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PROTECTIVE EFFECTS OF MUSA PARADISIACA FRUIT EXTRACT AGAINST REPRODUCTIVE TOXICITY IN FEMALE RATS EXPOSED TO BISPHENOL A (BPA)

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ABSTRACT

Bisphenol A (BPA) is a prevalent endocrine-disrupting chemical that poses risks to female reproductive health. In this study, we substantiated the traditional claim of the efficacy of Musa paradisiaca (banana) fruit plus peel in treating female reproductive system disorders. we investigated the potential protective effects of M. paradisiaca (banana) fruit extract against BPA-induced reproductive toxicity in female rats. The hydroalcoholic extract of M. paradisiaca fruit was administered to female Wistar rats exposed to BPA. The animals were divided into different groups: control, BPA-exposed, and Musa paradisiaca extract pre-treated followed by BPA exposure. Serum hormonal levels, histopathological changes in ovarian tissues, and estrous cyclicity were assessed to evaluate the protective effects. Our results revealed that BPA exposure led to disruptions in reproductive parameters, including altered hormonal profiles and abnormal estrous cycles. However, pre-treatment with M. paradisiaca fruit extract mitigated these adverse effects, restoring hormonal balance and promoting more regular estrous cycles. Histopathological examination showed improvements in ovarian tissue architecture in the M. paradisiaca extract pre-treated group compared to the BPA-exposed group. Our study provides supporting evidence for the traditional use of M. paradisiaca fruit plus peel in treating female reproductive system disorders, further highlighting the potential of natural remedies for female reproductive health. These findings suggest that M. paradisiaca fruit extract may possess protective properties against BPA-induced reproductive toxicity in female rats. The observed effects may be attributed to the presence alkaloids, terpenes, and flavonoids (phenolic compounds) in the extract. Our findings are consistent with previous research indicating that these compounds possess potential anti-infertility effects due to their radical scavenging and antioxidant properties. warranting further investigation to understand the underlying mechanisms and explore its potential therapeutic applications for female reproductive health.

INTRODUCTION

Reproductive health and fertility can be significantly influenced by exposure to environmental toxins, particularly Endocrine Disrupting Chemicals (EDCs). EDCs are external substances that, when encountered, especially during in utero or puberty stages, may contribute to infertility [1]. Bisphenol A (BPA) is one such EDC that humans are regularly exposed to through internal epoxy resin coatings of canned foods, polycarbonate and baby bottles, water bottles, and food storage containers, leading to potential health risks [2]. Previous research has provided substantial evidence

of the harmful effects of BPA on the female reproductive system, particularly its association with developmental toxicity and functional disturbances, ultimately contributing to female infertility [2]. Studies have also shown that BPA can interfere with sex hormone activities, leading to disruptions in the reproductive system [2]. Elevated levels of BPA have been detected in serum samples of infertile women, indicating its widespread presence and potential impact on reproductive health [3]. BPA-induced reproductive abnormalities include increased endometrial wall thickness, polycystic ovary syndrome, recurrent miscarriage, neonatal mortality, defective placental function, irregular menstrual cycles, and reduced primordial follicles [4,5,6,7,8,9,10,11]. M. paradisiaca, commonly known as banana, belongs to the Musaceae family and the genus Musa. As a staple crop, banana serves as a primary food source for populations in tropical regions. The term "banana" encompasses various hybrids from species within the Musaceae family, with M. paradisiaca being more prevalent among them[12]. Extensive studies on Musa species have revealed a plethora of biological properties associated with its compounds, including antioxidant, antidiabetic, cytotoxic and apoptotic, antimicrobial, anti-inflammatory, and cardiovascular protective effects [13]. Additionally, traditional Pakistani medicine has utilized extracts from different parts of M. paradisiaca to address various health concerns, such as inflammation, rheumatism, colic, diabetes, hypertension, and sepsis [14].

Furthermore, M. paradisiaca fruit plus peel has been traditionally used to address infertility [15]. The pharmacological properties of banana are largely attributed to its phenolic content [16]. In this study, we systematically investigate the potential protective roles of M. paradisiacaagainst the toxic effects of BPA on the female reproductive system. Female rats were concurrently treated with M paradisiaca extract and BPA via oral administration over a six-week period. Aim was to evaluate the protective effects of M. paradisiaca on follicular development, gonadotropin (17β-estradiol and progesterone) and sex steroid hormones (FSH and LH), as well as the pattern of the estrous cycle. Through this investigation, purpse was to uncover valuable insights into the potential therapeutic applications of M. paradisiaca in mitigating BPA-induced reproductive toxicity, with the ultimate goal of contributing to advancements in female reproductive health interventions.

METHODS

Chemicals and Drugs

The chemicals used in this study, including Bisphenol A, n-hexane, ethanol, dichloromethane, and water, were purchased from Sigma–Aldrich, Pakistan, and were of analytical grade.

The levels of serum FSH were measured using a Roche Elecsys FSH assay (Ref. 11775863122, Roche, Indianapolis, IN, Germany), the levels of serum LH were measured using a Roche Elecsys LH assay (Ref. 11732234122, Roche, Germany), the levels of serum progesterone were measured using a Snibe Maglumi Progesterone assay (Ref. 014210311, Snibe, Germany), and the levels of serum estradiol were measured using a Snibe estradiol Maglumi assay (Ref. 015220111, Snibe, Germany).

