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Honey Inhibits the Production of Pro-Inflammatory Cytokines and associated Neurobehavioral Impairment in Lead-exposed Male Wistar Rats

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ABSTRACT

The widespread neurotoxic impact of lead (Pb) continues to pose a threat to global health, primarily due to its ability to disrupt oxidative balance and induce neuroinflammatory effects. In response, the need for natural therapeutic agents is pressing. Honey is a well-known natural product with anti-inflammatory and antioxidant properties. This study investigated the neuroprotective effect of honey against lead-induced neurotoxicity in male Wistar rats. Male rats were randomly assigned to three groups (n = 7): the control, the Pb-treated, and the Pb + honey groups. Behavioral assessments were conducted using the Open Field Test (OFT), Elevated Plus Maze (EPM), and three-chamber sociability test to examine the anxiety and social behavior. Biochemical and hematological analyses were performed to determine the levels of interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α), as well as total antioxidant capacity (TAC), and hematological indices. The results revealed a significant increase in anxiety and a decrease in social behavior in the Pb-only group. Biochemically, there was a marked elevation in IL-1β and TNF-α, with a significant reduction in TAC in these rats. Rats co-treated with Pb and honey demonstrated reduced anxiety and improved social behavior, decreased levels of pro-inflammatory cytokines, restored antioxidant capacity, enhanced lymphocyte levels, and increased Neutrophil to Lymphocytes Ratio (NLR). These findings suggest honey has a neuroprotective effect against lead-induced neurotoxicity, via restoration of oxidative balance and suppression of inflammation.

Keywords: lead, neurotoxicity, inflammation, honey, neuroprotective

INTRODUCTION

Lead is a hazardous heavy metal derived from the Latin word *plumbum.* It is a widespread environmental poison whose use dates back to ancient times. Lead is a highly persistent toxin found in various sources, including contaminated drinking water, paints, batteries, water pipes, cosmetics, jewelry, tobacco smoke, fuel additives, lead crystal, contaminated candy, lead-glazed ceramics, and traditional folk remedies[1]. It is a non-biodegradable element[2] that exhibits a marked tendency to accumulate in soft tissues, particularly the brain, where it exerts deleterious effects[3,4].

Studies have indicated that lead exposure increases the risk of cancer[5]. Acute and chronic lead contact have been linked to neurological disorders, including learning and memory deficits, impaired neurobehavioral function, and motor function[4,6], as well as neurodegenerative changes in human and animal models (4). Additionally, alterations in cerebellar morphology have been recorded following lead intake[7].

The underlying mechanisms of lead-induced neurotoxicity are multifactorial[4,8]. The presence of lead in the brain has been linked with oxidative stress as well as activation of microglial cells, which precipitates inflammation and excessive generation of pro-inflammatory cytokines[9]. Another major target of lead is calcium signaling. By mimicking calcium ions, lead interferes with calcium-dependent cellular processes, including neurotransmitter release and synaptic plasticity[8,10]. All of these processes culminate in the detrimental effects of lead on brain functions.

Honey is a viscous semi-solid solution produced by honey bees - *Apis mellifera* - from floral nectar[11,12]. It is a complex, nutritious substance composed of 80-85% carbohydrates and 15-17% water, with protein and ash accounting for 0.3% and 0.2%, respectively[12,14]. Additionally, honey contains a modest amount of amino acids, phenols, pigments, vitamins, and trace elements such as calcium, magnesium, potassium, phosphorus, iron and manganese[12,15]. It is a natural sweetener that exhibits resistance to spoilage, likely due to its elevated level of sucrose[12].

Traditionally, honey has been used in various settings for the treatment of different conditions, including sore eyes, wounds, sunburn, coughs, tonsillitis, fatigue, and gastrointestinal infections[12]. Several studies in recent times have also highlighted the antioxidant and anti-inflammatory roles of honey. Honey contains phytochemicals with antioxidant properties, such as polyphenols, which are plant-based compounds with antioxidant, anti-inflammatory, and anti-cancer properties, as well as flavonoids, and enzymes like catalase and glucose oxidase that can help prevent oxidative stress[12,16,17].

