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Effect of Honey on Permethrin-induced Hepatotoxicity in Adult Male Wistar Rats

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ABSTRACT

This study examined the protective effects of honey on permethrin-induced hepatotoxicity in adult male Wistar rats. Forty Wistar rats were divided into four groups: Group A (control) received 0.1 ml of normal saline, Group B (permethrin only) was administered permethrin at 1000 mg/kg, Group C (honey only) was administered honey (0.7 ml/kg) and Group D received a combination of permethrin (1000 mg/kg) and honey (0.7 ml/kg) daily for 28 days. Following the administration, the rats were anesthetized, and liver tissues were harvested for histological, biochemical, and oxidative stress analyses. Key biomarkers, including superoxide dismutase, glutathione peroxidase, malondialdehyde, alanine aminotransferase, alkaline phosphatase, and aspartate aminotransferase, were assessed to evaluate oxidative stress, lipid peroxidation, and liver functions. Results revealed that permethrin exposure significantly elevated lipid peroxidation and depleted endogenous antioxidant levels. Co-administration of honey mitigated these effects, as evidenced by improved antioxidant status, reduced lipid peroxidation, and restoration of hepatic architecture. Honey exhibited a protective mechanism by counteracting oxidative damage and preserving liver morphology in permethrin-induced hepatotoxicity. This study highlights the potential of honey as a natural therapeutic agent against xenobiotic-induced liver damage.

Keywords: hepatotoxicity, honey, oxidative stress, permethrin

INTRODUCTION

The liver is a crucial organ in maintaining homeostasis and detoxifying harmful substances. Hepatotoxicity refers to liver toxicity that occurs when the liver is exposed to harmful substances or conditions that cause damage[1]. Hepatotoxicity, or liver toxicity, is a significant concern in both human and veterinary medicine due to the central role of the liver in metabolizing xenobiotics, including environmental pollutants, pharmaceuticals, and pesticides[2].

Various medications, including those commonly administered in research settings (in this case, permethrin), can lead to hepatotoxicity in Wistar rats. Some drugs cause direct toxic effects on liver cells, while others induce liver damage through metabolic or immune-mediated pathways [3,4]. Pesticides, particularly pyrethroids like permethrin, are widely used globally in agriculture, pest control, public health, and even in some therapeutic products [5]. Although these chemicals are effective in eliminating pests, their potential to induce toxicity in non-target species, including mammals, raises serious health concerns[6]. The hepatotoxic effects of chemical agents may involve different mechanisms of cytolethality[7]. These mechanisms may have either a direct effect on organelles like mitochondria, endoplasmic reticulum, the cytoskeleton, microtubules, and nucleus or an indirect effect on cellular organelles through the activation and inhibition of signaling kinases, transcription factors, and gene-expression profiles[1].

Permethrin is known to accumulate in the liver, leading to hepatotoxicity characterized by oxidative stress, inflammation, and cellular damage[8]. Significant changes, such as swelling in hyperchromatic nuclei, hepatic parenchymal cells, and the swelling of proximal tubules in the kidneys, have been observed in the tissue structures of the liver and kidneys[9]. Permethrin exposure has been associated with various adverse effects, including neurotoxicity, reproductive toxicity, and hepatotoxicity[10]. Histopathological changes such as hepatocellular degeneration, necrosis, and inflammation have also been reported[11].

Liver injury can be diagnosed by certain enzymes like alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and bilirubin. Elevations in these serum enzyme levels are taken as the relevant indicators of liver toxicity. In addition, several oxidative stress and inflammatory markers, such as malondialdehyde (MDA), glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase, etc., have been implicated in the diagnosis and explanation of hepatotoxicity.

Honey, a natural sweetener with ancient origins, has been valued by humans for millennia. Its antioxidant, anti-inflammatory, and antimicrobial properties have gained scientific attention for their protective effects against various forms of toxicity, including liver damage[12, 13]. However, no studies have specifically investigated its potential to counteract permethrin-induced liver damage.