The measurements of FSH, LH, progesterone, and estradiol levels were conducted using a fully-automated chemiluminescence immunology analyzer, Roche COBAS E411 (Roche Diagnostics, Basel, Switzerland) for FSH and LH, and Snibe Maglumi 800 (Snibe, Germany) for progesterone and estradiol. All the chemicals used in the standard grade were applied in this study.

Animals Used

Female Sprague-Dawley Rats were used for various research activities. All the animals were provided with standard housing conditions (NRC, 1996). Moreover, the Biosafety and Animal Ethical Review Committee, University of Sargodha, approved all the laboratory procedures for this research work (SU/ORJC/14009/09/2022).

Selection and collection of plant Materials

M. paradisiaca L. fruit with peel were selected and obtained from Tandojam Sindh (Pakistan).

Accurate identification of the plant specimens was carried out by Dr. Amin Shah, Associate Professor of Botany and Incharge of the Herbarium Department of Botany at the University of Sargodha, Pakistan. Herbarium specimens for M. paradisiaca fruits (Voucher No. MK-751) was deposited in the herbarium (SARGU) of the Department of Botany, University of Sargodha, Sargodha, ensuring its availability for future research references.

Extraction and Fractionation

Fruits with peel (15 kg) of M. paradisiaca were shade-dried and pulverized into a coarse powder for extraction. The refined powder was soaked in 3L of ethanol and water (70:30, v/v), then subjected to filtration with muslin cloth and filter papers. The soaking and filtration process were carried out three times, resulting in a 32 % yield of crude extract of M. paradisiaca. (MP).

For activity-guided fractionation, 200 g of crude extract of M. paradisiaca was mixed with distilled water, and solvent-solvent extraction was carried out using hexane and dichloromethane solvents. The obtained fractions were concentrated at 40°C using a rotary evaporator, resulting in 120g of the aqueous fraction and a negligible quantity of the hexane-soluble fraction. The dichloromethane-soluble fraction was obtained in a quantity of 72 g. All these samples were refrigerated at 4°C for further phytochemical and pharmacological studies [17].

Hexane-soluble fractions of M. paradisiaca were not used in any pharmacological study due to their insufficient quantity**.**

Pharmacological Studies on M. paradisiaca.

The experimental groups of female rats (n = 8) were defined as follows:

1) PC (placebo control) provided with vehicle (1.0 ml corn oil).
2) DC (disease control) provided with BPA incorporated in vehicle at 50 mg/kg body weight.
3) MP (M. paradisiaca) given 500 mg/kg b.w. of M. paradisiaca extract, half hour before they were administered with BPA (50 mg/kg b.w.).
4) MPC (M. paradisiaca control) given 500 mg/kg b.w. of M. paradisiaca extract.
5) MP2 (M. paradisiaca 2) given 250 mg/kg b.w. of M. paradisiaca extract, half hour before they were given BPA (50 mg/kg b.w.).
6) MPC2 (M. paradisiaca control 2) given 250 mg/kg b.w. o f M. paradisiaca extract.
7) MPDCM (M. paradisiaca Dichloromethane) given 150 mg/kg b.w. of M. paradisiaca Dichloromethane fraction half hour before they were given BPA (50 mg/kg b.w.).
8) MPDCMC (M. paradisiaca Dichloromethane control) given 150 mg/kg b.w. of M. paradisiaca Dichloromethane fraction.
9) MPAQ (M. paradisiaca aqueous) given 150 mg/kg b.w. of M. paradisiaca aqueous fraction half hour before they were given BPA (50 mg/kg b.w.).
10) MPAQC (M. paradisiaca aqueous control) given 150 mg/kg b.w. of M. paradisiaca aqueous fraction.

Different treatments were given once daily starting at 09:00 AM using oral gavage. The oral route was used to mimic the best possible route of exposure in humans for a six-week duration.

Histopathological Analysis

Ovaries were rapidly fixed in 10% buffered formalin for 24 to 48 hours for histologic examination. After fixation, each ovarian tissue sample was then routinely processed and embedded in paraffin. For routine histologic examination, 5-µm-thick sections were cut from paraffin-embedded tissue samples. After deparaffinization and rehydration, sections were stained with hematoxylin-eosin. All sections were studied and photographed using a light photomicroscope.

Assessment of Effects on Estrous Cycle Cyclicity

For estrous cycle assessment, daily vaginal smear was commenced in the rats between 09:00 and 10:00 AM. The methodology of Mayasari et al., was adopted for smear collection and for the

measurement of the proportion of three types of cells and identification of dominant cells [18]. Each rat was firmly but lightly grasped, keeping a free hand around the shoulders and speedily holding the neck scruff near the skull base between the forefinger and thumb. The tail was retracted and restrained backward with the help of the little finger. A sterile cotton swab was employed for smear collection. The swab was moistened with 0.9% NaCl, then inserted gently and quickly into the female rat's vagina and rotated against the vaginal wall. Epithelial cells were collected instantly and prepared by rotating the swab along a glass side. Giemsa stain was used for smear staining. After 10-20 minutes, the smear was washed with tap water. Slides were examined using ×10 and ×40 objective lenses of a light microscope. Estrous phase was characterized by cytological appearance as follows:

Proestrous: Predominantly involves nucleated epithelial cells.
Estrous: Predominantly involves anucleated cornified cells.
Metestrous: Predominantly involves equal parts of nucleated epithelial cells, leukocytes, and anucleated cornified cells.

