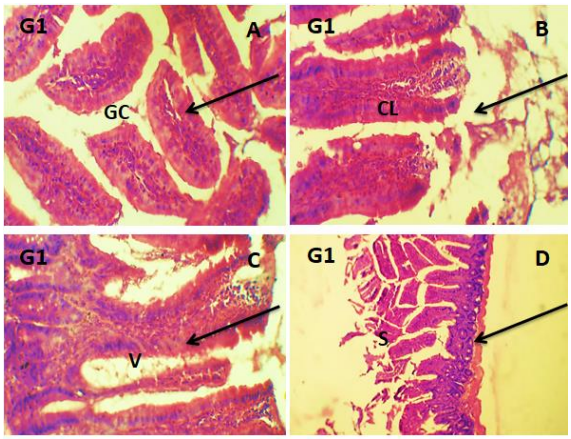

Figure 12 Photomicrographs (H&E; X40) of the H&E-stained slide sections of Small intestine tissue of Albino Mice in control group showed normal tissue structures like normal Villi (V), Goblet Cells (GC), normal Submucosa (S) and normal Crypts of Lieberkuhn (CL).

Group 2 (Control group Honey Bee)

Mice treated only with natural honey (G2) showed no significant histological damage. The villi remained intact, goblet cell distribution was normal, and there were no signs of tissue degeneration. This suggests that honey alone does not induce intestinal toxicity and may have protective properties.

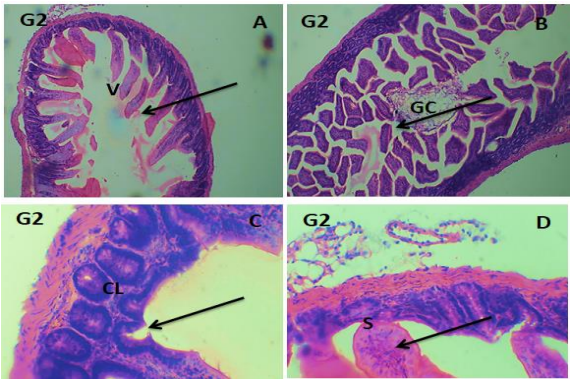

Figure 13 Photomicrographs (H&E; X40) of the H&E-stained slide sections of Small intestine tissue of Albino Mice in control group showed normal tissue structures like normal Villi (V), Goblet Cells (GC), normal Submucosa (S) and normal Crypts of Lieberkuhn (CL).

Group 3 (CPF) (3mg/Kg BW)

At a low dose (G3, 3 mg/kg CPF), minor histopathological alterations were observed, including slight villous shortening (VS), mild inflammatory infiltration (II), and a decrease in goblet cells (↓GC). The crypts of Lieberkühn showed early signs of degeneration (DCL), but the overall intestinal structure was still recognizable (Figure 14).

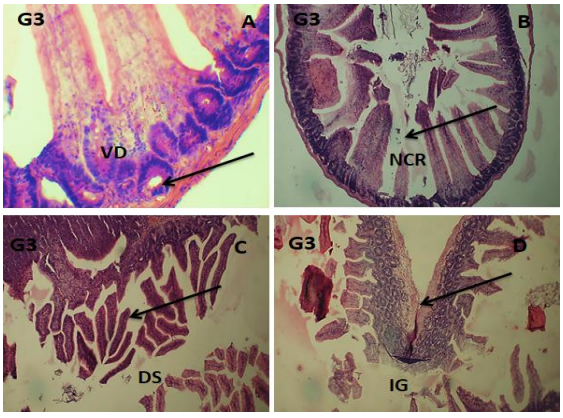

Figure 14 Photomicrographs (H&E; X40) of the H&E-stained slide sections of intestine tissues of Albino mice treated with medium dose (3mg/kg BW) of chlorpyrifos showing the histological abnormalities such as Degenerated Submucosa (DS), Distorted Cryptic Region (DCR), Inflamed Goblet cells (IG), and Degenerated Villi (DV).

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(1), 726-748 | ISSN:2251-6727

Group 4 (CPF) (5mg/Kg BW)

At a moderate dose (G4, 5 mg/kg CPF), more pronounced damage was noted. This included moderate villous atrophy (VA), degeneration of the submucosa (DS), cryptic distortion (CD), and inflammatory cell infiltration (ICI). Goblet cell reduction was more evident (↓GC) (Figure 15).

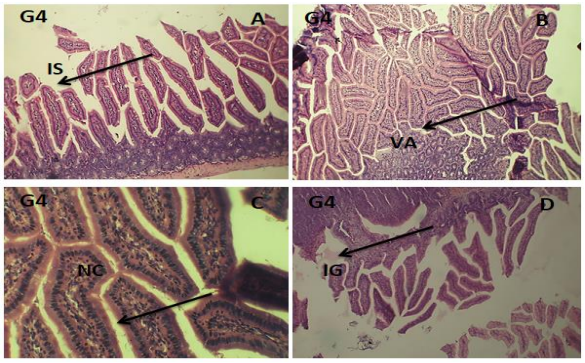

Figure 15 Photomicrographs (H&E; X40) of the H&E-stained slide sections of rat's intestine tissues treated with high dose (5mg/kg Body weight) of chlorpyrifos showed histological alterations such as Inflammatory Submucosa (IS), Villous Atrophy (VA), Inflamed Goblet cells (IG), and Necrosis at Crypt region (NC).

Group 5 (CPF) (8mg/Kg BW)

Mice receiving a high dose (G5, 8 mg/kg CPF) displayed severe histopathological changes. These included complete villous deformation (VD), severe cryptic necrosis (CN), and loss of goblet cells (↓GC). The submucosa showed inflammatory infiltration (II) and hemorrhagic lesions (HL), indicating intense oxidative stress and structural damage (Figure 16).

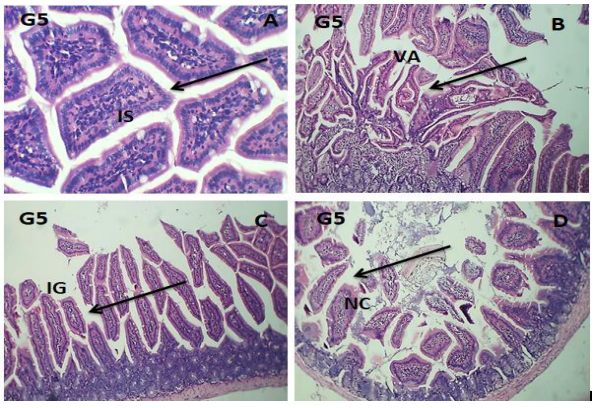

Figure 16 Photomicrographs (H&E; X40) of the H&E-stained slide sections of rat's intestine tissues treated with high dose (8mg/kg Body weight) of chlorpyrifosshowed histological alterations such as Inflammatory Submucosa (IS), Villous Atrophy (VA), Inflamed Goblet cells (IG), and Necrosis at Crypt region (NC)

Group 6 (CPF+Honey) (3mg/Kg BW+ Honey)

Mice treated with 3 mg/kg CPF along with honey (G6) showed a marked improvement in intestinal histology. While mild villous shortening (VS) and inflammatory cell infiltration (ICI) persisted, the overall tissue structure was significantly better compared to G3. Goblet cell loss was less prominent, and crypt degeneration was partially reversed, suggesting that honey ameliorated CPF-induced oxidative stress (Figure 17).

