

Ameliorative effects of banana peel extract against malathion-induced reproductive toxicity in female rats

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Abstract

Objective

Oxidative imbalance and reproductive dysfunction have been increasingly linked to exposure to malathion, a widely used organophosphate pesticide. This study evaluated the effects of subchronic oral malathion exposure on antioxidant status, reproductive hormones, and the histological architecture of ovarian and uterine tissues in female rats. Banana peel extract (BPE) was first characterized for antioxidant activity and selected phytochemical contents, and then assessed for its potential protective role against malathion-induced reproductive alterations.

Materials and methods

Forty adult nulliparous female rats (12 weeks old) were randomly assigned to eight groups (n = 5). The animals were orally treated for 30 consecutive days with distilled water (control), BPE (525 mg/kg), malathion at three doses (30, 90, or 150 mg/kg), or the corresponding malathion and BPE combinations. BPE was prepared using 50% acetone-water extraction and characterized by DPPH radical-scavenging activity and selected phytochemical measurements. Sampling was performed during the diestrus phase to reduce estrous cycle-related hormonal variation. Serum total antioxidant capacity (TAC), estradiol, and progesterone were measured, and ovarian and uterine tissues were examined histologically.

Results

BPE showed DPPH radical-scavenging activity, with an IC₅₀ value of 25.1 µg/mL, and contained measurable amounts of total tannins, total phenolics, total flavonoids, and dopamine. Malathion exposure induced dose-dependent reductions in serum TAC, estradiol, and progesterone. These changes were accompanied by ovarian and uterine histopathological alterations, including ovarian atrophy, stromal degeneration, hyalinization, epithelial desquamation, glandular alterations, and stromal thickening. Co-administration of BPE improved serum TAC, partially restored estradiol

and progesterone levels, and was associated with less severe histological alterations compared with the corresponding malathion-treated groups. This improvement was more evident at the low and moderate malathion doses, whereas the protective response was limited at the highest dose.

Conclusion

Subchronic malathion exposure was associated with impaired antioxidant status, reduced reproductive hormone levels, and structural damage in ovarian and uterine tissues of female rats. Co-administration of BPE provided partial protection against these changes, most likely through its antioxidant phytochemical constituents. The protective response appeared to vary according to malathion dose, suggesting that the effect of BPE was dose-dependent rather than complete across all exposure levels.

Keywords: banana peel extract, diestrus, estradiol, histopathology, malathion

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Introduction

Global statistics indicate that fertility rates have declined over the past few decades and continue to fall below the replacement rate in many populations. Although this decline may be influenced by economic and social factors, increasing evidence also points to biological and environmental factors that may reduce fertility (Bhattacharjee et al., 2024). Reproductive function in both sexes can be affected by environmental pollutants, including endocrine-disrupting chemicals (EDCs). Several studies have reported a decline in fertility parameters associated with these pollutants in both males and females, although the impact may be greater in females (Evgeni et al., 2014; Tricotteaux-Zarqaoui et al., 2024). Environmental pollutants, including pesticides, micro- and nanoplastic waste, and heavy metals, pose a major burden to human health. They may challenge reproduction by damaging tissues, impairing hormonal regulation, and causing oxidative imbalance (Ding et al., 2023). A recent review reported that repeated or long-term pesticide exposure is associated with cancer, neurological and respiratory diseases, and reproductive disorders. This exposure is sufficient to disrupt steroid hormone signaling, and such disruption is sufficient to impair reproductive function (Shekhar et al., 2024). The use of organophosphates is widespread and intensive in countries undergoing agricultural expansion,