The antioxidant effects of honey are primarily attributed to a diverse array of bioactive compounds, including Phenolic compounds, which are among the most significant contributors to the antioxidant capacity of honey. Phenolic acids and flavonoids, such as caffeic acid, p-coumaric acid, and pinocembrin, have been identified in various honey types and are known for their potent antioxidant properties[14, 15]. Enzymes, vitamins, organic acids, amino acids, and carotenoids are also found in honey and have been shown to enhance its antioxidative properties [15].

This study addresses this gap by evaluating the capacity of honey to ameliorate chronic permethrin-induced liver toxicity in Wistar rats, combining histopathological analysis with molecular assessments of oxidative stress. The investigation contributes to developing natural intervention strategies against pesticide-related hepatotoxicity.

MATERIALS AND METHODS

Laboratory animals and care

The study was approved by the University Ethical Review Committee. Forty (40) adult male Wistar rats weighing approximately 80-200 g were bought from the Veterinary Medicine Department, University of Ilorin. The animals were acclimatized for 14 days to reduce stress and allow them to adapt to the new hygienic environment. The rats were treated and maintained under standard environmental conditions (22-26◦C, 50-60% and 12-hour dark/light cycle).

Treatment of animals

Rambo insect powder (Rambo®; Gongoni Co. Ltd, Kano, Nigeria) containing 0.6% permethrin was used for this study. The animals were divided into 4 groups (A-D), each containing 10 rats. Treatment was for 28 consecutive days. Group A received a standard diet and was administered normal saline; Group B received a standard diet and was administered 1000 mg/kg of permethrin [16]; Group C received a standard diet and was administered 0.7 ml/kg; and Group D received a standard diet and was given both permethrin (1000 mg/kg) and honey (0.7 ml/kg).

Collection of samples

The weight of the animals was taken and recorded throughout the period of the experiment at weekly intervals. After 28 days of treatment, the animals were sacrificed after anaesthesia using a ketamine injection. The rats meant for biochemical studies were sacrificed by cervical dislocation. The liver of the animals was removed and weighed after the sacrifice.

Biochemical analysis

Homogenates of the liver were prepared using ice-cold 30% sucrose as the medium. The homogenates were carefully poured into 5 ml plain specimen bottles and then placed in the centrifuging tube and centrifuged at 3000 revolutions per minute for 5 minutes. Thereafter, the supernatant of the homogenates was decanted into separate plain specimen bottles to facilitate further biochemical analysis of oxidative markers (superoxide dismutase, glutathione peroxidase, and malondialdehyde) and liver enzymes (alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase) using appropriate biochemical kits.

The rats for histological studies were fixed in 10% formalin and processed for histology, embedded in paraffin, and sectioned at a thickness of 5 µm with the aid of a rotary microtome. The tissues were stained with hematoxylin and eosin (H&E) stain for general histology of the liver.

Data analysis

The data obtained were analyzed and expressed as mean ± standard error of mean (mean ± SEM). Means were compared by analysis of variance (ANOVA) followed by Bonferroni's Post hoc test. $P<0.05$ was taken as statistically significant.

RESULTS

The difference in initial and final weights of the animals was calculated for the control, permethrin only, honey only, and permethrin + honey groups. Also, the control group had the highest body weight gain difference, followed by the rats administered with honey only. The permethrin-only group had very low weight gain, while rats that received both permethrin and honey had the least weight gain (Table 1).

Table 1: **Body weight changes of Wistar rats**

Groups	Initial weight	Final weight	Weight difference
Control	152.63±6.66	223.0±15.12	70.4 ± 6.72
Permethrin	145.6±7.05	184.4±9.15	38.8± 4.70
Honey	148.7±7.44	214.5±8.63	65.8±4.64
Perm+ Honey	166.9±9.75	199.1±12.34	32.2± 6.47

The liver weight was significantly higher in the honey-treated group compared to other groups, with the permethrin-only group showing the lowest liver weight (Figure 1). The liver index showed the highest value in the honey group (Figure 2).