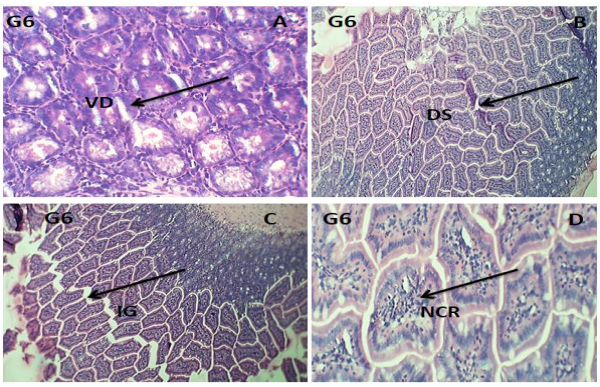

Figure 17 Photomicrographs (H&E; X40) of the H&E-stained slide sections of intestine tissues of Albino mice treated with low dose (3mg/kg BW) of chlorpyrifos along with honey showing the histological abnormalities such as Degenerated Submucosa (DS), Distorted Cryptic Region (DCR), Inflamed Goblet cells (IG), and Degenerated Villi (DV).

Group 7 (CPF+Honey) (8mg/Kg BW+ Honey)

In the highest CPF dose combined with honey (G7, 8 mg/kg CPF + Honey), intestinal histopathology showed partial recovery. Although villous deformation (VD) and crypt degeneration (CD) persisted, the presence of honey reduced inflammatory infiltration (↓II), prevented complete goblet cell depletion, and minimized hemorrhagic lesions (HL). The submucosal structure was more preserved compared to G5, indicating that honey provided a degree of protection against CPF-induced toxicity (Figure 18).

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(1), 726-748 | ISSN:2251-6727

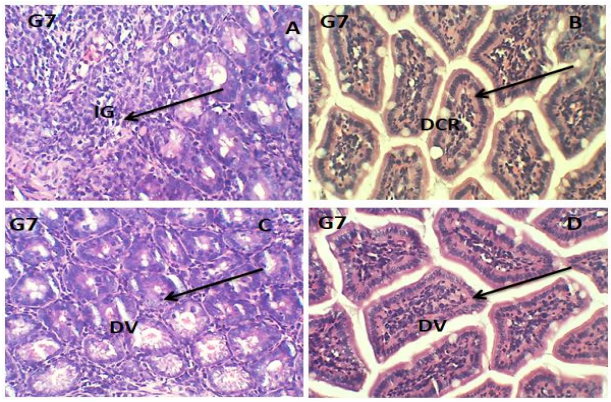

Figure 18 Photomicrographs (H&E; X40) of the H&E-stained slide sections of intestine tissues of Albinomice treated with High dose (8mg/kg BW) of chlorpyrifos along with honey showing the histological abnormalities such as Degenerated Submucosa (DS), Distorted Cryptic Region (DCR), Inflamed Goblet cells (IG), and Degenerated Villi (DV).

Histopathology of Spleen

Histopathology of Control Group in Spleen

First of all, observed the control group where animals were kept untreated under microscope at 40X. In Group 1 (Control), the spleen exhibited a normal histological structure, with well-defined white pulp and red pulp regions. The lymphoid follicles appeared intact, with a healthy population of immune cells, normal splenic cords, and a well-maintained vasculature. There were no signs of congestion, necrosis, or inflammatory infiltration, confirming an unaltered spleen architecture. Figure 19 showed the spleen of control group.

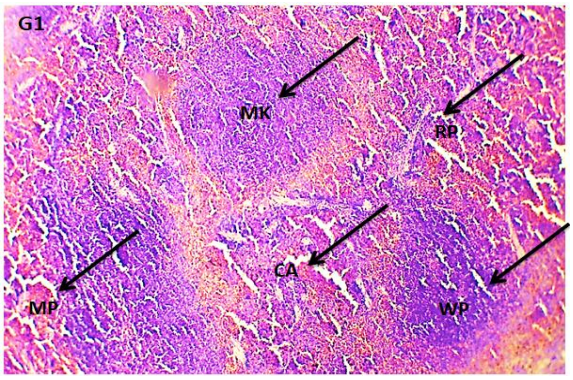

Figure 19 This figure showed Photomicrograph (H&E; X40) of spleen tissue from the control group (saline water) showing normal histological architecture, with well-organized white pulp (WP), red pulp (RP), and intact splenic cords. No histopathological abnormalities were observed

Histopathology of Control Group in Spleen

First of all, observed the control group where animals were kept untreated under microscope at 40X. In Group 2 (Natural Honey), the histological features were largely similar to those of the control group, showing normal tissue organization. However, a mild increase in lymphoid proliferation was observed, possibly indicating an immunomodulatory effect of honey. No signs of structural damage, congestion, or cellular degeneration were detected, reinforcing the non-toxic nature of honey on spleen tissues. Figure 20 showed the spleen of control group.

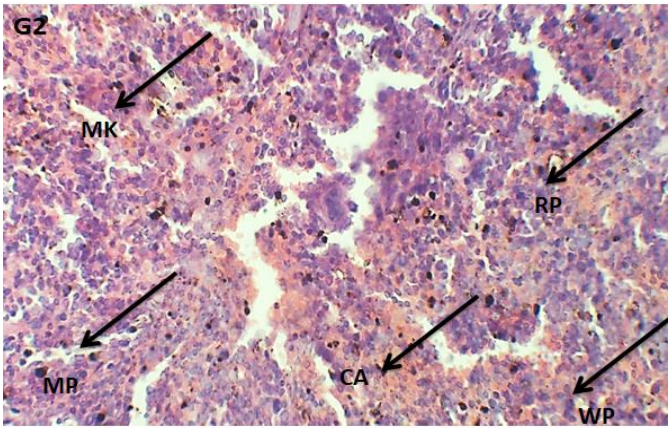

Figure 20 This figure showed Photomicrograph (H&E; X40) of spleen tissue from the natural honey-treated group showing a normal histological structure similar to the control. No significant pathological changes were noted, suggesting the protective role of honey.