which creates occupational and environmental risks to humans. They are considered among the most hazardous types of pesticides, as they may cause adverse effects even at low concentrations, particularly under chronic exposure conditions (Muñoz-Quezada et al., 2016). A recent meta-analysis showed the extent of exposure among agricultural workers in different regions of Iraq by measuring the reduction in acetylcholinesterase (AChE) activity, which is considered a main indicator of exposure to organophosphates. This confirms the presence of occupational exposure with health consequences (Mohammad et al., 2024). Malathion is one of the commonly used organophosphate compounds and is recognized in the literature for its toxic effects on both animals and humans. A recent systematic review indicated that malathion is associated with female reproductive disorders, as these disorders appeared in the form of ovarian hormonal disturbances, oxidative stress, and histological changes in the gonads (Barbosa et al., 2025). Malathion may contribute to different systemic disturbances because its exposure can induce oxidative stress, which in turn may cause a range of damage, such as lipid oxidation, DNA fragmentation, and stimulation of some inflammatory mechanisms. The resulting inflammation may further amplify oxidative damage, cause additional cellular injury and disrupt reproductive tissues (Lorke & Oz, 2025). Plant extracts have received wide attention due to their chemical diversity and biological activities in the field of biological treatments, including the mitigation of environmental toxicants. Among plant extracts, banana peel extract (BPE) has gained interest because it contains bioactive compounds that may contribute to antioxidant activity, especially phenolic and flavonoid compounds. These compounds provide the extract with activity against free radicals and contribute to reducing inflammation, thereby protecting sensitive tissues (Avram et al., 2022; Wani & Dhanya, 2025). BPE has demonstrated protective properties against oxidative and inflammatory damage induced by mixed heavy metal exposure in female albino rats (Eddie-Amadi et al., 2023). In addition, BPE attenuated carbendazim-induced toxicity, with treatment improving hepatic and renal function and reducing histopathological alterations (Abdel-Rahman et al., 2022). To date, limited studies have explored the ability of BPE to counteract malathion-induced reproductive toxicity, particularly in females. Accordingly, the current study was experimentally designed to assess whether BPE could mitigate reproductive toxicity caused by malathion.

Materials and methods

Experimental design and animals: Experimental procedures were reported according to ARRIVE 2.0 guidelines (Percie du Sert et al., 2020) and were generally aligned with OECD Test Guideline 407 (OECD, 2025), following approval from the Ethics Committee of the College of Science, University of Babylon, Iraq (Approval No. Z240602, dated June 12, 2024). Forty nulliparous female albino rats (12 weeks of age; 186.2 ± 6.18 g) were obtained from a local animal facility and housed under controlled environmental conditions (22 ± 2 °C, 50% relative humidity), and animals were maintained under a 12:12 h light-dark cycle with *ad libitum* access to standard chow and water and were acclimatized for 14 days before the start of the experiment. After acclimation, the rats were randomly divided into eight experimental groups ($n = 5$ per group) and treated for 30 consecutive days between May and June 2025. The evaluated endpoints included

serum total antioxidant capacity (TAC), histological examination of ovarian and uterine tissues, and assay of serum estradiol and progesterone levels.

Banana peel extract (BPE) preparation: Fresh banana peels (*Musa acuminata* L.) were washed and cut into relatively small pieces, then dried in an oven at 45°C. After that, they were ground into a fine powder and extracted using a 50% acetone–water solution (v/v) at a plant material-to-solvent ratio of 1:10 (w/v) under continuous mixing. The extraction method and solvents were used according to a previously described procedure (González-Montelongo et al., 2010). The extract was then filtered and concentrated by evaporation at 45 °C until a dry residue was obtained. The extraction yield in the present study was approximately 16%. The residue was preserved in airtight containers until use.

Antioxidant activity and BPE characterization: To evaluate the antioxidant activity of BPE, DPPH radical-scavenging activity was assessed colorimetrically at 516 nm using different BPE concentrations, based on the assay principle described by Brand-Williams et al. (1995). The antioxidant activity was expressed as the half-maximal inhibitory concentration (IC₅₀), which was calculated from the concentration-response curve. Total tannin, total phenolic, total flavonoid, and total dopamine contents were measured as part of the phytochemical characterization of BPE. Total tannin was measured at 540 nm using tannic acid as a standard (Crnkić & Klepo, 2020). Total phenolic content was determined using the Folin-Ciocalteu method at 765 nm and expressed as gallic acid equivalents (Dominguez-López et al., 2024). Total flavonoid was estimated using the aluminum chloride colorimetric method at 510 nm, with rutin used as a standard (Liu et al., 2017). Dopamine was quantified by HPLC with UV detection at 280 nm, using dopamine hydrochloride as the standard (Sultan Aydın & Bulduk, 2020).

Dosing protocol: The doses used were intended to produce a graded toxic effect and were based on subchronic models reported in previous studies on malathion toxicity. The selected BPE dose was based on previously validated effective ranges (Eddie-Amadi et al., 2025; Rezg et al., 2006). A total of eight groups (n = 5 rats per group) were included in the experiment. Groups received daily oral gavage for 30 days with distilled water (control), malathion (30, 90, or 150 mg/kg), BPE (525 mg/kg), or their respective combinations.