The patterns of estrous cycle were as follows:
Regular estrous cycle: When estrous phase was detected at least twice during a 4 to 5-day sampling period of the estrous cycle. Irregular estrous cycle: A fall in the frequency of estrus and proestrus phases and an increase in the frequency of metestrus and diestrus phases.

Effect of Extract/Fractions M. paradisiacaon Reproductive Hormones
On the last treatment day, the rats were anesthetized, dissected, and their blood samples were collected immediately. Blood was collected through cardiac puncture and centrifuged for 15 minutes at 3500 rpm. Then the serum was frozen and stored for further evaluation of hormones.

Investigation of Toxicity Potential of Extract of M. paradisiaca.
Evaluation of Acute Toxicity
Acute toxicity studies were performed as per OECD guidelines 425. The study was completed in three phases.
Phase 1: Rats were divided into four groups of two rats per group. Graded doses (500, 1000, 1500, 2000 mg/kg) of the plant extract were given to the rats orally. Rats were observed for the next 24 hrs. (0.25, 0.5, 1, 2, 4, 12 hrs.) for behavioral changes (dullness, restlessness, sedation, agitation), signs of toxicity, and mortality.
Phase 2: Based on the findings of Phase 1, three groups of rats (n = 2) were given the next doses (2500, 3000, 3500 mg/kg) of the plant extract orally. All the animals were continuously observed for general behavioral changes, symptoms of toxicity, and mortality during 24 hours—initially after every 15 minutes, then every 30 minutes, and then after 1, 2, 4, and 12 hours.
Phase 3: On the basis of the observations of Phase 2, after 24 hours, two groups of rats (n = 2) were administered the next higher doses (4000, 5000 mg/kg) of the plant extract orally. Rats were monitored for 24 hours post-treatment for behavioral changes (dullness, restlessness, sedation, agitation), signs of toxicity, and mortality [19].

Evaluation of Sub-acute Toxicity
OECD guidelines 407 were followed to perform sub-acute toxicity study with slight modifications (OECD, 2008). Although 1/5th of the highest dose from the acute toxicity test results could had been utilized for the current study, doses based on those previously employed in relevant research articles was selected and administered orally daily for 14 days to a rat's group (n = 6)[20]. For the same duration, the rat's control group (n = 6) was provided with the vehicle only. All experimental animals were kept under observation for any mortality, any abnormal clinical signs, and for the 14-day period of treatment. On the 14th day, rat's body weight was measured. Rats were anesthetized,

dissected, and blood samples were collected immediately with and without EDTA, for hematological and biochemical studies, respectively.

Phytochemical Analysis of Extract of M. paradisiaca

The aim of phytochemical analysis was to detect and identify the constituents present in the plant responsible for its pharmacological activities. GC–MS analysis was performed for the extracts of the plants using a Gas Chromatography equipment coupled with MS (5977B). Separation was performed using a 0.25 μm film DB-5 fused-silica capillary column (J&W Scientific, Folsom, CA). The mass spectrum was scanned (at a rate of 1.5 scans/s) from m/z 50 to 650, and peaks were identified using the Mass Hunter library, and retention time was used for the identification of the compounds.

Statistical Analysis

Results were presented as mean ± S.E.M. and for statistical analysis one-way ANOVA with post hoc Tukey test was used.

RESULTS

Effect of M. paradisiaca Extract/Fractions on Estrus Cycle Irregularity Induced by BPA:

A significant difference ($p < 0.05$) in the occurrence frequency of all estrus cycle phases was evident among groups administered with 500mg/kg and 250mg/kg M. paradisiaca extract (MP, MPC, MP2, MPC2) when compared to the disease control. In rats subjected to BPA administration, there was an elevated frequency of diestrus and metestrus phases, coupled with a reduction in the frequency of proestrus and estrus phases, contrasting with the placebo control (PC). Figure 1 shows representative photomicrographs of estrus cycle cytology of female rats treated with M. paradisiaca extract.

Table 1 presents, rats treated only with M. paradisiaca (MPC, MPC2) displayed estrus cycle cytology features that significantly different from those observed in the disease control (DC) rats. Similarly, rats administered only with the dichloromethane fraction of M. paradisiaca and the aqueous fraction (MPDCMC, MPAQC) also presented estrus cycle cytology features significantly different from those of the disease control (DC) rats. However, rats subjected to simultaneous treatment with BPA and the dichloromethane fraction or aqueous fraction of M. paradisiaca (MPDCM, MPAQ) did not show estrus cycle cytology features that were significantly different from those observed in the disease control (DC).