Histopathology of Treated Group with Low Dose in Spleen

In Group 3 (3mg/kg CPF-treated), mild histopathological changes were observed, including slight congestion in the red pulp and early signs of lymphocytic depletion. Although the general structure of the spleen remained largely intact, minor inflammatory infiltration was noted in some regions. These alterations suggest the initial stages of CPF-induced toxicity, though the damage was not severe at this dose.

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(1), 726-748 | ISSN:2251-6727

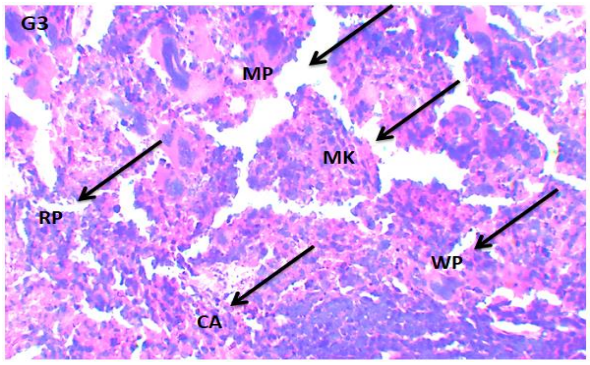

Figure 21 Photomicrograph (H&E; X40) of spleen tissue from the 3 mg/kg CPF-treated group showing mild histopathological changes, including mild congestion in the red pulp (CRP), slight lymphocytic depletion (LD), and early signs of structural disorganization.

Histopathology of Treated Group with Median Dose in Spleen

In Group 4 (5mg/kg CPF-treated), more noticeable histopathological changes were evident. There was a significant increase in congestion within the red pulp, accompanied by inflammatory cell infiltration and moderate lymphocytic depletion. The structural integrity of the white pulp began to show signs of disruption, indicating the progressive toxic effects of CPF on splenic tissue at this dose.

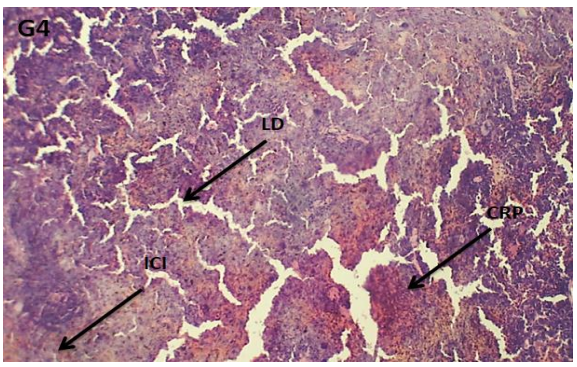

Figure 22 Photomicrograph (H&E; X40) of spleen tissue from the 5 mg/kg CPF-treated group displaying moderate histological damage, characterized by increased congestion in the red pulp (CRP), lymphocytic depletion (LD), and inflammatory cell infiltration (ICI).

Histopathology of Treated Group with High Dose in Spleen

In Group 5 (8mg/kg CPF-treated), severe histopathological alterations were observed. The spleen showed marked necrotic changes, extensive lymphoid depletion, and a disrupted architectural organization of both the white and red pulp. Heavy inflammatory infiltration and severe vascular congestion were present, along with degenerative changes in splenic cords. These findings suggest significant CPF-induced toxicity at higher doses, leading to substantial impairment of spleen function.

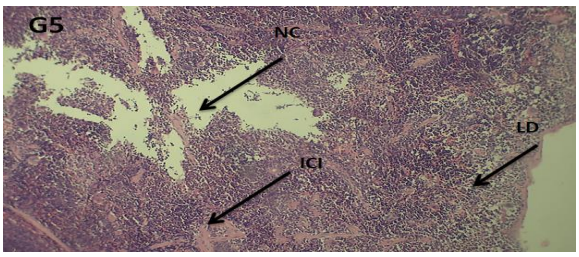

Figure 23 Photomicrograph (H&E; X40) of spleen tissue from the 8 mg/kg CPF-treated group exhibiting severe histopathological alterations, including pronounced lymphoid depletion (LD), necrotic changes (NC), increased inflammatory cell infiltration (ICI), and disrupted splenic architecture.

Histopathology of Treated Group with low Dose and Honey Bee in Spleen

In Group 6 (3mg/kg CPF + Honey-treated), histopathological alterations were less severe compared to the corresponding CPF-only group. The administration of honey appeared to reduce congestion and inflammatory infiltration. The white pulp structure remained relatively preserved, and there was a notable decrease in lymphocytic depletion compared to Group 3. These findings suggest that honey may provide a protective effect against CPF-induced toxicity at lower doses.

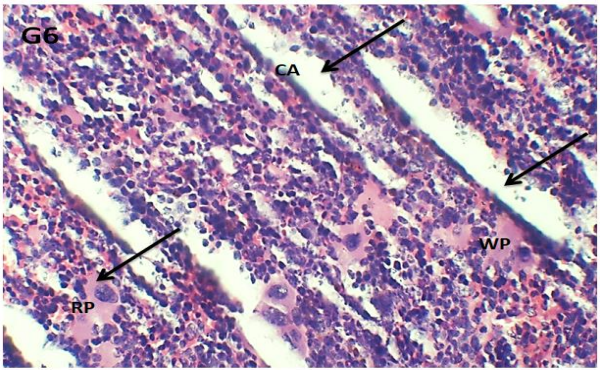

Figure 24 Photomicrograph (H&E; X40) of spleen tissue from the 3 mg/kg CPF + honey group demonstrating a relatively preserved structure compared

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(1), 726-748 | ISSN:2251-6727

to CPF-only treated groups. Mild congestion in the red pulp (CRP) and minimal inflammatory infiltration (ICI) were observed, suggesting a protective effect of honey.

Histopathology of Treated Group with High dose and Honey Bee in Spleen

In Group 7 (8mg/kg CPF + Honey-treated), partial protection against CPF toxicity was observed. Although necrotic changes and inflammatory infiltration were still present, they were significantly reduced compared to Group 5. Moderate vascular congestion and some degree of lymphoid depletion persisted, but the overall spleen architecture was better maintained than in the high-dose CPF-only group. The presence of honey appeared to mitigate some of the damaging effects of CPF, although it did not completely prevent histopathological alterations at this higher dose.