Studies have shown that honey scavenges free radicals, modulates inflammatory cytokine levels, and protects neuronal integrity in models of neurotoxicity and neurodegeneration[18,19]. A preliminary study conducted in our laboratory indicated that the administration of honey at high and low dosages increases the levels of superoxide dismutase (SOD), reduced glutathione (GSH), glutathione-s-transferase (GST), and catalase in rats exposed to lead[19]. The study also demonstrated that honey administration resulted in improved memory and locomotor activity, concomitant with reduced anxiety in the lead-exposed rats[19].

However, this pilot investigation had a limited scope in the outcome measures, justifying a more detailed exploration of the therapeutic effects of honey on lead-induced neurotoxicity. Therefore, this study seeks to examine the neuroprotective potential of honey beyond its established impacts on anxiety, cognition, and antioxidant activity. This research further examines its influence on social behavior, inflammatory responses, and hematological parameters in rats subjected to lead.

MATERIALS AND METHODS

Animals

Twenty-one (21) adult male Wistar rats with body weights ranging from 113 - 208 g were used for this study. The rats were obtained from McTemmy Concepts in Ogbomosho, Oyo State, Nigeria. The rats were housed in well-ventilated plastic cages with wire covers in a clean experimental environment within the physiology department of the University of Ilorin. All the animals were fed with standard rat pellets obtained from Ogo-oluwa feeds, Sango road, Ilorin, Kwara State, Nigeria, and allowed access to water *ad libitum*. The animals were allowed to acclimatize for 14 days before the commencement of the experiment. The experimental protocol and animal handling were conducted following the guidelines provided by the University of Ilorin Ethical Review Committee.

Experimental design

The rats were randomly divided into three groups (n = 7); group I (control) were allowed *ad libitum* access to tap water throughout the experiment; group II, designated as the 'lead-treated group', received water containing 0.5% lead for 29 days to induce lead neurotoxicity; while group III received water containing 0.5% lead with daily simultaneous administration of 0.2 ml of honey orally for 29 days. The drinking water was refilled upon exhaustion by the rats or replaced twice daily with fresh solution. At the end of the experiment, all rats were sacrificed after 29 days of maintenance on the test solutions.

Chemicals

Honey (UNILORIN Honey) was obtained from the Honey Research Institute, University of Ilorin, Ilorin, Kwara State, Nigeria, while Lead chloride was a product of Tianjin Kemio Chemical Reagent Co., Ltd. China.

Preparation of 0.5% Lead Solution

Using a well-calibrated, sensitive electronic weighing scale (Model: FA2104A**,** Shanghai Jingtian Electronic Instrument Co., Ltd., China), 5g of $PbCl_2$ was weighed and added to 1 liter of distilled water. The solution was thoroughly mixed. This procedure was done a number of times throughout the course of the experiment. This solution was administered orally via drinking water to rats in the experimental groups.

Behavioral Assessments

Behavioral assessments were carried out to evaluate the effects of lead exposure and honey intervention on various behavioral domains, including anxiety-like behavior and social interaction. These tests include the open field test (OFT), elevated plus maze (EPM), and novel object recognition test. The tests were conducted in a quiet, controlled environment under uniform lighting and temperature conditions. All tests were performed during the light phase of the light/dark cycle and 1hour post-administration for each rat to ensure consistency. Between trials, all apparatuses were cleaned thoroughly with 70% ethanol to eliminate olfactory cues and prevent influence from previous subjects.

Open Field Test

The Open Field Test (OFT) was conducted using the open-field box. The apparatus consisted of a square arena (typically 100 × 100 cm) with high opaque walls and a non-reflective floor divided into equal squares. Each rat was placed in the center and allowed to explore freely for 5 minutes. The activities of each rat were videotaped by a webcam for later analysis. Parameters observed included the number of line crossings, time spent in the center versus periphery, and grooming behavior.