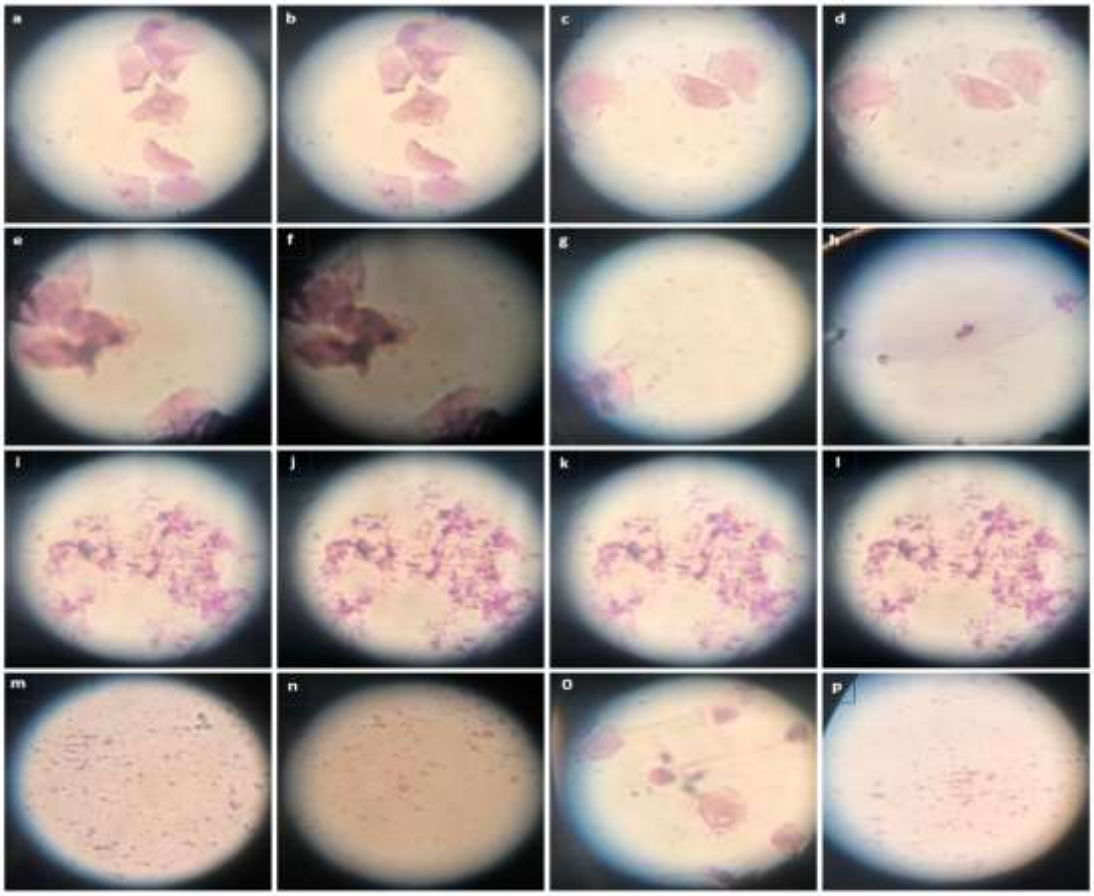


Figure No.1

Representative photomicrographs of estrus cycle cytology features at x10 magnification.The proestrus phase chiefly comprised of nucleated epithelial cells (a-d); The estrus phase chiefly comprised of anucleated cornified cells (e-h); The metestrus phase consisted of same part of the nucleated epithelial cells, anucleated cornified cells, and leukocytes (i-l); The diestrus phase chiefly consisted of of leukocytes (m-p).

Table 1: Mean number of days each phase of estrus cycle lasted per treatment group of *M. Paradisiaca* compared to disease control.

Group	Proestru	Estrus	Metestru	Diestrus
Placebo control	7.5± 0.5*	7.9± 1.1*	5.0 ± 0.7*	6.6 ± 1.1*
Disease control	2.9 ± 0.3	2.8 ± 0.7	8.3 ± 0.5	13.0 ± 1.0
MP	7.3 ± 1.0*	9.4 ± 0.9*	3.8 ± 0.9*	6.5 ± 0.4*
MPC	7.4 ± 0.2*	9.5 ± 1.0*	3.7 ± 0.2*	6.3 ± 0.3*
MP2	6.6 ± 1.7*	8.3 ± 0.4*	4.9 ± 1.3*	7.2 ± 1.5*
MPC2	6.1 ± 1.2*	9.0± 1.2*	4.5 ± 1.2*	6.9 ± 0.8*
MPAQ	3.9 ± 1.3 n.s.	3.6 ± 0.4 n.s.	7.1 ± 0.8 n.s.	12.4 ± 0.5 n.s.
MPAQC	6.0 ± 1.0*	7.7 ± 0.9*	5.2 ± 1.1*	7.0 ± 1.0*
MPDCM	4.1 ± 1.1 n.s.	3.5 ± 1.1n.s.	7.2 ± 0.3 n.s.	12.2 ± 0.3 n.s.
MPDCMC	6.1 ± 1.3*	7.5 ± 0.5*	5.5± 1.2*	7.2 ± 1.1*

*shows the values that are significantly different ($p \leq 0.05$) from Disease control.n.s. shows the values that are not significantly different ($P > 0.05$) from Disease control

Figure 2 Graphic presentation of mean duration of each phase of estrus cycle for each treatment group (M. paradisiaca extract/fractions).

Effect of M. Paradisiaca extract/fractions on BPA-Disrupted Female Sex Hormones.

