Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v31i6.7255

PROTECTIVE EFFECT OF HYDROALCOHOLIC EXTRACT FROM PSIDIUM GUAJAVA LEAVES ON BPA-INDUCED REPRODUCTIVE TOXICITY IN FEMALE RATS

Maryam Khan[1*], Hafiz M. Irfan[2], Taseer Ahmed Khan[3]

[1*,2]College of Pharmacy, University of Sargodha, 40100 Sargodha, Pakistan
[*]Email: maryamkhan59@yahoo.com; [2]Email: Muhammad.irfan@uos.edu.pk
[3]The Department of Physiology, University of Karachi, 75270 Karachi, Pakistan. Email: takhan@uok.edu.pk

***Corresponding Author:** Maryam Khan
*College of Pharmacy, University of Sargodha, Pakistan, Email: maryamkhan59@yahoo.com, Phone no: +923338602060.

ABSTRACT

Background: Psidium guajavaL. is a well-known plant traditionally used within the Pakistani community to address reproductive system irregularities. This study aims to assess the impact of various fractions of P. guajava L., derived from its hydroalcoholic extract, on female reproductive impairments induced by Bisphenol A (BPA).
Design: Animal study.
Setting: Conducted within animal and laboratory facilities at a university.
Animals: Eighty adult female Sprague Dawley rats.
Methods: The crude extract of the plant underwent acute and subacute toxicity studies to determine its safety profile. The extract was then subjected to activity-guided fractionation using solvents of differing polarities, including hexane, dichloromethane, and water. Dichloromethane and aqueous soluble fractions were used for pharmacological evaluations, while hexane-soluble fractions were omitted due to inadequate quantity. Eighty rats were divided into different groups based on the administered fraction. P. guajava L. leaves' hydroalcoholic extract and its fractions, along with Bisphenol A and the vehicle, were administered orally via gavage for six consecutive weeks. GC–MS analysis was conducted to detect and identify the plant's constituents responsible for its pharmacological effects. Main Outcome Measures: Parameters assessed included daily vaginal smears, gonadotropin and sexual-steroid hormone levels, as well as ovarian histopathology.
Results: Toxicity tests revealed the safety of the extract, with no mortality observed at doses as high as 5g/kg for P. GuajavaL. BPA-exposed animals exhibited disruptions in their estrous cycles, endocrine alterations, and adverse histopathological and morphological changes in rat ovaries. Animals treated with BPA in conjunction with ethanol-water extract displayed a dose-dependent reproductive protective effect in rats. Data from this study indicated that the plant's crude extract significantly increased the number of rats with normal estrous cycles, reduced the number of atretic follicles (as evidenced by histopathological examination of ovaries), and normalized levels of gonadotropin hormones (FSH and LH) and sexual steroid hormones (Estradiol and Progesterone), with the highest efficacy at 500 mg/kg for P. guajavaLethanol and water extract. GC-MS analysis of P. guajavaLethanol and water extract confirmed the presence of gallic acid, chlorogenic acid

(CGA), caffeic acid, catechin, epicatechin, epigallocatechin, and rutin in the plant's extract.Notably, published studies have reported the protective effects of rutin, gallic acid, and Kaempferol on reproductive disorders.

Conclusions: The data generated from this study underscore the valuable role of P. guajava L. in managing female reproductive system disorders. It is further concluded that no singular chemical entity can account for the reported pharmacological effects, as numerous phytochemicals have been identified in the plant, as revealed by the investigations mentioned above. Therefore, further research into the isolation of pure active principles and the elucidation of their precise mechanisms of reproductive protective and curative action is warranted.

INTRODUCTION

Reproductive health, including fertility, can be significantly impacted by exposure to environmental toxins, with Endocrine Disrupting Chemicals (EDCs) being a key concern. EDCs are external substances that, when encountered, particularly during in utero or puberty stages, may contribute to infertility [1].

Bisphenol A (BPA) is one such EDC to which humans are regularly exposed. It can leach into food from epoxy resin coatings of canned foods, polycarbonate and baby bottles, water bottles, and food storage containers. Previous research has established the harmful effects of BPA on the female reproductive system, making it a significant concern [2].

Studies have shown that BPA interferes with sex hormone activities, leading to developmental toxicity and functional disturbances in the reproductive system, ultimately contributing to female infertility [2]. High levels of BPA were detected in serum samples from infertile women, further highlighting its 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].