Elevated Plus Maze

The elevated plus maze (EPM) is a four-armed platform formed like a plus symbol and positioned above the ground. Two of the arms are open while the other two are closed. Each rat is placed in the center of the maze and allowed to move freely to

any of the arms for 5 minutes. The movements of each rat were recorded using a web camera mounted directly above the Elevated plus maze apparatus. The parameters evaluated from the recorded video are: times spent at the center, the open arms, and the closed arms.

Three-Chamber Box

This apparatus was used to evaluate social behavior in the rats. The apparatus used was made up of a rectangular box (60 cm × 40 cm × 22 cm) divided into three chambers of equal size (20 cm × 40 cm each) with two rectangular glasses of equal length and height. Each glass has a small doorway leading to each chamber. Two identical iron cages were placed in the two side chambers. The test was conducted in two phases. In phase 1, habituation was done by allowing the rat to freely explore all three empty chambers for 10 minutes to acclimate to the environment. Phase 2 involves the sociability test. A strange rat was enclosed in an iron cage in one side chamber, while the other side cage contained a small object. The test rat was reintroduced into the center chamber and allowed to explore for 10 minutes. The time spent in each chamber and the time spent sniffing each cage were recorded to evaluate how sociable the rat is. The sociability index was calculated using the formula:

$$\text{Sociability Index} = \frac{\text{Time spent with strange rat} - \text{Time spent with the object}}{\text{Time spent with strange rat} + \text{Time spent with the object}}$$

Sample collection

At the end of the experimental period, the animals were sacrificed on the 29th day. Each rat was anaesthetized with chloroform. The heart was exposed through a thoraco-abdominal cut, and blood samples were collected via cardiac puncture using a 5 mL sterile syringe and needle. The blood samples were collected into EDTA bottles and plain bottles for hematological parameters and biochemical analysis, respectively. The blood samples in the plain bottles were centrifuged at 3000 rpm for 15 minutes to separate the serum, which was stored at -20°C until used for further study.

Quantification of serum pro-inflammatory cytokines

The levels of serum Interleukin-1 beta (IL-1β and Tumor Necrosis Factor-alpha (TNF-α) were determined using the sandwich enzyme-linked immunosorbent assay (ELISA) technique using TNF-α ELISA Kit (Cat. No. E-EL-R0019) and IL-1β ELISA Kit (Cat. No. E-EL-R0012), both obtained from Elabscience Biotechnology Co., Ltd., Wuhan, China. Briefly, micro-ELISA plates pre-coated with monoclonal antibodies specific to rat TNF-α or IL-1β were used. Standards and serum samples were pipetted into the wells, allowing the cytokines to bind to the immobilized antibodies. A biotinylated detection antibody was then added, followed by an Avidin-Horseradish Peroxidase (HRP) conjugate. Following incubation and thorough washing to remove unbound components, a chromogenic substrate solution was added to each well. A blue color developed in wells containing the cytokine-antibody-HRP complex, which turned yellow after the addition of stop solution. The optical density (OD) was measured at 450 nm ± 2 nm using a microplate reader. The concentrations of TNF-α and IL-1β in the serum samples were calculated by comparing the OD values to a standard curve constructed from known concentrations of each cytokine.

Measurement of Serum Total Anti-Oxidant Capacity

The Total Antioxidant Capacity (TAC) of serum samples was measured using the Total Antioxidant Capacity Assay Kit (Cat. No. E-BC-K136) obtained from Elabscience Biotechnology Co.,

Ltd., Wuhan, China. This assay is based on the colorimetric principle in which antioxidants in the sample reduce ferric (Fe^{3+}) ions to ferrous (Fe^{2+}) ions. The Fe^{2+} then forms a stable complex with a chromogenic agent, producing a colored solution whose intensity is directly proportional to the antioxidant capacity of the sample. Serum samples were mixed with the chromogenic working reagent and incubated at the recommended temperature and time conditions. The resulting colored complex was measured spectrophotometrically at 520 nm using a microplate reader. A standard curve was generated using known concentrations of Trolox (a water-soluble vitamin E analog), and the TAC values of the samples were extrapolated and expressed in mmol Trolox equivalents per liter (mmol/L).