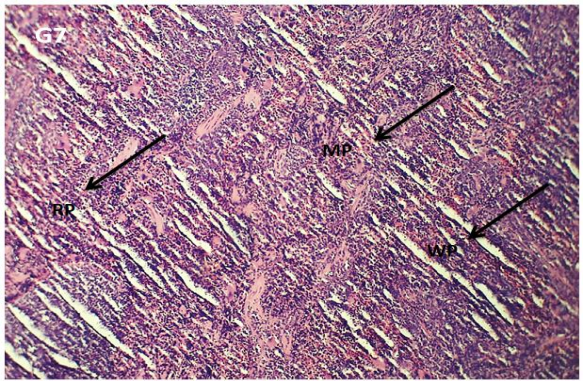

Figure 25 Photomicrograph (H&E; X40) of spleen tissue from the 8 mg/kg CPF + honey group showing moderate histological damage, including lymphocytic depletion (LD), inflammatory cell infiltration (ICI), and mild architectural distortion. However, the presence of honey appeared to reduce the severity of damage compared to the CPF-only group.

4.4.7 Comparative Study of Organ Spleen

When we observed the organ spleen comparatively the histopathological evaluation of spleen tissues in different treatment groups revealed a clear dose-dependent toxic effect of chlorpyrifos (CPF), with varying degrees of tissue damage. The addition of honey showed a protective role, reducing some of the degenerative changes caused by CPF exposure. A Comparative analysis of each group provides insights into the progression of splenic damage and the mitigating effects of honey.

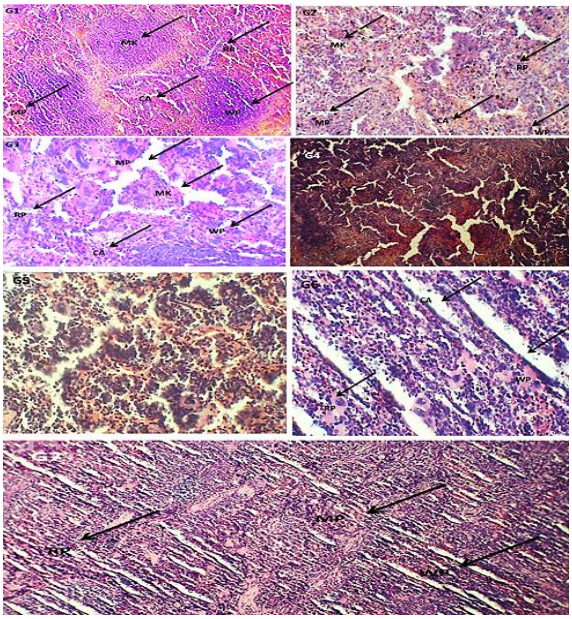

Figure 26 This comparative study demonstrates that CPF induces dose-dependent splenic damage, characterized by lymphoid depletion, vascular congestion, and necrotic changes. The co-administration of honey provides notable protective effects at lower CPF doses, helping to preserve tissue integrity and reduce inflammatory responses. However, at higher CPF concentrations, honey's protective benefits are limited, with only partial reduction of histopathological abnormalities. These findings highlight the potential role of honey as a mitigating agent against CPF toxicity, though its effectiveness may be dose-dependent.

DISCUSSION

Chlorpyrifos is an organophosphate insecticide widely used in agriculture to control pests, but it has raised significant health and environmental concerns, prompting regulatory scrutiny [9]. This insecticide operates by inhibiting the enzyme acetylcholinesterase, leading to the accumulation of acetylcholine at synaptic junctions, which can result in overstimulation of the nervous system in both insects and mammals [10]. Acute exposure to chlorpyrifos in humans may cause symptoms such as headaches, dizziness, nausea, and respiratory distress. Chronic exposure has been linked to developmental issues in children, potential neurodevelopmental disorders, and an increased risk of certain cancers [11]. Furthermore, studies on animal models indicate that chlorpyrifos may lead to long-lasting effects on neurobehavior and cognitive function, suggesting potential transgenerational impacts [12].

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(1), 726-748 | ISSN:2251-6727

Environmental health is also a concern, as chlorpyrifos persists in soil and water, affecting non-target species such as beneficial insects, aquatic life, and birds [13]. Its contamination of groundwater and surface water has been documented, resulting in broader ecological harm. In response to its harmful effects, chlorpyrifos has faced increasing regulatory action globally. The Environmental Protection Agency (EPA) in the United States has moved to restrict its use, acknowledging the risks it poses, particularly to children and sensitive populations [14]. Several states have enacted bans on chlorpyrifos, reflecting a shift toward safer pest management practices [15].

The mechanism behind chlorpyrifos-induced neurotoxicity involves oxidative stress and disruptions in cellular signaling pathways. Research has shown that exposure can elevate levels of reactive oxygen species (ROS), triggering apoptotic pathways and compromising cell integrity [16]. Additionally, alterations in neurotransmitter systems have been identified, which can lead to behavioral disturbances observed in both laboratory and field studies. Overall, chlorpyrifos presents significant risks to human health and the environment, necessitating careful consideration and management of its use [2].

This study examined the toxicological effects of chlorpyrifos (CPF) on albino mice over a 28-day sub-acute exposure period, focusing on behavioral changes, body weight fluctuations, organ weights, and histological parameters. The findings revealed significant differences among treatment groups, particularly in weight changes and organ-specific impacts, consistent with previous CPF toxicity research. Notably, no severe illness or mortality was observed in any treatment group, aligning with earlier studies suggesting that lower CPF doses may not cause acute toxicity (Fu et al., 2023). However, CPF-treated groups (G3, G4, G5, and G7) exhibited subtle behavioral changes, characterized by reduced activity levels, indicating potential mild neurotoxic effects that could impair locomotor activity and overall health. This observation is supported by [17], who noted similar impairments in locomotor activity in a rat model exposed to CPF. In contrast, groups treated with honey alone (G2) or a combination of CPF and honey (G6) showed no significant behavioral alterations, suggesting that honey may provide a protective effect against CPF's neurotoxicity, as indicated by [18, 19].

Body weight analyses revealed varying effects based on CPF and honey treatment. The control group (G1) exhibited the highest weight gain, while CPF-only groups (G3, G4, and G5) showed reduced weight gains, suggesting CPF-induced metabolic stress. This finding is corroborated by [18, 20], who reported weight reductions in CPF-treated animals. Interestingly, mice in the 8mg/kg CPF group (G5) experienced a notable weight increase, implying potential metabolic compensation at higher doses. Groups treated with honey alone (G2) and those receiving CPF with honey (G6 and G7) demonstrated moderate weight gains, with G7 showing a significant increase, indicating that honey may counteract some CPF-induced metabolic disruptions, a conclusion supported by [18, 21]. Analysis of organ weights revealed significant variations, particularly in brain weight. The 3mg/kg CPF group (G3) exhibited the highest brain weight, suggesting an increase in brain mass at lower CPF doses, while the 5mg/kg CPF group (G4) showed a decrease, indicating a dose-dependent neurotoxic effect consistent with [22]. The addition of honey in CPF-treated groups (G6 and G7) helped moderate these effects, with G7 displaying a higher brain weight than G6, supporting the hypothesis that honey may alleviate CPF-induced neural damage, as seen in research by [18].