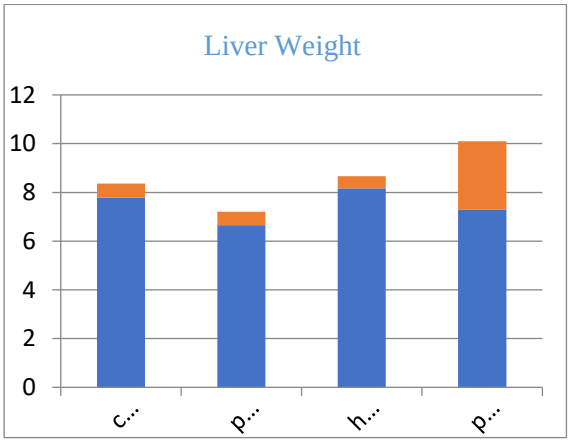

Figure 1: Effect of permethrin and honey on liver weig in adult male Wistar rats

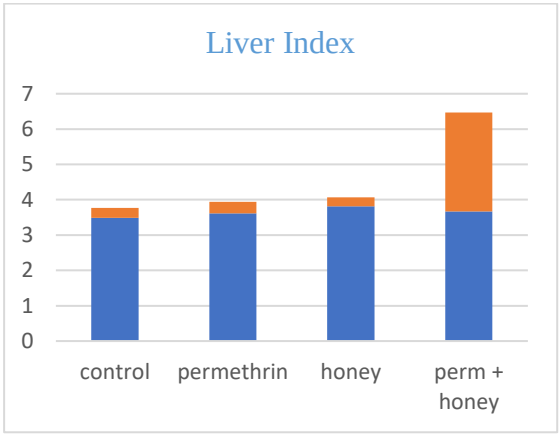

Figure 2: Effect of permethrin and honey on liver index in permethrin-induced hepatotoxicity in adult male Wistar rats.

Observation of the oxidative status of the liver following treatment

The lowest SOD level was observed in the permethrin group, indicating severe oxidative stress compared to the control group. However, the SOD level was increased significantly in the honey group compared to the permethrin group. The permethrin + honey group showed a slight increase compared to the permethrin group (Figure 3). There was a significant increase in MDA level in the permethrin group compared to the control group. The honey group showed the lowest MDA levels compared to the control, permethrin, and permethrin + honey groups. However, the permethrin + honey group also had a lower MDA level than the permethrin group (Figure 4). A significant decrease in GPx level was observed in the permethrin group compared to the control group. However, in the honey and permethrin + honey treated groups, a significant increase was observed (Figure 5).

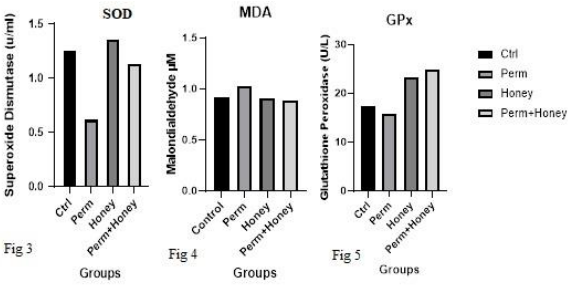

Figure 3: Effect of honey and permethrin on superoxide dismutase (SOD) in permethrin-induced hepatotoxicity in adult male Wistar rats. **Figure 4:** Effect of honey and permethrin on malondialdehyde (MDA) in permethrin-induced hepatotoxicity in adult male Wistar rats. **Figure 5:** Effect of honey and permethrin on glutathione peroxidase (GPx) in permethrin-induced hepatotoxicity in adult male Wistar rats.

Effects of honey on liver enzymes in permethrin-induced hepatotoxicity

Alkaline phosphate (ALP) was significantly increased in the permethrin group compared to the control, indicating liver damage. However, there was a significant reduction in the honey group and the permethrin + honey group (Figure 6). Alanine aminotransferase (ALT) was highest in the permethrin group compared to the others. However, the honey group showed similar ALT levels to the control group. The permethrin + honey group had the lowest ALT level (Figure 7). Aspartate Aminotransferase (AST) was significantly increased in the permethrin group compared to the control group. The lowest AST level was observed in the honey group. However,

the permethrin + honey had reduced AST levels compared to the permethrin group (Figure 8).