Hematological Analysis

Hematological parameters including red blood cell count (RBC), packed cell volume (PCV), mean corpuscular volume (MCV), white blood cell count (WBC), platelets (PLT), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were analyzed using an autohematology analyzer machine. Differential cell count was estimated using an electronic coulter (Advia-tm-60) according to Otitoloju *et al.* (2012).

Statistical Analysis

All experimental data were analyzed using GraphPad Prism software version 8.4.2 (GraphPad Software Inc., San Diego, California, USA). Results were expressed as mean ± standard error of the mean (SEM). Differences between groups were assessed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. A p-value less than 0.05 ($p < 0.05$) was considered statistically significant.

RESULTS

Administration of honey attenuates lead-induced anxiety-like behavior

The effect of honey lead exposure-induced anxiety-like behavior in was assessed using the Open Field Test (OFT) and Elevated Plus Maze (EPM). In the OFT, although no significant difference in line crossing frequency was observed among all groups, rats in the Pb-only group exhibited a significant increase ($p < 0.05$) in time spent at the periphery and grooming time, while also exhibiting a significant decrease in time spent at the center compared to the control group (Figure 1). Contrastingly, the rats administered with honey spent more time in the center and showed decreased peripheral and grooming time when compared to the rats treated with Pb only (Figure 1). This reflects a heightened anxiety-like behavior due to lead exposure, which was mitigated by honey.

Also, in the EPM test, our results show that lead administration significantly reduced ($p < 0.05$) the open arm exploration time and significantly increased ($p < 0.05$) the time spent at the center in rats treated with Pb only, compared to the control in the EPM test. Although no significant differences in time spent in the closed arms between the experimental groups were observed. Administration of honey significantly increased ($p < 0.05$) the time spent in the open arms and decreased the time spent at the center in the Pb + Honey group when compared to the Pb only group (Figure 2). Taken together, these results further support the role of honey in attenuating anxiety-like behavior induced by lead exposure

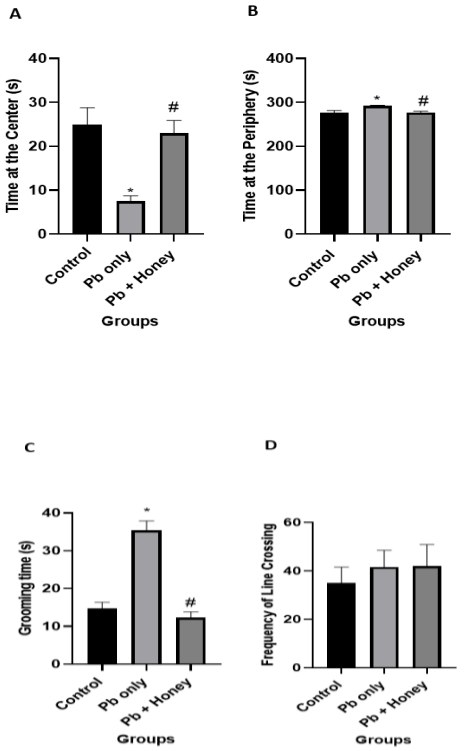


Figure 1: Open field behavioral test shows that honey reduces anxiety-like behavior in lead-exposed rats. (A) Average time spent at the center of the open field arena. (B) Average time spent in the periphery of the open field arena. (C) Average number of lines crossed within the open field arena. (D) The average number of times the rats showed grooming behavior. Each bar represents mean ± SEM (n = 7). *p < 0.05 vs control; #p < 0.05 vs Pb-only group.