Estrous staging and euthanasia: Beginning on day 23 of the experiment, estrous cycles were monitored daily by vaginal cytology using saline lavage and immediate wet-mount examination to identify the estrous-cycle stage (Goldman et al., 2007). After completion of the 30-day dosing period, necropsy was performed at the first diestrus stage detected after the final administration to standardize the cycle stage at sampling, following the approach described by Toyoda et al. (2000). Accordingly, necropsy was conducted between days 30 and 34, depending on the estrous cycle stage of each animal. Prior to tissue and blood collection, the rats were anesthetized with isoflurane and euthanized by cardiac exsanguination. Death was confirmed according to established recommendations for laboratory rodent euthanasia (Shomer et al., 2020).

Blood collection and processing: Blood samples (2-3 mL) were obtained via cardiac puncture under anesthesia. After allowing the samples to clot, they were centrifuged for 10 minutes at 3000 × g. The separated serum was kept at -20 °C until progesterone, estradiol, and total antioxidant capacity (TAC) were measured.

Serum total antioxidant capacity: TAC was measured using the CUPRAC method, as described by Apak et al. (2007). The results were expressed as μmol Trolox equivalents per liter ($\mu\text{mol/L}$), with absorbance read at 450 nm.

Hormonal assays: Serum estradiol and progesterone levels were measured using commercial ELISA kits from Sunlong Biotech, China (E₂: SL0268Ra; P₄: SL0596Ra), following the manufacturer's instructions and previously described procedures (Melekoglu et al., 2018; Youness et al., 2017).

Ovarian and uterine histology: Histological sections were prepared after fixation of tissues in 10% formalin. Routine tissue processing was then performed through dehydration in graded alcohols, followed by clearing in xylene. The tissues were subsequently embedded in paraffin wax and sectioned at a thickness of 4–5 μm . Conventional hematoxylin and eosin staining was used, and the sections were examined under a light microscope equipped with a camera for image acquisition and documentation (Alchalabi et al., 2016). The presented images were selected to represent the average histological changes observed during tissue examination.

Statistical analysis: The statistical analysis was performed with IBM SPSS Statistics version 29. The data were first checked for normal distribution and homogeneity of variance using the Shapiro-Wilk and Levene's tests. Group differences were then analyzed by one-way ANOVA, followed by Tukey's HSD test for multiple comparisons. The results are expressed as mean $\pm$ SD, and statistical significance was accepted at $p < 0.05$.

Results

BPE characterization: The extract showed a DPPH IC₅₀ value of 25.1 $\mu\text{g/mL}$, and selected phytochemical constituents were detected at measurable levels, as presented in Table 1.

Table 1. Measured phytochemical contents of banana peel extract

Phytochemical parameter	Concentration (mg/100 g dry weight)
Total tannins	1250
Total phenolics	196.9
Total flavonoids	125.8
Dopamine	11.3

Serum TAC: There were significant group differences in serum TAC (Figure 1, $p < 0.05$). Malathion-treated rats showed significantly lower TAC levels than the control group, with a progressive decrease as the malathion dose increased. The BPE-only group showed the highest TAC values, while co-treatment with BPE resulted in significantly higher TAC levels than the corresponding malathion-treated groups.

Serum hormone levels: Serum estradiol and progesterone differed significantly among groups (Figures 2 and 3, $p < 0.05$). Hormone levels decreased with increasing malathion dose, with the lowest values at 150 mg/kg. The BPE-only group showed higher estradiol levels than

control group, whereas co-treatment with BPE improved hormone levels at the moderate and high malathion doses compared with the corresponding malathion groups.

Histology: Histological sections of the uterus and ovaries showed varying degrees of degenerative damage after malathion exposure, whereas the control group showed normal histological architecture. Compared with the control group, the BPE-only group showed some differences in uterine and ovarian tissue appearance. Histological observations suggested less severe tissue alterations in the co-treated groups. Representative histological images are shown in Figure 4.