A significant increase was seen in the blood concentration of FSH, LH, 17β-estradiol and progesterone when animals exposed to 250 mg/kg b. wt. (low dose) of M. paradisiaca aqueous ethanol (70:30) extract (MP) as compared with the disease control group ($P<0.05$), as presented in Table 3.12. Increase in the dose of M. paradisiaca aqueous ethanol (70:30) extract (MP2) to 500 mg/kg b. wt. resulted an increase more significantly in serum FSH, LH, 17β-estradiol and progesterone levels. This increase was significant as compared with those in the disease control group ($P<0.05$). Administration of dichloromethane fraction of M. Paradisiaca (MPDCM) to animals for 6 weeks showed no significant increase in the blood concentration of
FSH, LH, 17β-estradiol and progesterone, also Administration of M. paradisiaca aqueous extract (MPAQ) to animals for 6 weeks showed no significant effect in the blood concentration of FSH, LH, 17β-estradiol and progesterone as compared with the disease control group.
Table 2 presents the comparison of the mean levels of FSH, LH, 17β-estradiol, and progesterone in each treatment group of *M. paradisiaca* compared to the disease control.

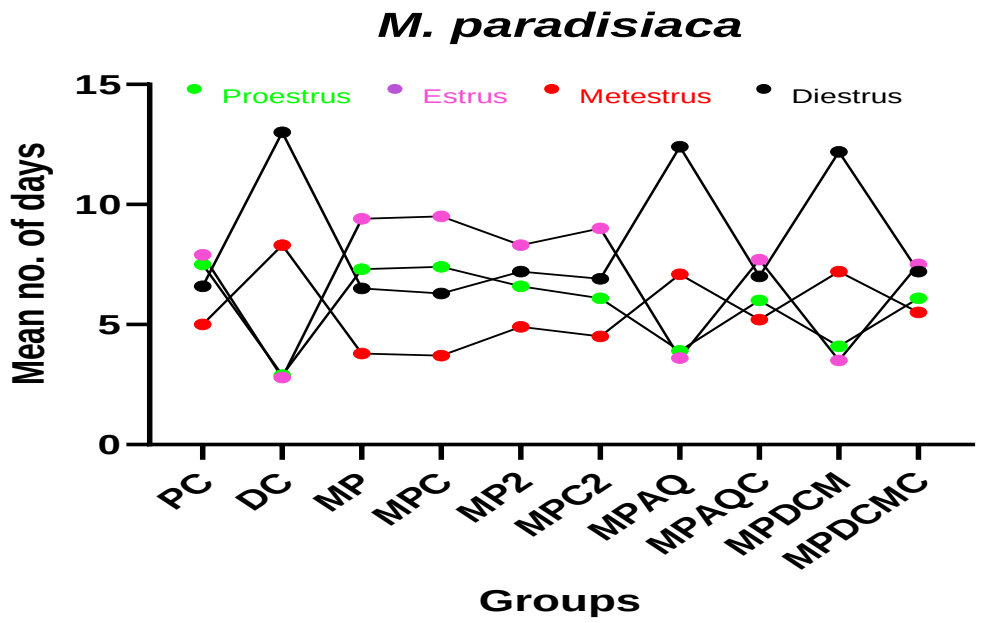


Figure 2

Figure 2 depicts the comparison of the mean number of days of each phase of the estrus cycle for each treatment group of *M. paradisiaca L* compared to thedisease control (DC).

Table 2: The levels of reproductive hormones in all *M. paradisiaca* treated groups (n = 8).

Group	FSH (mIU/ml)	LH (mIU/ml)	17β-Estradiol (pg/ml)	Progesterone (ng/ml)
Placebo control	10.81 ± 1.30*	15.50 ± 1.12*	351.3 ± 35.6*	14.44 ± 1.12*
Disease control	5.70 ± 1.54	8.02 ± 1.28	232.7 ± 11.7	6.63 ± 2.25
MP	8.8 ± 1.6*	12.3 ± 1.1*	318.8 ± 41.8*	11.7 ± 0.7*
MPC	8.9 ± 1.7*	15.8 ± 1.6*	348.1 ± 53.3*	14.8 ± 1.2*
MP2	9.6 ± 1.7*	11.1 ± 1.1*	306.9 ± 32.3*	12.7 ± 1.3*
MPC2	10.1 ± 1.7*	14.4 ± 1.5*	321.1 ± 50.1*	13.6 ± 0.8*
MPDCM	6.0 ± 1.3 n.s.	9.2 ± 2.2 n.s.	252.5 ± 46.2n.s.	7.7 ± 1.0n.s.
MPDCMC	9.8 ± 0.8 *	12.4 ± 1.2*	335.6 ± 39.2*	10.6 ± 0.6*
MPAQ	5.9 ± 1.0n.s.	8.8 ± 0.9n.s.	228.8 ± 40.2n.s.	8.7 ± 1.1n.s.
MPAQC	9.5 ± 1.7*	13.2 ± 1.1*	296.3 ± 39.6*	13.4 ± 1.3*

[*]00shows the values that are significantly different ($p \leq 0.05$) from Disease control.[n.s.] shows the values that are not significantly different ($P > 0.05$) from Disease control.

Effect of M. paradisiaca Extract/Fractions on BPA-Induced Follicular Disruption and Structural Changes in Rat Ovaries.