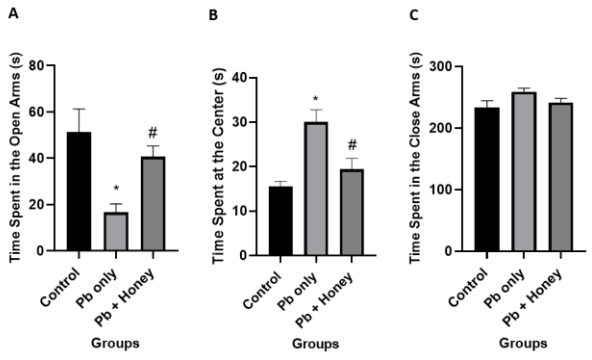
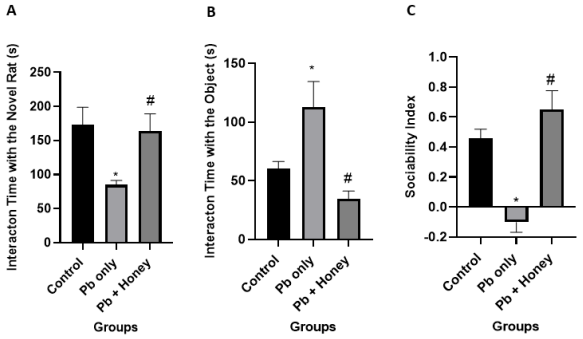


Figure 2: Behavioral test with elevated plus maze (EPM) indicated reduced anxiety-like behavior in lead-exposed rats treated with honey. (A) Average time spent in the open arm of the EPM. (B) Average time spent in the close arm of the EPM. (C) Average time spent at the center of the EPM. Each bar represents mean ± SEM (n = 7). *p < 0.05 vs control; #p < 0.05 vs Pb-only group.



Honey Ameliorates Lead Exposure-Induced Social Interaction Deficit

Assessment of social behavior across experimental groups using the three-chambered box is indicated in Figure 3. There was a significant decrease ($p < 0.05$) in time spent with the novel rat and in the novel rat chamber compared to the control group. conversely, the time spent with the object and in the novel object chamber was significantly increased ($p < 0.05$) in the Pb-treated rats when compared to the control rats. No marked changes were observed in the time spent at the center chamber across all groups. Lead administration significantly reduced ($p < 0.05$) the sociability index compared to the control rats. Honey administration resulted in contrasting effects in the Pb + honey group. Honey significantly increased the sociability index, the time spent with the novel rat and in the novel rat chamber. Similarly, a significant reduction ($p < 0.05$) in time spent with the novel rat and in the novel rat chamber was seen in the Pb + honey group. these findings indicate that honey has the potential to improve social behavior in rats exposed to lead.

Figure 3: Assessment of social behavior in control and treatment groups using a three-chamber box. (A) Time spent with a novel rat by the experimental rats. (B) Time spent with the object (C) Sociability index. (D) Time spent in the novel's rat's chamber. (E) Time spent in the center chamber by the experimental rats. (F) Time spent in the object chamber. Data presented as mean ± SEM (n = 7). *p < 0.05 vs control; #p < 0.05 vs Pb-only group.

Honey reduces the level of serum pro-inflammatory cytokines and increases total antioxidant capacity in lead-exposed rats

Lead exposure significantly increased the serum levels of IL-1β and TNF-α ($p < 0.05$), indicating a heightened inflammatory response due to lead exposure. Administration of honey significantly reduced the serum levels of IL-1β and TNF-α, suggesting an anti-inflammatory effect of honey. Additionally, serum TAC levels were significantly decreased ($p < 0.05$) in the Pb-only group compared to the control, reflecting a reduction in antioxidant levels. This was reversed in the Pb + honey group, where TAC levels significantly increased ($p < 0.05$) compared to the Pb-only group (Figure 4). These findings highlight the potential anti-inflammatory as well as antioxidant properties of honey in counteracting lead-induced toxicity.