Intestinal weight also increased in response to CPF exposure, particularly in the 8 mg/kg group (G5). The addition of honey in CPF-treated groups (G6 and G7) produced variable effects, indicating significant impacts of CPF on gastrointestinal physiology [1]. This aligns with findings by Yasmeen and Amir (2022), who noted alterations in intestinal weight and morphology in CPF-exposed animals, likely due to inflammatory responses. The regulatory influence of honey observed in this study suggests its beneficial role in mitigating adverse effects, consistent with earlier reports [23, 24].

Spleen weight analysis indicated a dose-dependent increase with CPF exposure, peaking in G6 (3mg/kg CPF + honey) and followed by G7 (8mg/kg CPF + honey). Control groups (G1 and G2) exhibited significantly lower spleen weights, while the increases in honey-supplemented groups suggest that honey may enhance immune responses or alleviate CPF-induced splenic stress, corroborated by research indicating honey's role in modulating immune parameters [25].

The findings highlight the toxic effects of CPF on body

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(1), 726-748 | ISSN:2251-6727

weight regulation, organ mass, and overall physiological health in albino mice. Co-administration of honey appears to mitigate some of these effects, showcasing its antioxidant and anti-inflammatory properties that may alleviate CPF-induced stress. This protective role is significant, as demonstrated in previous studies exploring honey's therapeutic potential against chemical toxicity [26]. The combination of CPF and honey in this study led to improved weight gain and moderated organ weight variations, suggesting potential protective or compensatory mechanisms [27]. Future research, including molecular and histopathological analyses, is necessary to further elucidate how honey mitigates CPF toxicity and to explore its potential therapeutic applications. The hematological assessment carried out over the 28-day period revealed significant alterations in blood parameters of mice treated with chlorpyrifos (CPF) in comparison to control and honey-treated groups, as detailed in Table 4.5. The increase in white blood cells (WBCs), mean corpuscular hemoglobin (MCH), and platelets (PLT) indicates an immune response or stress reaction due to CPF exposure. In contrast, the decreases in red blood cells (RBCs), hemoglobin (HB), mean corpuscular hemoglobin concentration (MCHC), and hematocrit (HCT) suggest potential anemia or suppression of bone marrow activity.

The marked increase in WBC counts (32.7±3.72 x 10^9/L in treated groups versus 9.7±1.12 x 10^9/L in controls) demonstrates reactive leukocytosis, consistent with Masad et al., (2021), who observed similar elevations in WBCs due to CPF-induced immune activation. This immune response may be associated with the inflammatory consequences of CPF, as noted by [28, 29] which documented similar alterations in hematological profiles indicative of toxic stress.

Conversely, the significant reduction in RBC count, hemoglobin levels, and HCT among CPF-treated groups points to anemia, possibly resulting from decreased erythropoiesis or increased hemolysis, a finding supported by [30] which indicates potential compromise of hematopoietic function due to CPF. Notably, the introduction of honey alongside CPF treatment improved these parameters, with hemoglobin levels in honey-treated groups rising to 12.30±1.073g/dL. This suggests that honey may mitigate the hematological changes induced by CPF toxicity, corroborating the protective effects of honey against toxic agents noted by [31].

The analysis of oxidative stress markers in the brain (Table 4.6) reveals significant differences among treatment groups, particularly regarding levels of catalase (CAT), glutathione (GSH), and malondialdehyde (MDA). The reduction in CAT levels with increasing CPF doses—except in honey-treated groups—indicates diminished antioxidant defenses due to CPF toxicity. For example, Group 2 (3 mg/kg BW) displayed the lowest CAT level (0.210±0.009 U/ml), suggesting compromised enzymatic activity in response to oxidative stress. This aligns with [32], linking CPF exposure to oxidative damage in neural tissues. On the other hand, honey co-administration led to increased CAT levels in Groups 5 (0.260±0.006U/ml) and 6 (0.250±0.007U/ml), emphasizing honey's protective role in bolstering antioxidant defenses, consistent with Mohamed et al., (2022), who found that honey supplementation reduces oxidative stress and enhances antioxidant capacity in models exposed to toxins [33].

GSH levels were lowest in Group 4 (0.070±0.003µM/g), reflecting heightened oxidative stress, a situation corroborated by previous studies indicating that chemical exposures can deplete vital antioxidants [34]. In contrast, honey supplementation restored GSH levels in Groups 5 (0.185±0.002µM/g) and 6 (0.172± 0.004µM/g), highlighting honey's ability to counter oxidative stress, supported by findings from [35]. MDA levels, which indicate lipid peroxidation and oxidative injury, peaked in Group 3 (0.072±0.005µM/g), suggesting considerable oxidative damage at this CPF dosage. Honey supplementation significantly lowered MDA levels in Groups 5 (0.035±0.002µM/g) and 6 (0.043±0.001µM/g), further reinforcing its protective properties against CPF-induced oxidative stress as noted by [36].

The analyses of hematological and oxidative stress markers reveal the substantial impact of CPF exposure on the blood parameters and antioxidant status in mice [37, 38]. Increased WBCs and platelets suggest a stress response, while decreased RBCs, HB, and HCT point to possible anemia. Importantly, honey supplementation appears to alleviate some adverse effects, improving hematological parameters and enhancing antioxidant defenses as demonstrated by elevated CAT and GSH levels alongside reduced MDA levels [39]. These findings not only support existing literature on CPF toxicity but also indicate that honey may function as an effective protective agent against the hematological and

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(1), 726-748 | ISSN:2251-6727

oxidative impairments associated with CPF exposure.

These results highlight the necessity for further investigation into the mechanisms through which honey and other natural antioxidants can provide protective effects against environmental toxins, potentially informing therapeutic strategies for addressing toxicological health risks.

The analysis of oxidative stress markers in the intestinal tissue revealed significant differences among treatment groups exposed to varying doses of chlorpyrifos (CPF) when compared to the control group. Catalase (CAT) levels were highest in Group 5 (8 mg/kg CPF) at 0.201±0.030 U/ml, indicating an enhanced antioxidant response. Conversely, Group 2 showed the lowest CAT levels at 0.095±0.011 U/ml, suggesting considerable oxidative stress (Table 4.7). Honey supplementation in Groups 6 and 7 resulted in moderate CAT levels of 0.135±0.009 U/ml and 0.180±0.014 U/ml, respectively, highlighting its protective role in bolstering intestinal antioxidant defenses [40]. Glutathione (GSH) levels also reflected similar trends, with the lowest levels in Group 2 (0.185±0.015µM/g) and a restoration to 0.320±0.004µM/g in Group 6 after honey supplementation, reinforcing honey's ability to enhance antioxidant capacity [41]. Malondialdehyde (MDA) levels, a marker of lipid peroxidation, were highest in Group 2 (0.670±0.013µM/g), indicating severe oxidative damage. Honey-treated groups showed lower MDA levels (0.410±0.009µM/g in Group 6 and 0.525± 0.018µM/g in Group 7), further demonstrating honey's effectiveness in alleviating oxidative damage caused by CPF exposure. These findings support previous studies emphasizing honey's antioxidative properties [42].