Rats administered with BPA alone (DC) showed more significant abnormalities in ovarian follicular structure compared to rats concurrently treated with M. paradisiaca(MP, MP2 group). The ovaries of BPA-treated rats exhibited a distinguished decrease in corpus luteum and antral follicles and an increase in cystic and atretic follicles. Histopathological evaluation of all ovaries from all experimental groups was done to find out the extent of these ovarian histopathological abnormalities (Fig. 3). The control groups (PC, MPC, MPC2, MPDCMC, MPAQC) showed significantly different morphology of ovaries as compared to the histological results of the ovaries in the disease control (DC) group. However, rats concomitantly treated with BPA and M. paradisiaca dichloromethane fraction or aqueous fraction (MPDCM, MPAQ) did not show significantly different morphology of ovaries as compared to the histological results of the ovaries in the disease control (DC) group. Figure 3 displays histopathological examinations of ovaries of rats treated with M. paradisiaca (extracts/fractions).

Figure 3

Histopathological examinations of ovaries of rats treated with *M. paradisiaca L*(extracts/fractions). In PC, normal histological appearance was examined (a-b). In BPA group, atretic follicles, stromal and follicular degenerations, and edema were seen (c-d). Normal histological features of the ovary with the presence of secondary and primary follicles in the ovarian cortex of BPA-exposed rats concomitantly treated with *M. paradisiaca* (MP, MP2 groups) (e-h). In rats treated with *M. paradisiaca* alone (MPC, MPC2, MPDCMC, MPAQC groups (i-p)), primary follicles were observed in the ovarian cortex. H&E. Histological appearance in the ovarian cortex of BPA-exposed rats concomitantly treated with M. paradisiaca dichloromethane extract and aqueous extract MPDCM, MPaq not significantly different to DC (q-t).

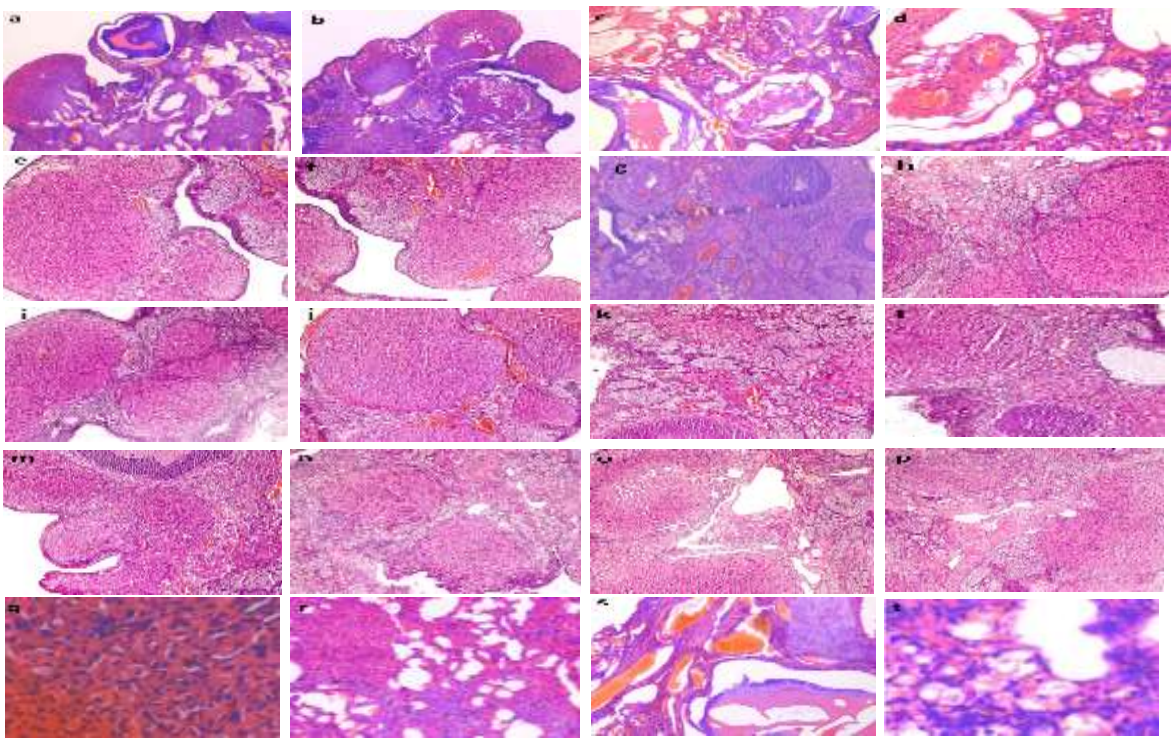


Figure 3

Safety Profile of *M. paradisiaca*: Evidence from Acute and Sub-Acute Toxicity Studies

M. paradisiaca ethanol aqueous (70:30) extract did not exhibit acute toxicity in Phase 1 and was observed to be safe when graded doses (0.5, 1.0, 1.5, 2.0 g/kg) of the plant were given orally to rats. No signs of mortality or changes in behavior or toxicity were detected during the next 24 hours (0.25, 0.5, 1, 2, 4, 12 hours) after treatment. Moreover, no toxic symptoms were observed immediately after dosing and throughout the 14-day period.

In sub-acute toxicity tests, there were no significant differences in the body weights between treated groups and the placebo control groupas shown in table 3. The data also presented the non-significant effect in serum levels of glucose as shown in table 4. Whereas, a significant decrease ($p< 0.05$) was detected in serum levels of, total cholesterol triglycerides and many liver enzymes (ALT, AST, and ALK Phosphatase).