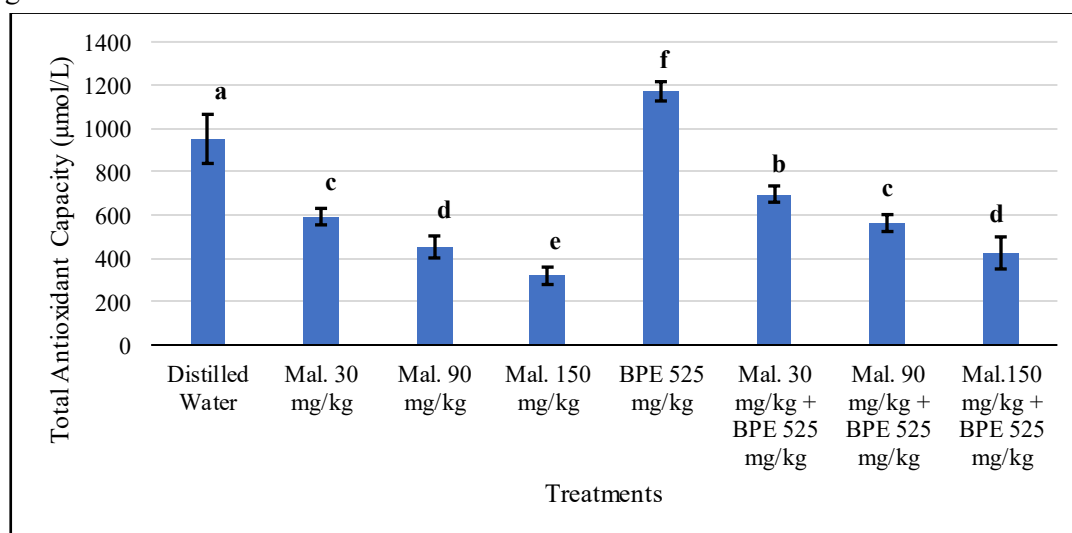


Figure 1. Female rats' serum total antioxidant capacity (TAC). The data are displayed as mean $\pm$ SD ($n = 5$). Significant differences exist between bars with various superscript letters ($p < 0.05$; Tukey's HSD test). Mal., malathion; BPE, banana peel extract

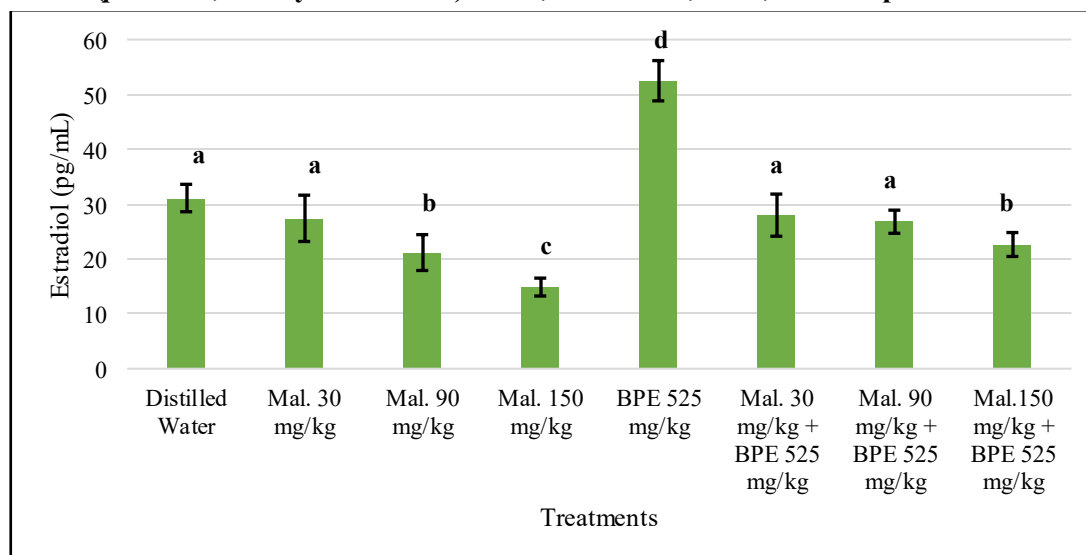


Figure 2. Female rats' serum estradiol levels. The results are displayed as mean $\pm$ SD ($n = 5$). Significant group differences are shown by different superscript letters ($p < 0.05$, Tukey's HSD test). Mal., malathion; BPE, banana peel extract

