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Ameliorative Effects of Honey on Petrol Vapor-Induced Oxidative Stress in the Reproductive System of Female Wistar Rats

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ABSTRACT

Background and Purpose: Oxidative stress and reproductive dysfunction have been linked to petroleum exposure. This study investigated the protective effects of honey on oxidative stress markers, reproductive hormones, and ovarian histology in female Wistar rats exposed to gasoline vapor.

Methods: Fifty-six female rats were randomly divided into seven groups including control, 6- or 12-weeks gasoline-exposed, and 6- or 12-weeks gasoline-exposed alongside honey at 330 mg/kg/day or 1000 mg/kg/day doses.

Results: Gasoline vapor exposure induced significant oxidative stress, evidenced by increased catalase and superoxide dismutase activities, and elevated malondialdehyde (MDA) levels. Hormonal analysis revealed significant alterations, particularly reduced luteinizing hormone (LH) estrogen and progesterone, and increased follicle-stimulating hormone (FSH) in exposed rats. Histology showed follicular degeneration and reduced corpora lutea. Honey treatment significantly restored antioxidant enzyme activity, normalized hormone levels, and improved follicular development and ovarian morphology.

Conclusion: These findings suggest that honey mitigates gasoline vapor-induced reproductive toxicity through its antioxidant and hormone-regulatory properties.

INTRODUCTION

Environmental pollution remains a significant public health concern globally, with petroleum and its derivatives ranking among the most common environmental contaminants (Pal and Sen, 2024). Petrol also called gasoline, a volatile hydrocarbon mixture widely used as fuel, poses serious health hazards when individuals are chronically exposed to its vapors (Abubakar *et al*., 2013a; Anigilaje *et al*., 2024). Such exposure commonly occurs in occupational settings (e.g., fuel stations, automobile mechanic workshops) and urban environments with high vehicular emissions (Saathoff *et al*., 2022). Inhalation of petrol vapor has been linked to systemic toxicity, particularly through the generation of reactive oxygen species (ROS), leading to oxidative stress (Ekpenyong and Asuquo, 2017).

Oxidative stress arises when the balance between the production of ROS and the body's antioxidant defense mechanisms is disrupted (Rotimi *et al*., 2024). This imbalance can result in lipid peroxidation, DNA damage, protein modification, and ultimately cellular dysfunction (Ekpenyong and Asuquo, 2017; Rotimi *et al*., 2024). Among various organ systems susceptible to oxidative damage, the reproductive system is particularly vulnerable (Madhu *et al*., 2022). In females, oxidative stress has been implicated in impaired folliculogenesis, hormonal imbalance, decreased fertility, and structural damage to reproductive organs such as the ovaries and uterus *(Dutta et al*., 2024). Several studies have established that petrol vapor exposure leads to reduced antioxidant enzyme levels, such as superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH), while increasing malondialdehyde (MDA), a marker of lipid peroxidation (Moronkeji *et al*., 2024). This oxidative imbalance contributes significantly to reproductive toxicity and infertility in females (Zargari *et al*., 2022).

In recent years, there has been increasing interest in the use of natural substances with antioxidant properties as therapeutic agents to combat oxidative stress-induced tissue damage (Marcucci *et al*., 2023). Honey, a natural product produced by *Apis mellifera*, has gained considerable attention due to its wide spectrum of biological and pharmacological properties, including antioxidant, anti-inflammatory, antimicrobial, and tissue-regenerating properties (de Souza *et al*., 2023). Honey is rich in flavonoids, phenolic acids, enzymes (e.g., glucose oxidase and catalase), vitamins (e.g., vitamin C and E), and trace elements (e.g., zinc and selenium), all of which contribute to its antioxidant capacity (Abubakar *et al*., 2013b; de Souza *et al*., 2023). Studies in animals have shown that honey can enhance endogenous antioxidant defense mechanisms, protect against oxidative organ damage, and improve reproductive function in various models of toxicity (Zaid *et al*., 2021).

Given the increasing environmental exposure to petrol vapor and the potential risk it poses to female fertility and reproductive function, it is crucial to explore safe, affordable, and natural therapeutic strategies. This study aimed to evaluate the ameliorative effects of honey on petrol vapor-induced oxidative stress in the reproductive system of female Wistar rats. The findings could provide valuable insights into the development of natural preventive or therapeutic measures for women at risk of hydrocarbon exposure, thereby promoting reproductive health and reducing the burden of associated infertility and reproductive disorders.

MATERIALS AND METHODS

Animals

A total of 56 female Wistar rats weighing 180-200 (9-11 weeks old) were purchased from the Animal House of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria. The animals were maintained on a standard and balanced rat diet and had free access to water. The rats were housed in well-ventilated cages and were acclimatized to the animal room conditions for one week prior to the experiment. An ethical clearance was obtained from Usmanu Danfodiyo University, Sokoto, Nigeria, ethical clearance committee (clearance number PTAC/Ho/(He)OT/72-24) prior to the commencement of the study. The animals were handled according to the international protocols for the use of animals in experiments (Kiani *et al*., 2022).