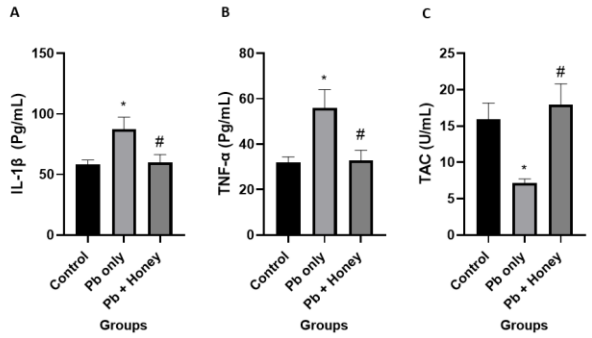


Figure 4: Evaluation of serum levels of interleukin-1 Beta (IL-1β), Tumor Necrosis Factor-alpha (TNF-α), and Total Anti-oxidant Capacity (TAC) in experimental rats. (A) serum level of IL-1β. (B) serum level of TNF-α. (C) serum total antioxidant capacity. Data presented as mean ± SEM (n = 7). *p < 0.05 vs control; #p < 0.05 vs Pb-only group.

Honey reduces the neutrophil-lymphocyte ratio in lead-exposed rats

The effect of lead and honey administration on hematological indices in experimental rats is represented in Table 1. No significant difference in red blood cell (RBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets (PLT), white blood cells (WBC), and some of its differential count, such as eosinophils, basophils, monocytes, and neutrophil was observed between the rats in all experimental groups. However, there was a marked increase ($p<0.05$) in lymphocytes in the rat cotreated with Pb and honey in comparison to the rats treated with Pb only. The neutrophil-lymphocyte ratio (NLR) in the Pb + honey group also shows a significant reduction when compared to the Pb-only group. These show that honey can modulate immune responses in rats exposed to lead.

Table 1: Hematological parameters

Groups/Index	Control	Pb Only	Pb + Honey
RBC ($\times 10^{12}$/L)	5.48 ± 0.65	5.45 ± 0.52	5.96 ± 0.59
HB (g/dL)	10.93 ± 1.15	9.996 ± 0.52	11.13 ± 1.07
PCV (%)	32.33 ± 3.49	31.80 ± 2.18	34.60 ± 3.00
MCV (fL)	59.32 ± 2.45	59.10 ± 2.59	58.38 ± 1.86
MCH (Pg)	20.03 ± 0.38	18.76 ± 1.28	19.10 ± 1.00
MCHC (g/dl)	33.98 ± 1.07	31.58 ± 0.99	32.66 ± 0.77
WBC ($\times 10^{9}$/L)	8.59 ± 0.60	6.52 ± 0.98	51.02 ± 45.00
NEUT (%)	37.33 ± 1.65	40.60 ± 1.40	34.40 ± 2.09
LYM (%)	59.83 ± 1.33	55.40 ± 0.87	63.40 ± 1.91$^{\#}$
MON (%)	2.40 ± 0.24	1.80 ± 0.37	1.50 ± 0.29
EOS (%)	1.25 ± 0.25	1.00 ± 0.00	1.25 ± 0.25
PLT ($\times 10^{9}$/L)	353.00 ± 64.78	272.20 ± 61.54	369.8 ± 80.54
NLR	0.64 (0.54, 0.72)	0.75 (0.68, 0.78)	0.60 (0.44, 0.64)$^{\#}$

Notes: n = 7; *p < 0.05 vs control; #p < 0.05 vs Pb-only group. Abbreviations: RBC, red blood cells; HB, hemoglobin; PCV, packed cell volume; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; WBC, white blood cell; NEUT, neutrophil; LYM, lymphocytes; MON, monocytes; PLT, platelets; NLR, neutrophil-to-lymphocyte ratio. Continuous variables with normal distribution are presented as mean ± standard error of mean (SEM); non-normal variables, e.g., NLR, are expressed as median (interquartile range).

DISCUSSION

The present study revealed that honey has protective potential against lead-induced neurobehavioral deficits by decreasing anxiety, improving social behavior, reducing pro-inflammatory cytokines, enhancing total antioxidant capacity, and modulating immunity by increasing the lymphocyte level and decreasing the NLR. Anxiety is a complex neurological behavior that involves the coordinated activity of different brain regions, including the amygdala, hippocampus and prefrontal cortex[20], together with the monoaminergic signaling pathways[20]. In the OFT test conducted, rats subjected to lead only exhibited a significant increase in time spent at the periphery and grooming time, accompanied by a considerable reduction in time spent at the center, indicating increased anxiety in these rats relative to the control group. Similarly, rats treated with lead only spent considerably less time in the open arms and significantly increased the time spent exploring the center of the EPM. These buttress the impact of lead on anxiety behavior and agree with previous studies that have recognized lead as a potent neurotoxicant implicated in the disruption of brain regions like the amygdala[21,22] and brain systems associated with anxiety behavior, such as the serotonergic[22] and dopaminergic systems and the hypothalamic-pituitary-adrenal (HPA) axis[23 – 25]. However, co-administration of Pb and honey produced opposite effects, further confirming the anxiolytic impact of honey on lead-induced neurotoxicity stated in our previous study[19].