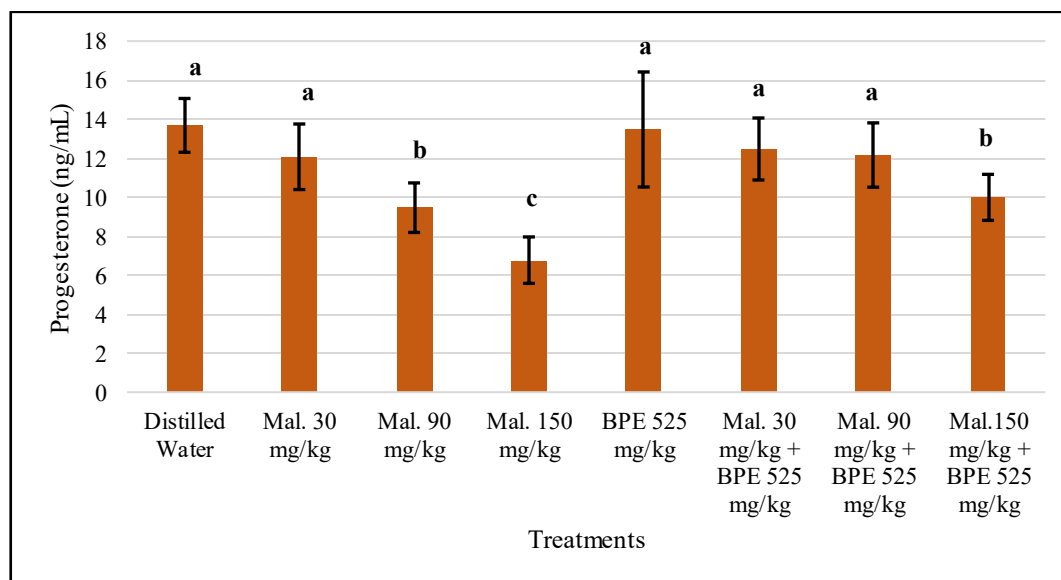


Figure 3. Female rats' serum progesterone levels. The findings are displayed as mean $\pm$ SD (n = 5). Statistically significant differences between groups are indicated by superscript letters above bars ($p < 0.05$, Tukey's HSD). Mal., malathion; BPE, banana peel extract

Discussion

The DPPH assay showed that BPE had free radical-scavenging activity, with an IC_{50} value of 25.1 μ g/mL, indicating marked antioxidant activity. This activity may be related, at least in part, to the antioxidant phytochemical constituents measured in the extract, including total tannins, total phenolics, total flavonoids, and dopamine, as presented in Table 1. Similar antioxidant-related constituents have been reported in peel-derived extracts (Islam et al., 2023). Differences between the present findings and previous reports on banana peel extracts may be attributed to variations in cultivar, maturity stage, and extraction procedures (Bashmil et al., 2021). Cellular damage and impaired fertility are associated with oxidative stress, a condition that occurs when free radicals overwhelm the body's ability to counteract them (Agarwal et al., 2012; Zhang et al., 2021). In the present study, malathion exposure resulted in a significant dose-dependent decrease in serum TAC (Figure 1), suggesting reduced systemic antioxidant capacity and possible oxidative stress. This finding is supported by earlier studies suggesting that reduced antioxidant capacity may result from impairment of endogenous antioxidant defense mechanisms, particularly enzymes such as superoxide dismutase and catalase (Possamai et al., 2007). However, direct oxidative stress markers were not assessed, and acetylcholinesterase activity, a relevant marker of organophosphate toxicity, was not measured in the present study.