Source of Honey

The honey used was purchased from the Botanical Garden, Department of Biological Sciences, Faculty of Sciences, Usmanu Danfodiyo University, Sokoto.

Experimental Design

Experimental design was done according to Abubakar *et al*. (2013; Suku *et al*., 2023) with some modification in the treatment groups.

The 56 female rats were randomly allotted to 7 groups (n=8) using Decision Analyst (Stats 2.0) computer software as follows:

Group 1: Normal control group (NCG), given distilled water 10 mL/kg body weight daily.

Group 2: Gasoline-exposed control group 1 (PCG-1), exposed to gasoline vapor (11.1±1.1 mL/h, 6 h daily, 6 days per week) + distilled water (10 mL/kg) daily.

Group 3: Gasoline-exposed control group 2 (PCG-2), exposed to gasoline vapor (11.1±1.1 mL/h, 6 h daily, 6 days per week) + distilled water (10 mL/kg daily).

Group 4: Honey test group 1 (HTG-1), exposed to gasoline vapor (11.1±1.1 mL/h, 6 h daily, 6 days per week) + honey (330 mg/kg/day) + distilled water (10 mL/kg/day).
Group 5: Honey test group 2 (HTG-2), exposed to gasoline vapor (11.1±1.1 mL/h, 6 h daily, 6 days per week) + honey (330 mg/kg/day) + distilled water (10 mL/kg/day).
Group 6: Honey test group 3 (HTG-3), exposed to gasoline vapor (11.1±1.1 mL/h, 6 h daily, 6 days per week) + honey (1000 mg/kg/day) + distilled water (10 mL/kg/day).
Group 7: Honey test group 4 (HTG-4), exposed to gasoline vapor (11.1±1.1 mL/h, 6 h daily, 6 days per week) + honey (1000 mg/kg/day) + distilled water (10 mL/kg/day).

Groups 2, 4 and 6 were sacrificed after 6 weeks, while groups 1, 3, 5 and 7 were sacrificed after 12 weeks. Distilled water served as the vehicle for oral administration and was given uniformly to all groups to maintain consistency in dosing volume.

Source of Gasoline
Petrol/gasoline (PMS) was purchased daily from A.A. Rano filling station, located at Gidan Man Ada roundabout, Sokoto, Nigeria.

Exposure to Gasoline Vapor
The animals in groups 2 to 6 were exposed to gasoline according to a technique described by Abubakar *et al.* (2013). Briefly, the animals were housed in their cages (8 per cage). The cages were then placed in an exposure chamber measuring 100 cm × 90 cm × 150 cm (2 cages per chamber). Four calibrated 1000 mL beakers containing 500 mL of gasoline each were placed in the exposure chamber. The test animals were allowed to inhale the evaporating petrol in the chambers during the exposure period. An exposure period of 6 h (9:00 am to 3:00 pm) daily, 6 days per week, was adopted. The initial and final volumes of the liquid gasoline were respectively recorded before and after daily exposure. The daily differences in volume were used to estimate the relative concentrations of vapors used in the experiment. After the daily exposure, the animals were returned to vapor-free section of the experimental animal house.

Sample Collection
At the end of the experimental period, the animals were sedated using chloroform, blood samples were immediately taken by cardiac puncture into plain tubes for assay of the oxidative stress marker malondialdehyde (MDA) and hormones. Each blood sample was centrifuged, and serum was collected. Both ovaries were excised and washed in ice-cold normal saline. One of the ovaries was fixed in Bouin's solution for histopathological examination, while the other was homogenized and used for assessment of oxidative stress associated enzymes.

Antioxidant Assays
Superoxide dismutase (SOD) activity
Superoxide dismutase activity was determined in the ovaries using the pyrogallol method as described by Marklund and Marklund (1974), Magnani *et al.* (2000), and Hamed *et al*., (2024). Principle of the method is based on the competition between the pyrogallol (1,2,3-trihydroxybenzene) autoxidation by superoxide and dismutation of the radicals by SOD enzyme.

Catalase (CAT) activity
Catalase activity was determined in the rats ovaries according to the method described by Beers and Sizer, (1952), Kadhum and Hadwan, (2021). The method is based on the breakdown of hydrogen peroxide by the enzyme and the disappearance of peroxide is followed spectrophotometrically at 240 nm. One Unit is equal to one µmol of hydrogen peroxide decomposed per minute at 25^{o}C.

Lipid peroxidation assay
Malondialdehyde (MDA) was determined in the serum of the animals as an indicator of lipid peroxidation using thiobarbituric acid method (Ohkawa *et al*., 1979; Draper *et al*., 1993; Zeb and Ullah, 2016; Wu *et al*., 2024).

Assay for Progesterone, Estrogen, Follicle Stimulating Hormone and Luteinizing Hormone
Progesterone
The serum concentration of progesterone was determined using rat progesterone ELISA kit (PARS BIOCHEM, 188, Jiangning, Nanjing, China) according to the manufacturer's instruction (Conley *et al*., 2023). The concentration of progesterone in ng/ml was read on the microplate ELISA reader.

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The serum concentration of progesterone was determined using rat progesterone ELISA kit (PARS BIOCHEM, 188, Jiangning, Nanjing, China) according to the manufacturer's instruction (Conley *et al*., 2023). The concentration of progesterone in ng/ml was read on the microplate ELISA reader.