Table 3 Effect of *M. paradisiaca.* on the weight of different organs in sub-acute toxicity studies.

Parameter	Control	500 mg/kg	250 mg/kg
Body weight (g)	234.38 ± 5.63	223.13 ± 7.85$^{n.s}$	225.00 ± 6.81$^{n.s}$
Heart weight (g)	1.44 ± 0.03	1.43 ± 0.04$^{n.s}$	1.44 ± 0.14$^{n.s}$
Kidney weight (g)	2.83 ± 0.11	2.84 ± 0.11$^{n.s}$	2.74 ± 0.13$^{n.s}$
Liver weight (g)	12.54 ± 0.39	12.24 ± 0.42 $^{n.s}$	11.59 ± 0.32$^{n.s}$

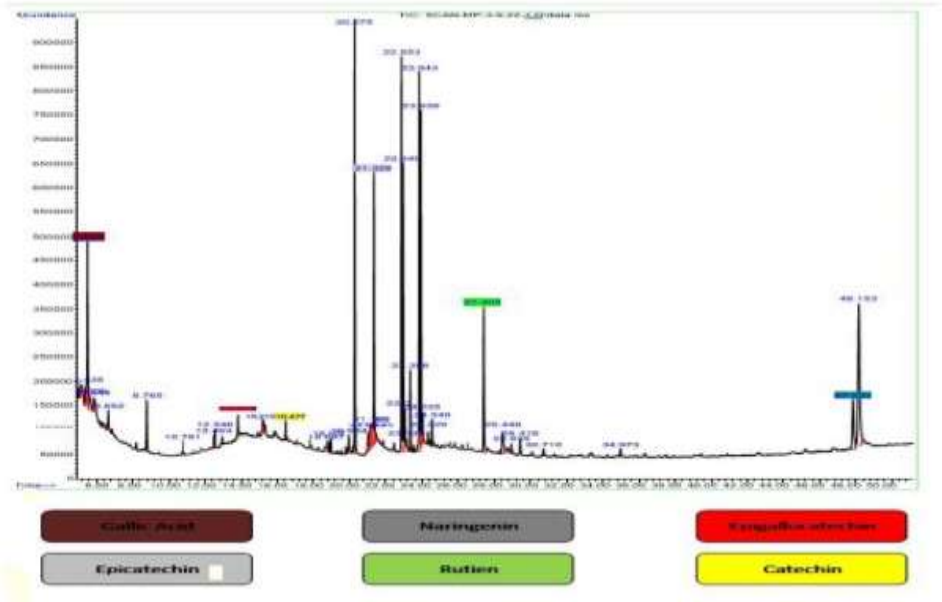
The data is presented as means ± standard error of the mean (S.E.M.). * shows the values that are significantly different ($p \leq 0.05$) from control. n.s. shows the values that are not significantly different ($P > 0.05$) from control

Table 4: Effect of *M. Paradisiaca*on biochemical parameters in sub-acute toxicity studies.

Parameter	Control	500 mg/kg	250 mg/kg
Glucose (mmol/L)	7.38 ± 0.35	7.15 ± 0.25 $^{n.s.}$	6.99 ± 0.32 $^{n.s.}$
Total cholesterol (mg/dl)	68.75 ± 3.87	63.63 ± 3.40*	52.38 ± 4.18*
Triglycerides (mg/dl)	91.25 ± 3.50	76.25 ± 6.73*	68.75 ± 4.30*
SGPT(ALT) U/L	61.50 ± 3.37	49.38 ± 3.33*	47.50 ± 3.27*
SGOT(AST) U/L	133.75 ± 6.80	111.88 ± 7.32*	103.75 ± 7.60*
ALK Phosphatase U/L	145.00 ± 5.67	121.25 ± 8.75*	113.75 ± 7.30*

The data is presented as means ± standard error of the mean (S.E.M.). * shows the values that are

Figure 4: GC-MS profile of M. paradisiaca



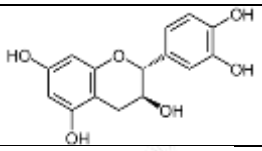
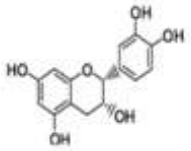
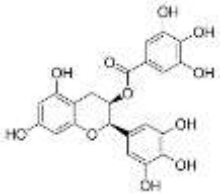
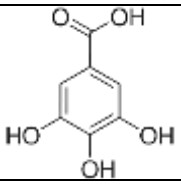
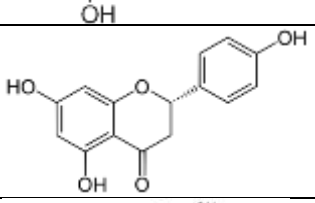
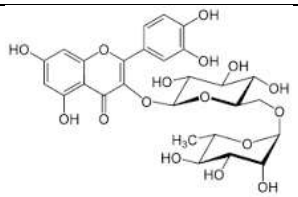
significantly different ($p \leq 0.05$) from control. n.s. shows the values that are not significantly different ($P > 0.05$) from control

Significant Phytochemical Analysis of *M. paradisiaca* Reveals Vital Compounds Beneficial for the Female Reproductive System

GC-MS analysis of *M. paradisiaca* was demonstrated in Figure 4 Graph confirms the presence of gallic acid, catechin, epicatechin, epigallocatechin, rutin, naringenin in *M. paradisiaca* extract.