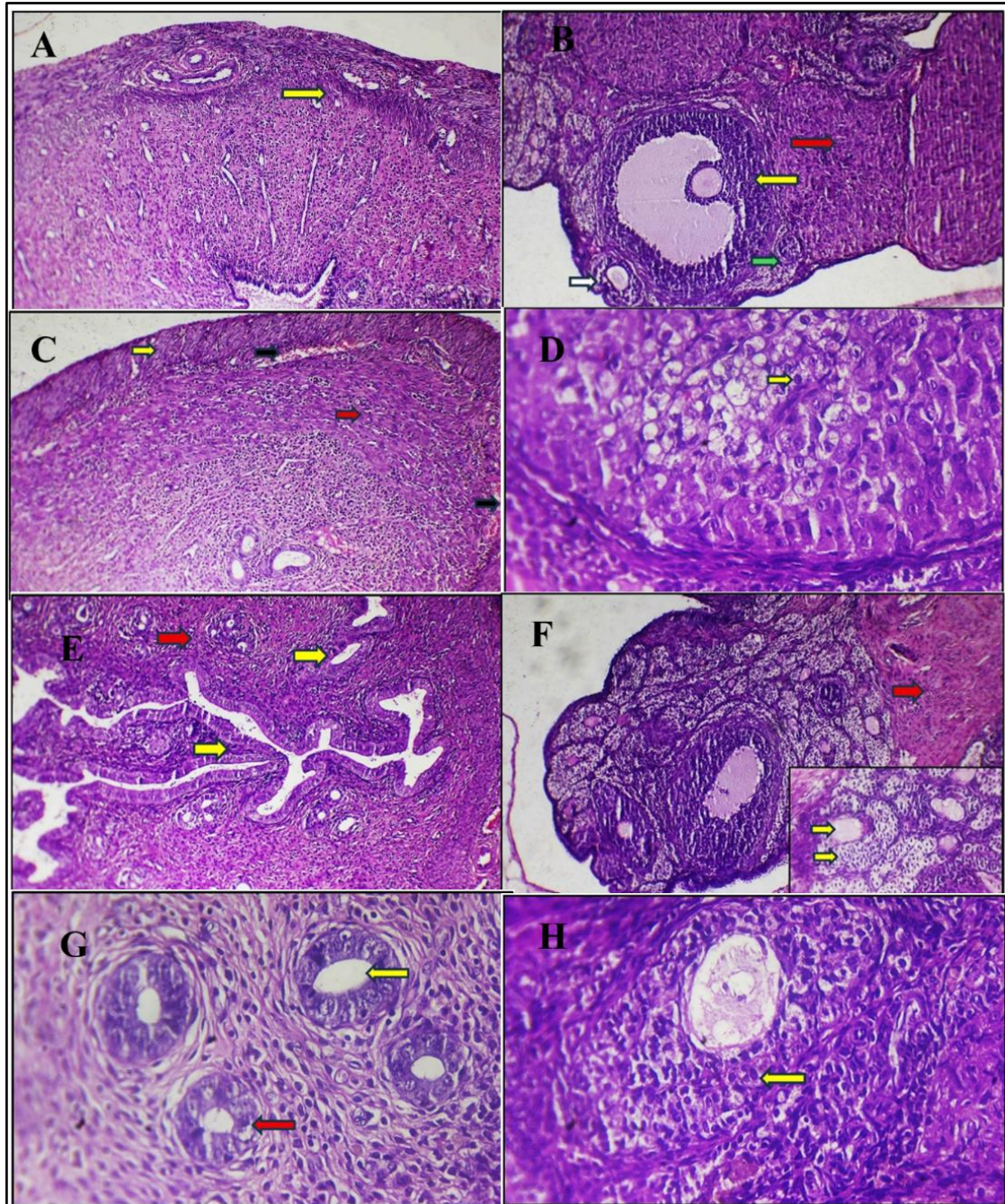


Figure 4. Representative histological sections of ovarian and uterine tissues (H&E stain). (A) Control uterus showing normal myometrial smooth muscle bundles (yellow arrow); $\times 10$. (B) Control ovary showing normal follicles at different developmental stages (green, white, and yellow arrows) and corpora lutea (red arrow); $\times 10$. (C) BPE-treated uterus showing perimetrial thickening (yellow arrow), myometrial hypertrophy (red arrow), and vascular congestion (black arrow); $\times 10$. (D) BPE-treated ovary showing follicular cell vacuolization and darkly stained nuclei (yellow arrow); $\times 40$. (E) Uterus of rats treated with malathion (90 mg/kg) showing epithelial desquamation and glandular alterations (yellow arrow), with stromal thickening (red arrow); $\times 10$. (F) Ovary of rats treated with malathion (90 mg/kg) showing ovarian atrophy, stromal degeneration, and hyalinization (yellow arrow), with medullary fibrosis (red arrow); $\times 10$, $\times 40$. (G) Uterus of rats co-treated with malathion (90 mg/kg) and BPE showing relatively preserved glandular architecture (yellow arrow) with reduced epithelial alterations (red arrow); $\times 40$. (H) Ovary of rats co-treated with malathion (90 mg/kg) and BPE showing improved granulosa cell organization with mild vacuolization and nuclear pyknosis (yellow arrow); $\times 40$.