Estrogen
The serum concentration of estrogen was determined using rat estradiol (E2) ELISA kit (PARS BIOCHEM, 188, Jiangning, Nanjing, China) according to the manufacturer's instruction and applying similar principle as in progesterone (Gholizadeh *et al*., 2021).

Follicle stimulating hormone
The serum concentration of follicle stimulating hormone (FSH) was determined using rat FSH ELISA kit (PARS

BIOCHEM, 188, Jiangning, Nanjing, China) according to the manufacturer's instruction and applying similar principle as in progesterone (Ongaro *et al*., 2021).

Luteinizing hormone

The serum concentration of luteinizing hormone (LH) was determined using rat LH Elisa kit (PARS BIOCHEM, 188, Jiangning, Nanjing, China) according to the manufacturer's instruction and applying similar principle as in progesterone (Bernard and Ongaro, 2022).

Ovarian Tissue Preparation and Histology

Ovarian tissue samples were identified, labelled, and grossed before being placed in labelled tissue cassettes. The cassettes were then transferred into an automated tissue processor for 24 h, where the tissues underwent standard processing steps including dehydration (using graded alcohols), clearing (with xylene), and paraffin wax infiltration (Aziz and Zeman-Pocrnich, 2022).

Processed tissues were embedded in paraffin to form tissue blocks, which were sectioned at 5 µm thickness using a microtome. Sections were mounted on clean glass slides and placed on a hot plate to adhere and remove excess moisture. Slides were dewaxed, stained with hematoxylin and eosin (H&E), and dried. The stained slides were mounted with a coverslip using a mounting medium and air-dried (Aziz and Zeman-Pocrnich, 2022). Microscopic examination was carried out at ×100 magnification using Olympus CX23 Binocular Microscope. Photomicrographs were taken using Euromex CMEX- 12f pro 12 Megapixel camera and analyzed by a histopathologist.

Statistical Analysis

Data were analyzed using One-way ANOVA followed by Tukey-Kramer test for multiple comparison (GraphPad Prism version 10). Differences were considered significant at $P < 0.05$. The data are reported as mean ± SEM (standard error of mean).

RESULTS

Effects of Honey on Oxidative Stress Markers of Rats Exposed to Petrol

Exposure to gasoline vapor for 6 weeks resulted in a statistically significant increase in catalase (CAT) activity when compared to the normal control group (P=0.0002; Figure 1A). In contrast, a 12-week exposure produced only a slight, non-significant elevation in CAT activity relative to control (Figure 1B). Treatment with honey at 330 mg/kg for 6 weeks elicited a mild, non-significant reduction in CAT activity. However, administration of honey at 1000 mg/kg significantly decreased CAT activity (P<0.0001) compared not only to the 6-week gasoline-exposed group but also to the group treated with the lower honey dose (Figure 1A).

Similarly, SOD activity was significantly elevated following 6 weeks of gasoline vapor exposure compared to normal controls (P=0.0005; Figure 2A). The 12-week exposure resulted in a modest, non-significant increase in SOD activity (Figure 2B). Treatment with honey for 6 weeks led to a dose-dependent attenuation of SOD activity. Notably, both the 330 mg/kg (P = 0.0184) and 1000 mg/kg (P=0.0007) honey-treated groups exhibited significantly reduced SOD activity compared with the 6-week gasoline-exposed group. Furthermore, the high-dose honey treatment group demonstrated significantly lower SOD activity than the low-dose group (P=0.0009; Figure 2A).

Lipid peroxidation, assessed by malondialdehyde (MDA) levels, was significantly increased in rats exposed to gasoline vapor for 12 weeks (P=0.0395) compared to the normal control group (Figure 3B). Although treatment with graded doses of honey produced a dose-related reduction in MDA levels, the changes did not reach statistical significance relative to the untreated 12-week gasoline vapor-exposed group.