Previous studies have demonstrated the deleterious influence of lead on social behavior [26,27]. For instance, Cutler (1977) found that male and female offspring of mice exposed to lead exhibited pronounced reduction in social and sexual behaviors, in contrast to the controls[26]. This may be due to an impaired ability of olfactory perception, an essential tool for social behavior in mice, resulting from lead exposure[26,27]. In the current

study, rats treated with only lead demonstrated a less significant interaction with the novel rat and spent significantly less time in the novel rat chamber, while exhibiting an appreciable interaction with the object and a markedly increased duration spent in the object chamber. Furthermore, the sociability index, which is used to measure the tendency to engage in social interactions, was significantly reduced in these Pb-treated rats. This is consistent with the observed effect in the previous study[26]. Contrastingly, rats cotreated with lead and honey showed a reversal in these parameters. This explicitly highlights the potential influence of honey in the improvement of social behaviors, consistent with a previous study that highlighted the mitigating effect of honey on lead-induced social deficits[28].

Disruption of the body's oxidative balance is a well-known consequence of lead poisoning. Lead causes excessive generation of oxidants and depletion of antioxidants, resulting in an oxidative burden that contributes to neuronal dysfunction and damage to cellular components such as lipids, proteins, and nucleic acids[8,10]. Total Antioxidant Capacity is a measure of both non-enzymatic antioxidants and enzymatic defenses in biological systems. A significant reduction in TAC following lead exposure was observed in this study, aligning with earlier reports that associated lead toxicity with decreased antioxidant reserves[8,10,19,29]. Oppositely, honey restored TAC in rats exposed to lead and honey, thus agreeing with our previous study that indicated that honey can increase the activities of antioxidant enzymes – SOD, GSH, and GST[19].

Disruption of oxidative balance has been intricately linked to neuroinflammation, with ROS acting as signaling molecules that promote the production and release of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6[9,30,31]. In this study, lead exposure resulted in significantly elevated levels of pro-inflammatory cytokines - IL-1β and TNF-α, in conformity with past reports[9,32,33]. In contrast, administration of honey markedly reduced levels of TNF-α and IL-1β. This is in line with a study that examined the ability of honey flavonoid extract in modulating the release of pro-inflammatory mediators in N13 microglial cells activated by lipopolysaccharide[34]. Findings from this study indicated that HFE notably suppresses the release of TNF-α and IL-1β while also acting as a strong suppressor of microglial activation, thereby positioning honey as a protective agent against neuroinflammation[34].

Furthermore, results from the present study showed no notable differences in lymphocyte or neutrophil levels and the neutrophil-to-lymphocyte ratio (NLR) in the rats that were subjected to lead alone. Conversely, rats given lead and honey exhibited a pronounced increase in the lymphocyte level and a sharp reduction in NLR, indicating an enhanced immunoinflammatory balance. This is in agreement with reports of the immunomodulatory properties of honey that documented the impact of honey in enhancing lymphocyte proliferation[35] and in lowering NLR[36]. However, no notable change was observed in the neutrophil levels of these rats, suggesting that the lymphocyte boost was the major contributor to the observed decline in NLR. In consideration of the findings from this study, it is evident that honey attenuated the deleterious neurological effects induced by lead toxicity, possibly due to its rich antioxidative and immunomodulatory properties. Therefore, this study demonstrated the ameliorative potential of honey against lead-induced neurobehavioral deficits in Wistar Rats.

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