The reduction in estradiol and progesterone levels was associated with a decrease in serum TAC (Figures 2 and 3). This indicates a reduction in ovarian function in the production of steroid hormones. Similar reductions in hormone levels have previously been observed as a result of severe oxidative stress, which led to degenerative changes in the ovarian follicles responsible for hormone production (Ruder et al., 2008). These hormonal changes were supported by descriptive histopathological observations, although the histological changes were not scored, as shown in Figure 4. The malathion-treated groups exhibited ovarian atrophy, degeneration of granulosa cells, and disruption of connective tissue. Such structural alterations may impair follicular development, leading to reduced estradiol production and impaired corpus luteum formation, which may consequently decrease progesterone synthesis (Al-Gubory et al., 2012). An increase in serum TAC was observed in the group that received the BPE alone. This increase may be attributed to the presence of bioactive compounds in the extract, as shown in its characterization in Table 1. A similar finding was reported in a previous study in which banana peel powder was used and resulted in an enhancement of the body's antioxidant defenses (Chueh et al., 2019). Estradiol levels were also significantly higher in the BPE-only group than in the control group. This increase may be attributed to the effect of the phytochemical compounds present in the extract on the hormonal activity of the ovaries, although specific indicators of ovarian activity were not measured in the present study. A previous systematic review indicated that flavonoids may contribute to enhancing the hormonal secretory activity of the ovary and may also contribute to the protection of ovarian tissues (J. Zhang et al., 2022). Furthermore, phytoestrogenic compounds in BPE may contribute to increased estradiol levels, as reported in a similar extract (Pratama et al., 2011). The increase in estradiol levels was accompanied by changes in uterine tissues, including myometrial hypertrophy, increased uterine wall thickness, and vascular congestion. These findings may reflect the uterine response to elevated estradiol levels. This needs further research to understand the mechanism through which BPE exerted its effects. Co-administration of BPE significantly improved antioxidant status in co-treated groups, which was associated with partial restoration of estradiol and progesterone levels. These hormonal improvements were associated with histological observations showing partial restoration of ovarian and uterine architecture, particularly improved organization of granulosa cell layers and reduced degenerative changes. The observed partial improvement following BPE co-administration may be partly attributed to its antioxidant phytochemicals, which may support antioxidant defenses and help maintain tissue integrity (Avram et al., 2022; Wani & Dhanya, 2025). The apparent protective effect of BPE varied according to the malathion dose. At lower exposure levels, the possible disturbance in antioxidant status was relatively limited, leaving a narrow range for detectable improvement. On the other hand, at the medium and high doses, malathion exerted an effect on oxidative status, which allowed BPE to exhibit its antioxidant activity in an ameliorative manner. However, the effect of the extract was less evident at the 150 mg/kg malathion dose (Arab et al., 2018).

Conclusion: The findings of the present study indicate that malathion produced reproductive toxicity during the subchronic exposure period. It also affected oxidative balance in a dose-dependent manner through the reduction of TAC. This reduction may indicate the presence of

oxidative stress, and it was accompanied by decreases in estradiol and progesterone levels, as well as degenerative changes in the ovaries and uterus. Administration of BPE was associated with improved antioxidant status, partial restoration of hormonal levels, and attenuation of ovarian and uterine tissue alterations. Disturbance of oxidative balance may be involved in the reproductive toxicity of malathion. The apparent effect of BPE, owing to its antioxidant nature, may also have contributed to the attenuation of malathion toxicity associated with oxidative imbalance. However, the effect of the extract appeared to be dependent on the malathion dose, as it was less evident at higher doses.

Limitations: The study was limited to measuring TAC as an indicator of oxidative balance disturbance without measuring direct markers of oxidative stress. In addition, acetylcholinesterase activity was not measured as an indicator of malathion toxicity. Five animals were used in each experimental group, which may reduce the strength of the statistical analysis. A single dose of BPE was used.

Author contributions

Both authors confirm contribution to the paper equally.

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Data availability statement

The datasets of the current study are available from the corresponding author on reasonable request.

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Conflict of Interest:

The author declares no conflict of interest.