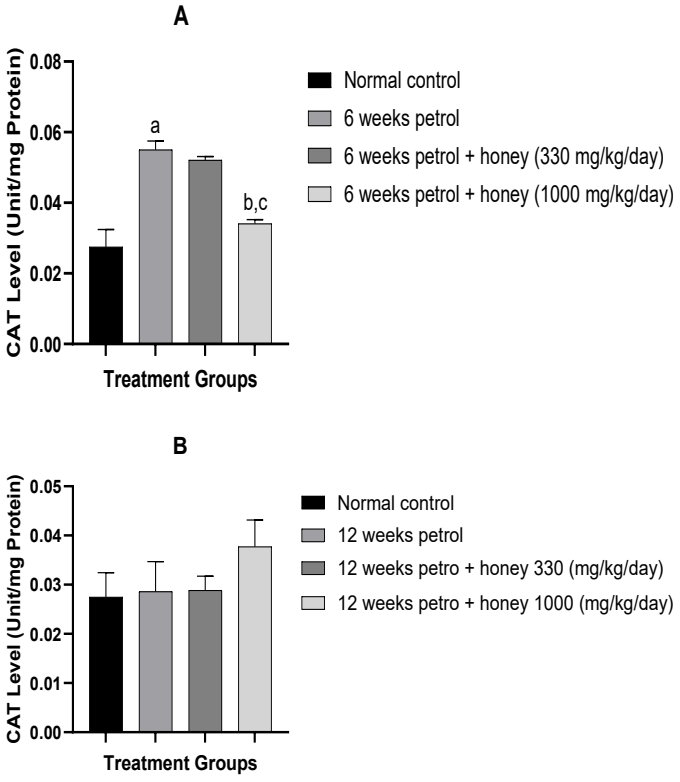

Figure 1: Effects of honey on catalase (CAT) of rats exposed to petrol vapor. Results were analyzed with one-way ANOVA followed by Tukey-Kramer post hoc test. Values are expressed as mean ± SEM, n=8, a P<0.05 compared with normal control, b P<0.05 compared with 6-week petrol vapor-exposed + 330 mg/kg honey group, c P<0.05 compared with 6-week petrol vapor-exposed group. A=6-week exposure and treatment, B=12-week exposure and treatment, CAT=catalase.

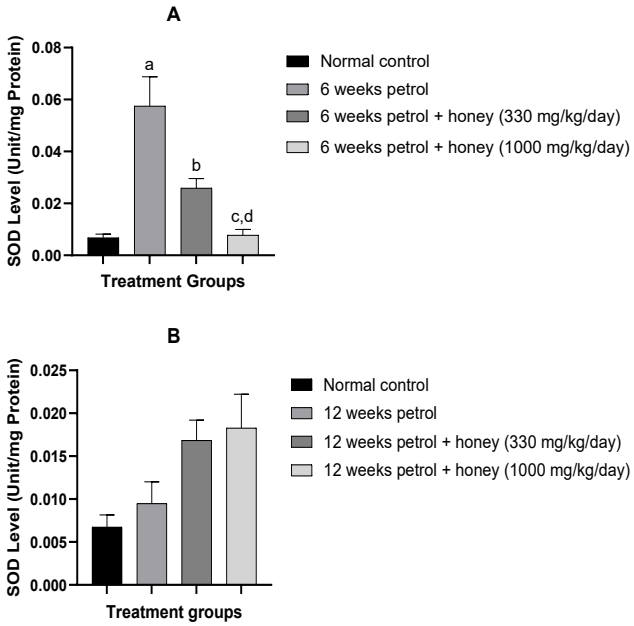

Figure 2: Effects of honey on superoxide dismutase (SOD) of rats exposed to petrol vapor. Results were analyzed with one-way ANOVA followed by Tukey-Kramer post hoc test. Values were expressed as mean ± SEM, n = 8, a $P<0.05$ compared with normal control, b $P<0.05$ compared with 6-week petrol exposed, c $P<0.05$ compared with 6-week petrol vapor-exposed + 330 mg/kg honey group, d $P<0.05$ compared with 6-week petrol vapor-exposed group. A=6-week exposure and treatment, B=12-week exposure and treatment.

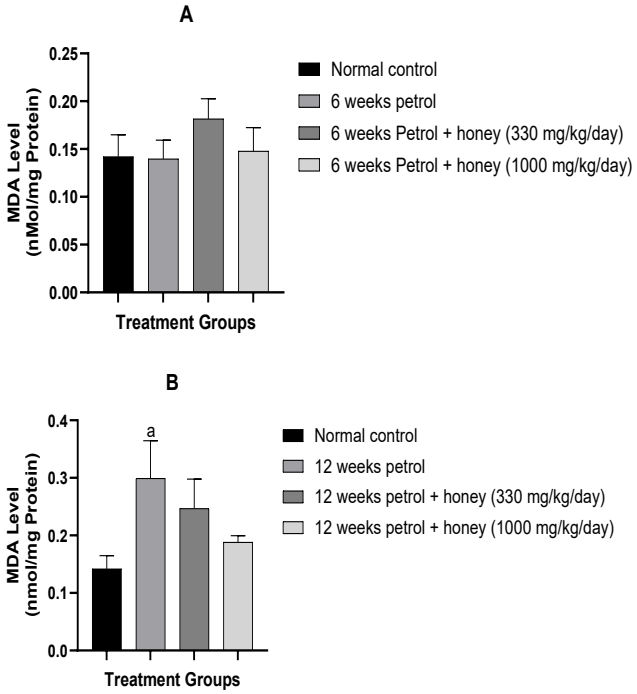

Figure 3: Effects of honey on malondialdehyde (MDA) of rats exposed to petrol vapor. Results were analyzed with one-way ANOVA followed by Tukey-Kramer post hoc test. Values were expressed as mean ± SEM, n = 8, a $P<0.05$ compared with normal control. A=6-week exposure and treatment, B=12-week exposure and treatment.

Effects of Honey on Fertility Hormones of Rats Exposed to Petrol

Figure 4 illustrates the serum levels of follicle stimulating hormone (FSH) in female Wistar rats exposed to petrol vapor for 6 weeks (Figure 4A) and 12 weeks (Figure 4B). A 6-week exposure to petrol vapor resulted in a significant elevation in FSH levels compared to the normal control (P=0.0006). Treatment with honey at a dose of 330 mg/kg significantly attenuated the FSH increase (P=0.0226) relative to the petrol-exposed group (Figure 4A). After 12 weeks of exposure, the same trend of increased FSH with petrol vapor and its reduction with honey treatment was observed; however, these changes were not statistically significant (Figure 4B).