In the spleen, significant differences were also noted concerning oxidative stress markers (Table 4.9). CAT levels increased with higher CPF doses, peaking at 0.700± 0.004 U/ml in Group 7 (8mg/kg CPF + honey), indicating improved antioxidant defenses supported by literature on honey's ability to enhance enzyme activity [43]. However, GSH levels decreased with increasing CPF doses, reaching a low of 1.100±0.02100µM/g in Group 5, signifying reduced antioxidant defenses [44]. Groups 6 and 7 that received honey supplementation exhibited improved GSH levels (2.650±0.03700µM/g and 2.300±0.02800µM/g, respectively), reaffirming honey's effectiveness in restoring antioxidant balance [45].

MDA levels mirrored these changes, with the highest concentration in Group 5 (0.720±0.00410µM/g), indicating significant oxidative stress. The control group had the lowest MDA level (0.410±0.00280µM/g), while honey-treated groups showed significantly lower levels (0.310±0.00260µM/g in Group 6 and 0.440±0.00300µM/g in Group 7), reinforcing the protective role of honey against oxidative damage [46]. Collectively, findings from both intestinal and splenic analyses highlight the detrimental effects of CPF on oxidative stress markers, with honey emerging as a promising protective agent against oxidative damage.

The 28-day histological study of Albino mice evaluated the effects of chlorpyrifos (CPF) exposure across various organs, including the brain, intestine, and spleen. The control group displayed normal histological architecture without pathological changes. However, increased CPF doses led to significant structural alterations. High CPF doses caused noticeable brain damage, including axonal dystrophy and vacuolation. Moderate doses resulted in neurophagia and edema, while lower doses caused severe necrosis and distortion of the pyramidal nucleus. In the intestine, high doses induced villous atrophy and inflammatory changes in the submucosa, while moderate doses caused degeneration of crypt areas. The spleen exhibited lymphoid depletion and congestion at high doses, whereas moderate doses resulted in mild hemosiderosis and disorganization of white pulp. Lower CPF doses revealed lymphocytic depletion and increased red pulp area, indicating overall heightened toxicity across all examined tissues [47]. Further examination of the brain's histological changes across treatment groups clarified CPF's neurotoxic effects. The control group (Group 1) treated with saline water showed normal brain characteristics, while Group 2, which received only honey, exhibited well-developed neural tissues suggesting neuroprotective.

A deeper examination of histological changes in the brain across different treatment groups further elucidated the neurotoxic effects of CPF. Group 1, served as the control group treated with saline water, displayed normal brain characteristics, including well-structured glial cells, neuronal cells, and organized arrangements of neurons (Figure 1). In contrast, Group 2, which received only natural honey, demonstrated well-developed neural tissues with mature neuronal cells and prominent glial cells, suggesting that honey supports neuronal integrity and may confer neuroprotective benefits [48]. In Group

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(1), 726-748 | ISSN:2251-6727

3, mice administered a low dose of CPF (3 mg/kg body weight) exhibited mild histopathological changes such as neuronal shrinkage and gliosis, indicating early signs of oxidative stress and neurotoxicity while still maintaining some structural integrity (Figure 3). Conversely, mice in Group 4, treated with a moderate dose of CPF (5mg/kg), showed more severe neurotoxic effects, including neurophagia and the presence of pyknotic nuclei, reflecting a significant immune response to cellular injury (Figure 4). Group 5, which received a high dose of CPF (8mg/kg), displayed extensive neuronal necrosis and severe axonal degeneration, highlighting the profound neurotoxic impact of CPF on brain architecture (Figure 5).

Groups 6 and 7 were particularly significant as they assessed the protective effects of honey against CPF-induced neurotoxicity. In Group 6, mice exposed to low-dose CPF alongside honey showed reduced histological damage, with less vacuolation and improved neuronal health, suggesting honey's capacity to mitigate CPF-induced neurotoxicity (Figure 6). Similarly, Group 7, receiving high-dose CPF combined with honey, exhibited reduced neuronal necrosis and limited perineuronal edema, indicating that honey supplementation can significantly alleviate oxidative stress and neuronal injury even at higher CPF doses (Figure 7). These findings align with existing literature, which emphasizes honey's neuroprotective properties against oxidative damage [18, 49]. Overall, the histopathological assessment confirmed that CPF exposure causes significant tissue damage in multiple organs, and honey may serve as a beneficial adjunct therapy to limit these adverse effects.

The histopathological effects of chlorpyrifos (CPF) on the small intestine of Albino mice were assessed over a 28-day period, using Hematoxylin and Eosin (H&E) staining to evaluate tissue changes in relation to both CPF exposure and the potential protective role of honey. The control group (Group 1) exhibited normal intestinal histology, characterized by well-organized villi, intact goblet cells, a structurally sound submucosa, and properly arranged crypts of Lieberkühn, with no signs of inflammation or necrosis (Figure 4.21) [50, 51]. Similarly, Group 2, which received only natural honey, displayed no significant histological damage, indicating that honey itself does not induce intestinal toxicity and may confer protective benefits [23].

Mice in Group 3, treated with a low dose of CPF (3mg/kg BW), exhibited minor histopathological changes, including slight villous shortening, mild inflammatory infiltration, and a reduction in goblet cells, suggesting early signs of degeneration (Figure 4.22). The structural integrity of the intestinal tissue remained largely intact. In contrast, Group 4, which received a moderate dose (5mg/kg BW) of CPF, displayed more pronounced damage, including moderate villous atrophy, degeneration of the submucosa, cryptic distortion, and increased inflammatory cell infiltration (Figure 4.23). Mice in Group 5, administered a high dose of CPF (8mg/kg BW), showed severe histopathological alterations, characterized by complete villous deformation, severe cryptic necrosis, loss of goblet cells, and significant inflammatory infiltration with hemorrhagic lesions (Figure 4.24). These findings align with existing literature indicating that CPF exposure leads to significant oxidative stress and structural damage in intestinal tissues [52].