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اثرات بهبوددهنده عصاره پوست موز در برابر سمیت تولیدمثلی ناشی از مالاتیون در موش‌های صحرایی ماده

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چکیده

هدف: عدم تعادل اکسیداتیو و اختلالات تولیدمثلی به‌طور فزاینده‌ای با مواجهه با مالاتیون، یکی از آفت‌کش‌های ارگانوفسفره پرکاربرد، مرتبط شناخته شده‌اند. هدف این مطالعه بررسی اثرات مواجهه خوراکی نیمه‌مزمّن با مالاتیون بر وضعیت آنتی‌اکسیدانی، هورمون‌های تولیدمثلی و ساختار بافت‌شناسی تخمدان و رحم در موش‌های ماده بود. همچنین، عصاره پوست موز (BPE) ابتدا از نظر فعالیت آنتی‌اکسیدانی و برخی ترکیبات فیتوشیمیایی مورد ارزیابی قرار گرفت و سپس نقش حفاظتی احتمالی آن در برابر تغییرات تولیدمثلی ناشی از مالاتیون بررسی شد.

مواد و روش‌ها: در این مطالعه، ۴۰ سر موش صحرایی ماده بالغ و فاقد سابقه زایمان (۱۲ هفته‌ای) به‌صورت تصادفی در هشت گروه (هر گروه ۵ حیوان) تقسیم شدند. حیوانات به مدت ۳۰ روز متوالی به‌صورت خوراکی با آب مقطر (گروه شاهد)، عصاره پوست موز (۵۲۵ میلی‌گرم بر کیلوگرم)، مالاتیون در سه دوز ۳۰، ۹۰ و ۱۵۰ میلی‌گرم بر کیلوگرم، یا ترکیب هر یک از دوزهای مالاتیون همراه با عصاره پوست موز تیمار شدند. عصاره پوست موز با استفاده از حلال استون-آب ۵۰ درصد تهیه و از نظر فعالیت مهار رادیکال آزاد DPPH و برخی ترکیبات فیتوشیمیایی ارزیابی شد. نمونه‌برداری در مرحله دی‌استروس چرخه فحلی انجام گرفت تا تغییرات هورمونی مرتبط با چرخه تولیدمثلی به حداقل برسد. ظرفیت تام آنتی‌اکسیدانی سرم (TAC)، استرادیول و پروژسترون اندازه‌گیری شد و بافت‌های تخمدان و رحم از نظر بافت‌شناسی مورد بررسی قرار گرفتند.

نتایج: عصاره پوست موز فعالیت مهارکنندگی رادیکال آزاد DPPH را با مقدار IC_{50} برابر با ۲۵/۱ میکروگرم بر میلی‌لیتر نشان داد و حاوی مقادیر قابل اندازه‌گیری تانن کل، ترکیبات فنولی کل، فلاونوئیدهای کل و دوپامین بود. مواجهه با مالاتیون باعث کاهش وابسته به دوز در ظرفیت تام آنتی‌اکسیدانی سرم، استرادیول و پروژسترون شد. این تغییرات با ضایعات بافت‌شناسی در تخمدان و رحم همراه بود که شامل آتروفی تخمدان، تخریب استروما، هیالینیزاسیون، ریزش اپی‌تلیوم، تغییرات غددی و ضخیم‌شدن استروما می‌شد. تجویز همزمان عصاره پوست موز موجب بهبود ظرفیت تام آنتی‌اکسیدانی سرم، بازگشت نسبی سطوح استرادیول و پروژسترون و کاهش شدت تغییرات بافتی در مقایسه با گروه‌های دریافت‌کننده مالاتیون شد. این اثر محافظتی در دوزهای پایین و متوسط مالاتیون آشکارتر بود، در حالی که در بالاترین دوز، میزان حفاظت محدودتر مشاهده شد.

نتیجه‌گیری: مواجهه نیمه‌مزمین با مالاتیون با کاهش وضعیت آنتی‌اکسیدانی، افت سطح هورمون‌های تولیدمثلی و آسیب ساختاری در بافت‌های تخمدان و رحم موش‌های ماده همراه بود. تجویز همزمان عصاره پوست موز تا حدودی از این تغییرات جلوگیری کرد که احتمالاً ناشی از ترکیبات آنتی‌اکسیدانی و فیتوشیمیایی موجود در آن است. به نظر می‌رسد اثر حفاظتی عصاره پوست موز به دوز مالاتیون وابسته بوده و در تمامی سطوح مواجهه به‌طور کامل یکسان نبوده است.

کلمات کلیدی: آسیب‌شناسی بافتی، استرادیول، دی‌استروس، عصاره پوست موز، مالاتیون

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