As shown in Figure 5A, petrol vapor exposure for 6 weeks significantly suppressed serum luteinizing hormone (LH) levels in comparison with the control group (P<0.0001). Administration of honey at 330 mg/kg/day resulted in a significant increase in LH levels (P=0.0008) relative to the 6-week petrol-exposed group.

Exposure to petrol vapor for 6 weeks also caused a significant reduction in serum estrogen levels (P = 0.0002) when compared with normal controls (Figure 6A). Treatment with honey led to a mild, though statistically non-significant, increase in estrogen concentration relative to the petrol-exposed group.

As illustrated in Figure 7A, serum progesterone levels were significantly reduced following 6 weeks of petrol vapor exposure (P=0.0004) in comparison with the control. Honey treatment at 330 mg/kg significantly elevated serum progesterone levels (P= 0.0290) when compared with the untreated petrol-exposed group.

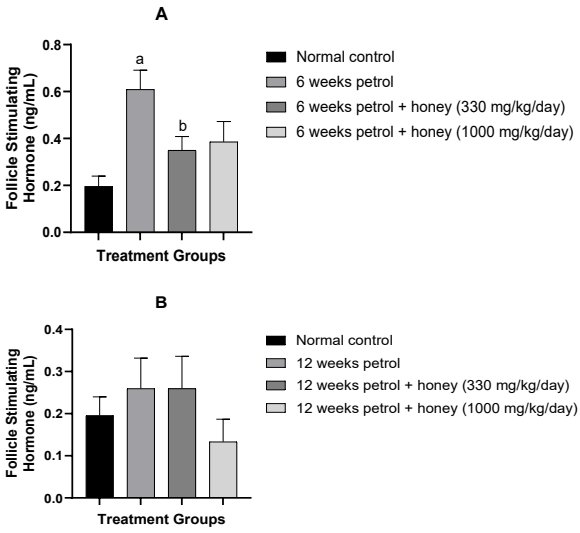

Figure 4: Effects of honey on follicle stimulating hormone (FSH) of rats exposed to petrol. Results were analyzed with one-way ANOVA followed by Tukey-Kramer post hoc test. Values are expressed as mean ± SEM, n=8, a $P<0.05$ compared with normal control, b $P<0.05$ compared with 6-week petrol exposed. A=6-week exposure and treatment, B = 12-week exposure and treatment.

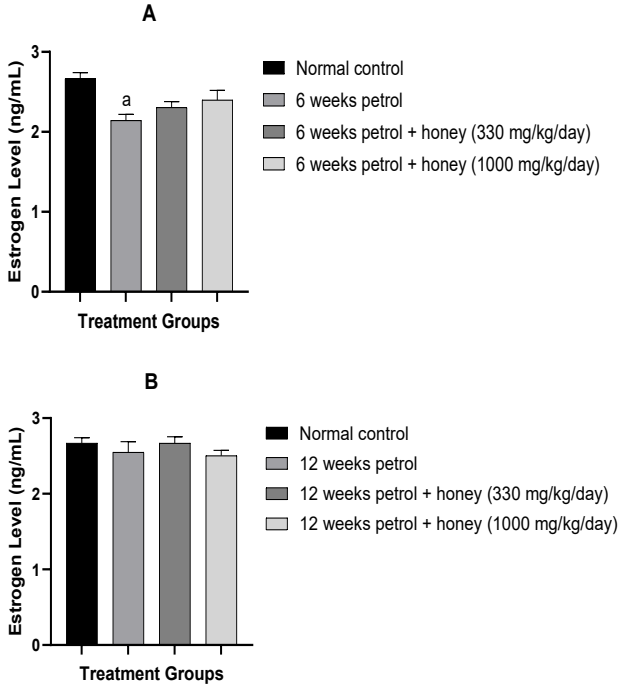

Figure 6: Effects of honey on estrogen of rats exposed to petrol. Results were analyzed with one-way ANOVA followed by Tukey-Kramer post hoc test. Values are expressed as mean ± SEM, n=8, a $P<0.05$ compared with normal control. A=6-week exposure and treatment, B = 12-week exposure and treatment.