GC-MS analysis indicated that *M. paradisiaca*extracts contains several chemical components eluted between 5.438 and 48.153 min (Table 5).

Table 4 GC-MS analysis of *M. paradisiaca* extract

Sr. No	Chemical Name	R.T	Mol. Formula	Mol. Mass (g/mol)	Structure
1	Catechin	16.477	$C_{15}H_{14}O_6$	290	
2	Epicatechin	21.329	$C_{15}H_{14}O_6$	290	
3	Epigallectocatechin	13.819	$C_{22}H_{18}O_{11}$	458	
4	Gallic acid	5.491	$C_7H_6O_5$	170.12	
5	Naringenin	47.882	$C_{15}H_{12}O_5$	272.25	
6	Rutin	27.405	$C_{27}H_{30}O_{16}$	610	

Discussion

The recent rise in associations between adult reproductive physiology and elevated body burdens of Bisphenol A (BPA) has garnered substantial attention due to the potential impact on fertility and overall reproductive health [8,9]. As BPA is an estrogenic endocrine-disrupting chemical, concerns about its adverse effects on various body organs are well-founded. However, despite the investigation of potential molecular mechanisms underlying BPA-induced toxicity, specific targeted treatments for BPA-induced toxicity in humans remain indescribable. This has led to the exploration of therapeutic alternatives that can counteract BPA's negative impacts. Natural compounds, especially those derived from plants, have demonstrated promising potential in treating

diseases[21,22]. Plant extracts like P. integerrima, green tea, soy-rich diets, Gb, KRG, and ginseng have shown considerable promise in alleviating BPA-induced toxicity, likely due to their intricate blend of bioactive compounds. The significance of these compounds becomes even more evident in the context of female reproductive health. Chlorogenic acid (CGA), catechins, Epigallectocatechin (EGC), gallic acid, rutin, and naringeninhave been identified as vital bioactive compounds withinM. paradisiaca. These compounds have shown potential benefits for female reproductive system disorders. For instance, a study demonstrated the efficacy of CGA in improving the in vitro development and growth of ovarian follicles in mice by boosting antioxidant capacity [23]. Similarly, catechins, such as those found in M. paradisiaca., have been highlighted for their antioxidant properties that could potentially enhance reproductive performance [24]. Another study identified EGCG's ability to inhibit infertility of oocytes and germ cells, offering a promising recommendation for enhancing fertility in animals and humans [25].

The alteration of estrous cyclicity induced by BPA in rodent models is well-documented [26,27]. BPA exposure leads to persistent diestrus, estrus, or even acyclicity in estrous cycles, reflecting disruptions in the hypothalamic-pituitary axis and the normal secretion of gonadotropins [28]. In the context of this study, the administration of M. paradisiacademonstrated an improvement in the percentage of normal estrous cycles in BPA-exposed rats. This improvement can be attributed to the normalization of FSH and LH hormone levels, reflecting the restoration of GnRH production in the brain. These observations align with the morphological improvements in ovarian follicles, which could be linked to the presence of alkaloids, terpenes, and flavonoids in M. paradisiaca.[29,30].

Specific flavonoids, like those identified in M. paradisiaca have demonstrated anti-infertility effects due to their radical scavenging and antioxidant properties [29,30]. Compounds such as gallic acid and rutin have been shown to counteract the detrimental effects of oxidative stress on ovarian histopathological structure [31,32]. These findings collectively underscore the potential of naturally occurring compounds to mitigate reproductive system diseases and disorders. The administration of M. paradisiaca to BPA-exposed rats led to a significant improvement in the morphological abnormalities of ovarian follicles, resulting in reduced atretic follicles and a higher percentage of animals exhibiting normal estrous cycles. These findings suggest that M. paradisiaca. possesses detoxifying properties, capable of mitigating ovarian toxicity induced by BPA. This substantiates its role as a protective agent against toxic effects triggered by BPA exposure. In conclusion, the bioactive compounds found in M. paradisiaca, such as chlorogenic acid, catechins, and epigallocatechin,gallic acid and rutin hold significant promise in addressing female reproductive system disorders. Their ability to counteract BPA-induced disruptions suggest their potential utility in reproductive health interventions. These findings provide insights into the development of novel therapeutic strategies for mitigating the toxic impacts of BPA on the female reproductive system. Further research is warranted to fully understand the underlying mechanisms and potential clinical applications of these compounds.

Overall, these findings underscore the crucial role of naturally occurring compounds in safeguarding and improving female reproductive health in the

face of environmental toxicants like BPA. Further exploration of these compounds could lead to innovative interventions for reproductive health disorders and may offer potential solutions to the rising concerns of infertility and reproductive dysfunction.

Conclusions

In conclusion, the hydroalcoholic extract derived from the fruit plus peel of M. paradisiaca L. exhibits a promising capacity to mitigate BPA-induced reproductive toxicity. This is evident through its ability to ameliorate the aberrations in ovarian follicle morphology, restore a regular estrous cycle, and normalize the levels of sexual-steroid hormones. These findings underscore the potential utility of M. paradisiacaas a therapeutic agent for countering the detrimental impacts of BPA on the reproductive system.

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