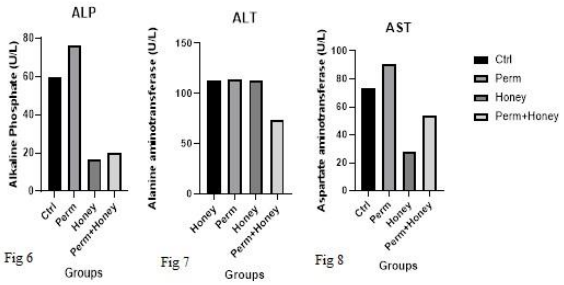

Figure 6: Effect of honey and permethrin on alkaline phosphate (ALP) in permethrin-induced hepatotoxicity in adult male Wistar rats. **Figure 7:** Effect of honey and permethrin on alanine aminotransferase (ALT) in permethrin-induced hepatotoxicity in adult male Wistar rats. **Figure 8:** Effect of Honey and Permethrin on aspartate aminotransferase (AST) in permethrin-induced hepatotoxicity in adult male Wistar rats.

Microarchitectural observation

The liver histology in the control group showed well-preserved hepatic architecture. Hepatocytes were well arranged with prominent nuclei and normal cytoplasm, and sinusoids appeared normal, maintaining proper spacing and integrity. The central vein and portal triad were intact, without congestion or inflammation. The permethrin group demonstrated evidence of hepatocyte degeneration with loss of normal cellular architecture, increased cytoplasmic vacuolization indicating fatty changes (steatosis), cellular swelling and ballooning degeneration suggesting toxic damage, sinusoidal dilation and congestion due to hepatic stress, and blood pooling and possible necrosis or apoptosis characterized by cell shrinkage, nuclear fragmentation, and loss of structure. There was a rupture of the bile ducts, with the contents leaking into the surrounding parenchyma; central vein borders appeared fragmented, and sinusoids exhibited abnormal widening.

The liver of the rats administered with honey showed minimal to no histological damage. The hepatocytes were well-arranged with normal nuclei and cytoplasm; normal sinusoids with no congestion or dilation; no inflammatory cell infiltration nor necrotic areas. The permethrin + honey group showed an elongated bile duct lumen without signs of rupture. Other observations included reduced hepatocyte damage compared to the only group, less vacuolization and steatosis, lower inflammatory cell infiltration, mild sinusoidal congestion, and partial hepatocyte regeneration. At high magnification, hepatocellular integrity was assessed to determine viable and damaged hepatocytes with the presence of nuclear distortion and compromised cell boundaries. The Permethrin group exhibited the highest prevalence of damaged hepatocytes, characterized by irregular nuclear morphology and disrupted cellular membranes (Figure 9).

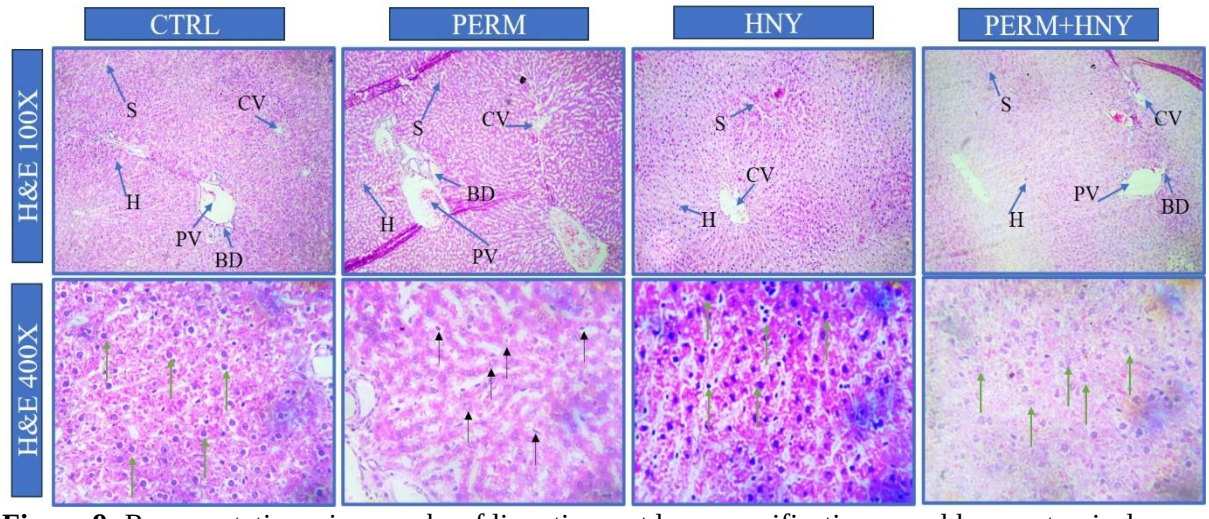

Figure 9: Representative micrographs of liver tissue at low magnification reveal key anatomical