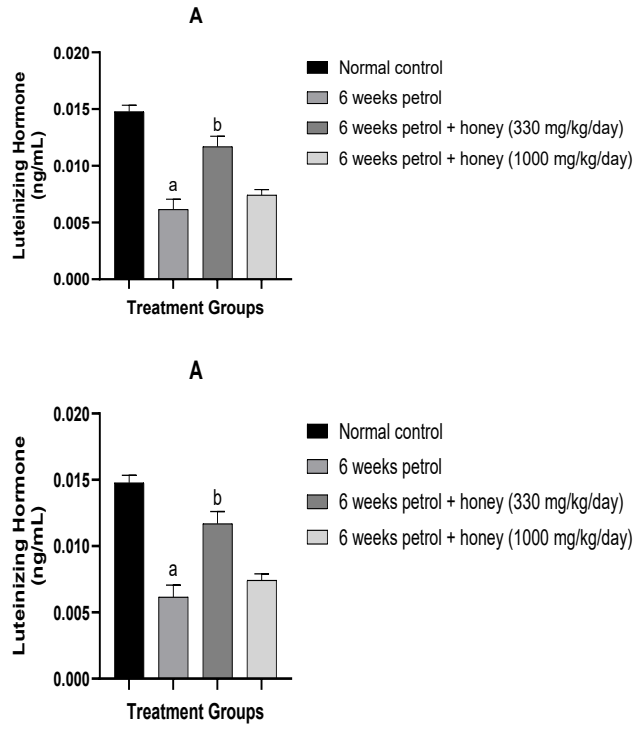

Figure 5: Effects of honey on luteinizing hormone (LH) of rats exposed to petrol vapor. Results were analyzed with one-way ANOVA followed by Tukey-Kramer post hoc test. Values were expressed as mean ± SEM, n=8, a $P<0.05$ compared with normal control, b $P<0.05$ compared with 6-week petrol exposed. A=6-week exposure and treatment, B=12-week exposure and treatment.

Effects of Honey on the Histology of Ovaries of Rats Exposed to Petrol

Table 1 and Figure 8 present the histomorphological findings in the ovaries of female Wistar rats exposed to petrol vapor, with or without honey treatment. The histological evaluation included quantification of ovarian structures such as primordial follicles, Graafian follicles, other developing follicles, and corpora lutea. Exposure to petrol vapor for 6 or 12 weeks did not result in statistically significant alterations in the number of follicles or corpora lutea compared to the normal control group. However, a qualitative reduction in follicular density was observed in photomicrographs of the petrol-exposed groups, suggesting possible subtle histological disruptions. In contrast, honey treatment, particularly at a dose of 1000 mg/kg/day, was associated with an increase in the number of observed follicles. The group treated with 1000 mg/kg/day honey for 12 weeks demonstrated a statistically significant increase in total follicular count (P=0.0056) compared with the 12-week petrol vapor-exposed group without treatment. This effect was most notable in the number of developing and Graafian follicles, indicating enhanced folliculogenesis (Table 1, Figure 8).

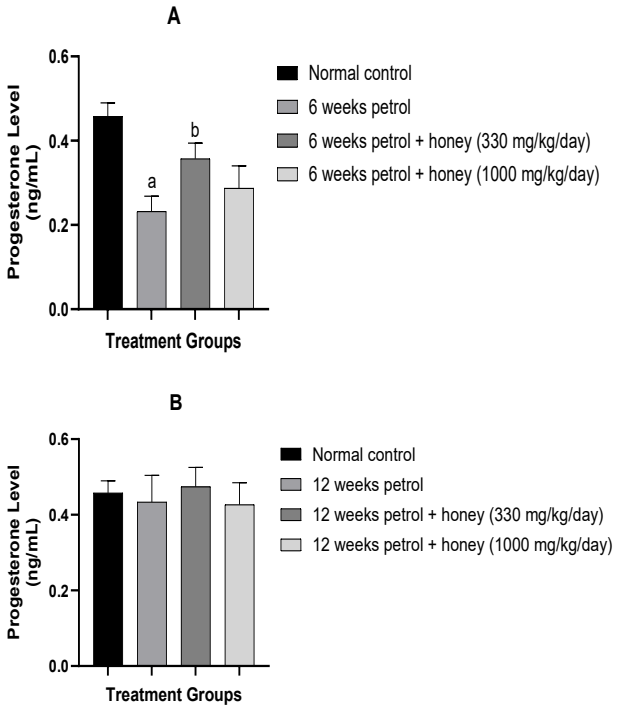

Figure 7: Effects of honey on progesterone of rats exposed to petrol. Results were analyzed with one-way ANOVA followed by Tukey-Kramer post hoc test. Values were expressed as mean ± SEM, n=8, a $P<0.05$ compared with normal control, b $P<0.05$ compared with 6-week petrol exposed. A=6-week exposure and treatment, B=12-week exposure and treatment.

Table 1: Number of follicles per photomicrograph representing groups 1-7.

Groups	Primordial Follicle	Graafian follicle	Other follicles	Corpus luteum	Total follicles	Mean ± SEM
NC	4	6	3	6	19	4.750±0.750
PCG-1	3	2	7	10	22	5.500±1.848
PCG-2	7	6	4	5	22	5.500±0.646
HTG-1	5	4	2	11	22	5.500±1.936
HTG-2	7	5	5	18	35	8.750±3.119
HTG-3	2	3	1	6	12	3.000±1.080
HTG-4	15	10	21	20	66	16.500±2.533**

Quantification of the number of follicles were done by visual microscopic examination under ×100 magnification using Olympus CX23 Binocular Microscope. Results were analyzed with one-way ANOVA followed by Tukey-Kramer post hoc test. Values are expressed as mean ± SEM, n = 8, ***P*=0.0056 when compared with 12 weeks gasoline vapor-exposed group. NC: Normal control, PCG-1: 6 weeks gasoline vapor-exposed group, PCG-2: 12 weeks gasoline vapor-exposed group, HTG-1: 6 weeks gasoline vapor + low dose honey (330 mg/kg/day) group, HTG-2: 12 weeks gasoline vapor + low-dose honey group, HTG-3: 6 weeks gasoline vapor + high dose honey (1000 mg/kg) group, 12 weeks gasoline vapor + high dose honey group.