In Groups 6 and 7, where CPF was co-administered with honey, notable improvements in histological outcomes were observed. Group 6, receiving a low dose of CPF alongside honey, showed marked improvement in intestinal structure, with reduced villous shortening and partial recovery of goblet cells and crypts (Figure 4.25). This suggests that honey effectively mitigates CPF-induced oxidative stress. Similarly, in Group 7, which received a high dose of CPF combined with honey, histopathological damages were reduced compared to Group 5. Although some degree of villous deformation and crypt degeneration persisted, honey limited inflammatory infiltration and preserved the submucosal structure, indicating a protective effect against CPF-induced toxicity (Figure 4.26). These results support the notion that honey can play a crucial role in protecting intestinal integrity against the detrimental effects of CPF exposure [21, 53]. This study confirms that exposure to chlorpyrifos (CPF) causes significant toxicological effects, including neurotoxicity, metabolic disturbances, organ weight changes, and oxidative stress in albino mice. Behavioral, hematological, and histological analyses further highlight CPF's harmful impact, particularly on the nervous and immune systems. Notably, CPF-induced oxidative stress is evident through increased malondialdehyde (MDA) levels and decreased antioxidant enzyme activity, emphasizing its role in cellular damage [54].

However, honey supplementation exhibited a protective effect against CPF-induced toxicity by reducing oxidative stress, enhancing antioxidant enzyme activity, and alleviating histopathological damage in key organs such as the brain, intestine, and spleen. Improvements in weight regulation, hematological parameters, and tissue integrity suggest honey's potential as a therapeutic agent in counteracting CPF-related toxicity. Given CPF's widespread agricultural use and associated health risks, Furthermore, continued research into natural antioxidants like honey may offer valuable strategies for mitigating environmental toxicant exposure and developing potential therapeutic interventions.

REFERENCES

1. El-fakharany, Y. and O. Abdel Hamid, *Toxic effects of chronic chlorpyrifos exposure on jejunum of adult male albino rats and the possible ameliorative role of propolis.* Ain Shams Journal of Forensic Medicine and Clinical Toxicology, 2017. **28**(1): p. 28-41.
2. Nandhini, A., M. Harshiny, and S.N. Gummadi, *Chlorpyrifos in environment and food: a critical review of detection methods and degradation pathways.* Environmental Science: Processes & Impacts, 2021. **23**(9): p. 1255-1277.
3. Boyina, M.B., *ENVIRONMENTAL TOXINS.* Principles of Medical Toxicology, 2025: p. 193.
4. Zhou, W., M. Li, and V. Achal, *A comprehensive review on environmental and human health impacts of chemical pesticide usage.* Emerging Contaminants, 2025. **11**(1): p. 100410.
5. Ma, J., et al., *Environmental impacts of chlorpyrifos: Transgenerational toxic effects on aquatic organisms cannot be ignored.* Science of the total environment, 2023. **905**: p. 167311.
6. Alipanah, H., et al., *Subacute toxicity of chlorpyrifos on histopathological damages, antioxidant activity, and pro-inflammatory cytokines in the rat model.* Environmental Toxicology, 2022. **37**(4): p. 880-888.
7. Akpa, A.R., et al., *Protective effect of fisetin against subchronic chlorpyrifos-induced toxicity on oxidative stress biomarkers and neurobehavioral parameters in adult male albino mice.* Toxicological research, 2021. **37**(2): p. 163-171.
8. Council, N.R., et al., *Guide for the care and use of laboratory animals.* 2010.
9. Sellers, C., et al., *History of Risk Assessments of the Organophosphate Pesticide Chlorpyrifos at the US Environmental Protection Agency, 1980–2024.* American Journal of Public Health, 2025. **115**(7): p. 1074-1084.
10. Kawyn, M., *Pyrethroid and Chlorpyrifos Exposure and IQ of Children from the Salud Ambiental Montevideo (SAM) Study in Uruguay*. 2020, State University of New York at Buffalo.
11. Bemanalizadeh, M., et al., *Parental occupational exposure and neurodevelopmental disorders in offspring: a systematic review and meta-analysis.* Current Environmental Health Reports, 2022. **9**(3): p. 406-422.
12. De Felice, A., et al., *Sex-dimorphic effects of gestational exposure to the organophosphate insecticide chlorpyrifos on social investigation in mice.* Neurotoxicology and teratology, 2014. **46**: p. 32-39.
13. Mikušková, N., *Effect of pesticides and their metabolites on aquatic organisms.*
14. Veal, L., *United States environmental protection agency.* US Environmental Protection Agency (EPA)(2005) National Management Measures to Control Non-Point Source Pollution for Urban Areas, 2021.
15. Kyriakopoulos, G.L. and I. Sebos, *Marketable and banned pesticides in agriculture: Categorization, simulation, and crystallography review.* International Journal of Molecular Sciences, 2024. **25**(22): p. 11885.
16. Agarwal, A., et al., *Oxidative stress and assisted reproduction: a comprehensive review of its pathophysiological role and strategies for optimizing embryo culture environment.* Antioxidants, 2022. **11**(3): p. 477.
17. Amani, N., et al., *Chlorpyrifos toxicity in mouse cultured cerebellar granule neurons at different stages of development: Additive effect on glutamate-induced excitotoxicity.* Cell Journal (Yakhteh), 2016. **18**(3): p. 464.
18. Tanvir, E., et al., *Honey has a protective effect*

against chlorpyrifos-induced toxicity on lipid peroxidation, diagnostic markers and hepatic histoarchitecture. European Journal of Integrative Medicine, 2015. **7**(5): p. 525-533.