structures, including portal veins (PV), central veins (CV), hepatocytes (H), sinusoids (S), and bile ducts (BD) across different experimental groups: Control (CTRL), Permethrin-treated (PERM), Honey-treated (HNY), and Permethrin + Honey (PERM+HNY). At high magnification, green arrows denoted viable hepatocytes and black arrows showed damaged hepatocytes with nuclear distortion and compromised cell boundaries; Hematoxylin & Eosin.

DISCUSSION

This study revealed significant variations in body weight, liver weight, and liver index across the experimental groups. Permethrin-induced weight loss was reported, similar to an earlier observation that it causes dose-dependent reduction in body weight[17, 18]. The reduction in liver weight in permethrin-exposed rats, compared to other groups, was a pointer to the structural and biochemical damage in the organ. An increase in basal metabolic rate is a possible explanation for the loss in weight observed in this study[19]. According to Kondo *et al.* [20], a decrease in body weight and liver weight suggests that permethrin exposure impairs normal growth and reduces liver mass, likely due to oxidative stress-induced cellular damage. Meanwhile, Himtas *et al*. [21] reported an increase in body weight following permethrin exposure. The rats that received honey concurrently with permethrin demonstrated increased body weight and liver weight, indicating that honey mitigates the adverse effects of permethrin on growth and liver health. These findings align with previous studies showing that antioxidant-rich diets improve liver function and organ-to-body weight ratios by reducing oxidative damage[22, 23].

Permethrin exposure resulted in a reduction in SOD and GPx activity while elevating MDA. This is an indication of oxidative imbalance. Elevated MDA signifies an increase in lipid peroxidation, which is detrimental to liver function and the body in general. Similar findings have been reported in other studies linking pesticides to oxidative stress[9, 24]. Himtas *et al*. [21] also reported a 75% increase in MDA level in permethrin-treated mice, indicating the generation of reactive oxygen species (ROS), which depleted endogenous antioxidants. Honey treatment, as shown in the current study, significantly improved oxidative stress markers by increasing SOD and GPx activity and reducing MDA levels. These antioxidant effects can be due to the rich phenolic compounds, such as flavonoids, that can be found in honey[15]. However, the combination of honey with permethrin also showed partial improvements in these parameters, although not as pronounced as honey alone.

As part of the clinical and diagnostic determination of liver functions, some enzymes that are usually assessed are alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST). Hepatocellular injury is characterized by elevation of ALT and AST, with ALT being more liver-specific, while an increase in ALP is a pointer towards cholestasis[25]. These enzymes are important markers that indicate hepatocellular injury, necrosis, and hepatocytolysis[26]. We reported that permethrin exposure led to an increase in ALT, AST, and ALP, while administration of honey significantly reduced the level of these enzymes in the liver, even to levels lower than the control. Previous studies revealed that permethrin causes hepatotoxicity through DNA damage and mitochondria disruption[18, 27, 28].

The results of the oxidative damage and biochemical changes in the liver manifest morphologically in the organ, and these were demonstrable histologically in the current study. Extensive alterations were demonstrable in the liver structure in the rats exposed to permethrin, suggestive of tissue injury. Degenerative changes in hepatocytes, loss of cellular microarchitecture, nuclear fragmentation, vacuolization, sinusoidal dilation, ballooning degeneration, and rupture of bile ducts were some of the features in the liver. By reducing

lipid peroxidation and improving the oxidative status of the liver structure, administration of honey substantially mitigated permethrin-induced hepatotoxicity in rats.

CONCLUSION

This study concludes that honey effectively mitigates permethrin-induced hepatotoxicity by improving body weight gain, enhancing antioxidant defenses, reducing lipid peroxidation, and stabilizing liver enzyme levels. These findings highlight the therapeutic potential of natural antioxidants like honey in managing pesticide-induced liver damage while also emphasizing the need for further research into optimal dosing strategies.

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