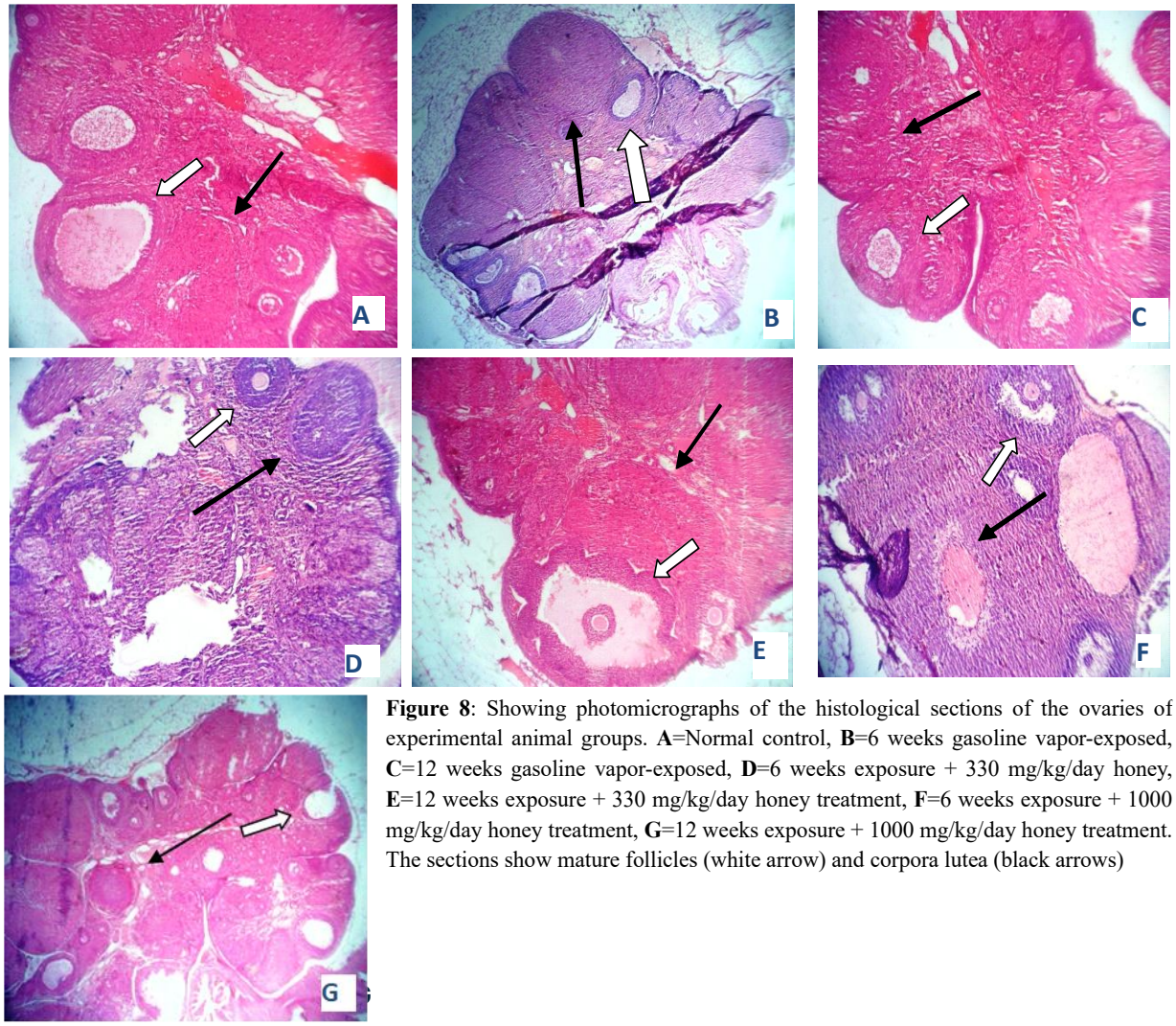

Figure 8: Showing photomicrographs of the histological sections of the ovaries of experimental animal groups. **A**=Normal control, **B**=6 weeks gasoline vapor-exposed, **C**=12 weeks gasoline vapor-exposed, **D**=6 weeks exposure + 330 mg/kg/day honey, **E**=12 weeks exposure + 330 mg/kg/day honey treatment, **F**=6 weeks exposure + 1000 mg/kg/day honey treatment, **G**=12 weeks exposure + 1000 mg/kg/day honey treatment. The sections show mature follicles (white arrow) and corpora lutea (black arrows)

DISCUSSION

The present study elucidates the protective effects of honey on oxidative stress markers, reproductive hormone levels, and ovarian histomorphology in female Wistar rats exposed to gasoline vapor. Chronic exposure to environmental pollutants such as gasoline vapor has been associated with the induction of oxidative stress and endocrine disruption (Bus *et al*., 2022), both of which pose significant threats to female reproductive health (Tricotteaux-Zarqaoui *et al*., 2024). The findings of this study provide compelling evidence that honey, particularly at higher doses, may mitigate these deleterious effects.