19. Panase, A. and M. Thirabunyanon, *Effects of dietary synbiotics on growth performances and immune improvement in Nile tilapia (Oreochromis niloticus)*. 2023, Maejo University.
20. Yang, Y., et al., *Effects of three common pesticides on survival, food consumption and midgut bacterial communities of adult workers Apis cerana and Apis mellifera.* Environmental Pollution, 2019. **249**: p. 860-867.
21. Yaman, T., Z. Yener, and I. Celik, *Histopathological and biochemical investigations of protective role of honey in rats with experimental aflatoxicosis.* BMC complementary and alternative medicine, 2016. **16**(1): p. 232.
22. RAVIKUMAR, Y., *CADMIUM (Cd) AND CHLORPYRIFOS (CPF) INDUCED TOXICOPATHOLOGY OF ENDOCRINE ORGANS IN MALE RATS.* 2020.
23. Ranneh, Y., et al., *Honey and its nutritional and anti-inflammatory value.* BMC complementary medicine and therapies, 2021. **21**(1): p. 30.
24. El Mileegy, I.M., et al., *Protective Effects of Curcumin, Coumarin and Honey against Diclofenac Sodium-induced Testicular Dysfunction in Adult Mice.* Journal of Advanced Veterinary Research, 2023. **13**(1): p. 110-122.
25. Salyha, Y.T., *Chlorpyrifos leads to oxidative stress-induced death of hippocampal cells in vitro.* Neurophysiology, 2013. **45**(3): p. 193-199.
26. Bose, D., et al., *The therapeutic mechanisms of honey in mitigating toxicity from anticancer chemotherapy toxicity: A review.* Journal of Xenobiotics, 2024. **14**(3): p. 1109-1129.
27. El Sabbouri, H.E.K., *Impacts of a combined exposition to a pesticide, Chlorpyrifos (CPF), and a High-Fat Diet on the contractile properties of the gastrointestinal smooth muscle and striated diaphragm muscle*. 2019, Université de Picardie Jules Verne; Université Libanaise.
28. Roshanravan, B., et al., *Protective effect of chrysin against chlorpyrifos-induced hepatotoxicity in rats: biochemical and histopathological approaches in a sub-chronic study.* Current Pharmaceutical Biotechnology, 2023. **24**(10): p. 1291-1296.
29. Rajabi, S., et al., *The Effect of Chrysin-nanocrystal on Oxidative Stress Indices and Histopathological changes in Kidney Tissue of Rats Exposed to Chlorpyrifos.* Current Nutrition & Food Science, 2024. **20**(8): p. 1006-1012.
30. Shokry, A., et al., *Acute toxicity assessment of polyaniline/Ag nanoparticles/graphene oxide quantum dots on Cypridopsis vidua and Artemia salina.* Scientific Reports, 2021. **11**(1): p. 5336.
31. Alhumaydhi, F.A., *Biochemical studies on the protective effect of honey against doxorubicin-induced toxicity in BALB/C mice.* International Journal of Health Sciences, 2020. **14**(6): p. 31.
32. Albrakati, A., *Quercetin mitigates glyphosate kidney toxicity Protective Effects of Quercetin Against Glyphosate-Induced Nephrotoxicity in Rats: Role of Oxidative Stress, Inflammatory Response, and Apoptotic Pathways.* Frontiers in Veterinary Science, 2025. **12**: p. 1624763.
33. Machado, A., et al., *Selected honey as a multifaceted antimicrobial agent: review of compounds, mechanisms, and research challenges.* Future Microbiology, 2025. **20**(7-9): p. 589-610.
34. Detcheverry, F., et al., *Changes in levels of the antioxidant glutathione in brain and blood across the age span of healthy adults: A systematic review.* NeuroImage: Clinical, 2023. **40**: p. 103503.
35. Tlak Gajger, I., et al., *Antioxidant capacity and therapeutic applications of honey: Health benefits, antimicrobial activity and food processing roles.* Antioxidants, 2025. **14**(8): p. 959.
36. Alijaniha, F., et al., *Protective effects of Melissa officinalis against Chlorpyrifos-induced hepatotoxicity in rat: a dose-dependent evaluation of biochemical, histological, and oxidative stress markers.* Toxicology Research, 2025. **14**(5): p. tfaf114.
37. Mansour, S.A. and A.-T.H. Mossa, *Oxidative damage, biochemical and histopathological alterations in rats exposed to chlorpyrifos and the*

antioxidant role of zinc. Pesticide Biochemistry and Physiology, 2010. **96**(1): p. 14-23.

38. Ambali, S., et al., *Evaluation of subchronic chlorpyrifos poisoning on hematological and serum biochemical changes in mice and protective effect of vitamin C.* The Journal of Toxicological Sciences, 2007. **32**(2): p. 111-120.
39. Erejuwa, O.O., et al., *Honey supplementation in spontaneously hypertensive rats elicits antihypertensive effect via amelioration of renal oxidative stress.* Oxidative medicine and cellular longevity, 2012. **2012**(1): p. 374037.
40. Rani, A. and S. Verma, *Phytochemical Composition, Antioxidant, and Antimicrobial Potential of Honey: A Comprehensive Review.* INTERNATIONAL JOURNAL OF PLANT AND ENVIRONMENT, 2025. **11**(01): p. 10-19.
41. Obia, O. and E. Asuquo, *Endogenous Antioxidant Responses to Dietary Honey Supplementation in Alloxan induced Diabetic Wistar Rats.* IOSR Journal of Nursing and Health Science, 2018: p. 37-40.
42. M Alvarez-Suarez, J., F. Giampieri, and M. Battino, *Honey as a source of dietary antioxidants: structures, bioavailability and evidence of protective effects against human chronic diseases.* Current medicinal chemistry, 2013. **20**(5): p. 621-638.
43. Saad, B., *Immunomodulatory and Anti-Inflammatory Properties of Honey and Bee Products.* Immuno, 2025. **5**(2): p. 19.
44. Gok, G., et al., *Evaluation of Glutathione (GSH) System in Porcine Saliva: Validation and Application of Colorimetric Method.* Antioxidants, 2024. **13**(10): p. 1231.
45. Neeta Dave, N.D., P.S. Pritika Sanadhya, and V. Patel, *Effect of feeding honey on the serum antioxidant status of young females.* 2014.
46. Abdallah, F.B., et al., *Dimethoate-induced oxidative stress in human erythrocytes and the protective effect of vitamins C and E in vitro.* Environmental toxicology, 2011. **26**(3): p. 287-291.
47. Mesquita, B.R., et al., *LYMPHOCYTE LOSS AND PLASMACYTOSIS ARE ASSOCIATED WITH IL-6- AND TNF-PRODUCING CELLS IN THE SPLEENS OF FATAL COVID-19 CASES.* Frontiers in Cellular and Infection Microbiology. **15**: p. 1645378.
48. IGADO, O.O., *AMELIORATIVE EFFECT OF MORINGA OLEIFERA LEAVES ON VANADIUM-INDUCED NEUROTOXICITY IN MICE*. 2018.
49. Rehman, S. and M. Waliullah, *Chlorpyrifos-induced neuro-oxidative damage in bee.* Toxicology and Environmental Health Sciences, 2012. **4**(1): p. 30-36.
50. Nolte, T. and J.E. Markovits, *Gastrointestinal System*, in *Toxicologic Pathology*. 2024, CRC Press. p. 38-81.
51. Camin, F., *DEVELOPMENT OF A NEXT-GENERATION IN VITRO PLATFORM FOR IN-DEPTH EVALUATION OF ALTERNATIVE SUSTAINABLE FISH FEED FORMULATIONS.* 2025.
52. El-Bendary, H., et al., *Histopathological changes associated with exposure of male mice to profenofos and chlorpyrifos.* Annual Research & Review in Biology, 2014. **4**(5): p. 766.
53. Ezz El-Arab, A.M., et al., *Effect of dietary honey on intestinal microflora and toxicity of mycotoxins in mice.* BMC Complementary and Alternative Medicine, 2006. **6**(1): p. 6.
54. Esmaeili, A. and O. Ahmadi, *Oxidative Stress and Endocrine Disruption Following Exposure to Chlorpyrifos.* Caspian Journal of Health Research, 2025. **10**(2): p. 93-100.