Exposure to gasoline vapor for 6 weeks significantly elevated the activities of catalase (CAT) and superoxide dismutase (SOD), two key antioxidant enzymes involved in cellular defense against reactive oxygen species (ROS). This elevation suggests an adaptive response to increased oxidative burden triggered by inhaled hydrocarbons. Interestingly, this effect was more pronounced at 6 weeks than at 12 weeks, with the latter showing only non-significant changes. This pattern may reflect a temporal adaptation or a potential exhaustion of antioxidant reserves following prolonged exposure (Sharifi-Rad *et al*., 2020). Administration of honey resulted in a dose-dependent attenuation of both CAT and SOD activities, with the 1000 mg/kg dose producing significant reductions. This effect may be interpreted in two ways: firstly, as a direct antioxidant action of honey that neutralizes ROS, thereby reducing the need for endogenous enzymatic defense; secondly, it could reflect an overall reduction in oxidative stress due to honey's bioactive components, such as flavonoids, phenolic acids, and vitamins, which are known to modulate redox homeostasis (Andrés *et al*., 2024). Lipid peroxidation, a downstream consequence of oxidative stress, was significantly increased after 12 weeks of gasoline vapor exposure, as indicated by elevated malondialdehyde (MDA) levels. Although honey treatment led to a dose-dependent decrease in MDA, the reductions were not statistically significant. This discrepancy may be due to variability in individual responses or an insufficient treatment window to reverse established lipid peroxidation. Nevertheless, the downward trend aligns with the antioxidant potential of honey observed in enzymatic assays.

Gasoline vapour exposure significantly disrupted key reproductive hormones, suggesting interference with the hypothalamic–pituitary–gonadal (HPG) axis. A marked increase in follicle-stimulating hormone (FSH) and concomitant suppression of luteinizing hormone (LH), estrogen, and progesterone were observed after 6 weeks of exposure. This hormonal profile is indicative of disrupted ovarian feedback, likely due to compromised follicular development and luteal insufficiency. The increase in FSH levels may be interpreted as a compensatory mechanism secondary to diminished ovarian function. The reduction in LH and steroid hormones such as estrogen and progesterone further supports the notion of impaired folliculogenesis and ovulation. These findings are consistent with previous reports demonstrating that exposure to petroleum hydrocarbons impairs reproductive endocrine signaling and ovarian steroidogenesis through oxidative damage and hormonal imbalances (Gautam *et al*., 2024). Treatment with honey at 330 mg/kg/day effectively attenuated the elevation in FSH and significantly restored LH and progesterone levels, suggesting a partial recovery of ovarian function. Although the increase in estrogen levels following honey administration was not statistically significant, the upward trend is biologically relevant and indicative of improved granulosa cell function. These hormonal modulations may stem from honey's ability to enhance ovarian antioxidant status, reduce oxidative insult to endocrine tissues, and support enzymatic pathways involved in steroid biosynthesis (Fouad *et al*., 2022).

Histological analyses provided structural support for the biochemical and hormonal findings. While petrol vapor exposure did not significantly alter the follicle counts in statistical terms, qualitative histological examination revealed reduced follicular density and morphological evidence of degeneration. This observation aligns with the biochemical indications of follicular dysfunction, such as reduced estrogen and progesterone levels. Importantly, honey treatment, especially at the 1000 mg/kg dose for 12 weeks, significantly increased the number of ovarian follicles, particularly the developing and Graafian follicles. This suggests that honey not only mitigates oxidative damage but may also stimulate folliculogenesis. The observed enhancement in follicular counts and morphology is consistent with the hormonal data, indicating improved ovarian responsiveness and potential restoration of fertility. The histological recovery may be attributed to honey's rich phytochemical content, which includes flavonoids, phenolic compounds, and micronutrients known to promote cellular proliferation, inhibit apoptosis, and modulate growth factor signaling within the ovary (Barreiros *et al*., 2024; de Lima *et al*., 2025). These effects collectively support tissue regeneration and preservation of ovarian reserve under toxicant-induced stress.

CONCLUSION

Collectively, the results of this study demonstrate that honey confers protective effects against gasoline vapor-induced oxidative stress, endocrine disruption, and ovarian damage in female Wistar rats. The dose-dependent efficacy, particularly at 1000 mg/kg/day, highlights the potential of honey as a natural therapeutic agent for mitigating reproductive toxicity associated with environmental pollutants. The observed improvements in antioxidant markers, hormonal balance, and ovarian histoarchitecture provide a holistic view of honey's restorative potential.

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Not applicable.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHORS' CONTRIBUTION

The paper is an excerpt of an MSc project by RZA who also prepared the manuscript. SOB, AC and MBA supervised the MSc project. MU reviewed and interpreted histology slides. All Authors read the manuscript and approved its submission.

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AUTHORS' DECLARATION

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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