In the quest for possible treatment, natural products (NPs) have emerged as a promising avenue. P. guajava L., commonly known as guava, is a tropical tree extensively cultivated for its fruit [12]. With recognized pharmacological properties, guava leaves are valued as both food and traditional medicine, owing to their high content of bioactive compounds, particularly phenolic compounds, responsible for their antioxidant and anti-inflammatory activities [13]. Notably, quercetin is among the potent antioxidants found in guava leaves [13,14]. Traditional uses of guava leaves encompass treating gastrointestinal issues (diarrhea, stomach pain, gastroenteritis, indigestion, and dysentery) and dermatological problems (skin infection, skin aging, and ulcers) [15]. Additionally, in Pakistan, fresh guava leaves have been traditionally employed for addressing reproductive system irregularities, particularly menstrual problems [16].

In this study, the potential protective effects of P. guajava L. against the toxic effects of BPA on the female reproductive system was systematically explored. Through a six-week concurrent treatment of female rats with P. guajava L. and BPA via oral gavage, the protective impact on follicular development, gonadotropin (17β-estradiol and progesterone) and sex steroid hormones (FSH and LH), and the pattern of the estrous cycle were assessed. This

investigation may shed light on the role of P. guajava L. as a potential therapeutic agent for mitigating BPA-induced reproductive toxicity and could contribute to advancements in female reproductive health interventions.

METHODS

Chemicals and Reagents

The chemicals employed in this investigation, including Bisphenol A, n-hexane, ethanol, dichloromethane, and water, were procured from Sigma–Aldrich, Pakistan, and held to analytical grade standards.

Experimental Animals

Female Sprague-Dawley Rats were selected for this study, and they were housed under standard conditions as outlined by NRC, 1996. Ethical approval for the laboratory procedures was granted by the Biosafety and Animal Ethical Review Committee of the University of Sargodha (Approval No.: SU/ORJC/14009/09/2022).

Plant Material Selection and Collection

P. guajava L.leaves were chosen and collected from Sargodha, Punjab, 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 P. gujava L. leaves (Voucher No. MK-748) were deposited in the herbarium (SARGU) of the Department of Botany, University of Sargodha, Sargodha, ensuring their availability for future research references.

Extraction and Fractionation

Leaves (15 kg) of P. guajava L. were shade-dried and transformed into coarse powder for extraction. The powdered material was soaked in a mixture of ethanol and water (70:30, v/v), followed by filtration using muslin cloth and filter papers. This extraction process was repeated thrice, yielding a 25 % crude extract of P. guajava L. (PG).

For activity-guided fractionation, 200 g of the crude P. guajava L. extract was mixed with distilled water, and solvent-solvent extraction was carried out using hexane and dichloromethane solvents. The fractions obtained were concentrated at 40°C using a rotary evaporator, leading to the generation of a 105g aqueous fraction and a limited quantity of hexanefraction. The dichloromethanefraction was produced in a quantity of 48g. These samples were stored at 4°C for subsequent phytochemical and pharmacological evaluations [17]. The hexanefractions of P. guajava L. were excluded from pharmacological investigations due to their limited availability.

Pharmacological Studies on P. guajava L.

Experimental groups of female rats (n = 8) were categorized as follows:

1) PC (placebo control) provided with the vehicle (1.0 ml corn oil).
2) DC (disease control) provided with BPA incorporated in vehicle at 50 mg/kg b.w.
3) PG (P. gujava L.) extract was given 500 mg/kg b.w. half hour before BPA (50 mg/kg b.w.).
4) PGC (P. gujava L. control) was given 500 mg/kg b.w. of P. gujava L. extract.
5) PG2 (P. gujava L.2) given 250 mg/kg b.w. of P. gujava L. extract, half hour before they were given BPA (50 mg/kg b.w.).
6) PGC2 (P. gujava L. control 2) given 250 mg/kg b.w. of P. gujava L. extract.
7) PGDCM (P. gujava L. dichloromethane) was given 150 mg/kg b.w. of P. gujava L. Dichloromethane fraction half hour before BPA (50 mg/kg b.w.).
8) PGDCMC (P. gujava L. dichloromethane control) was given 150 mg/kg b.w. of P. gujava L. Dichloromethane fraction.
9) PGAQ (P. gujava L. aqueous) was given 150 mg/kg b.w. of P. gujava L. aqueous fraction half hour before BPA (50 mg/kg b.w.).
10) PGAQC (P. gujava L. aqueous control) was given 150 mg/kg b.w. of P. gujava L. aqueous fraction.

Effect of P. guajava L. Extract/Fractions on Reproductive Hormones

On the final day of treatment, rats were anesthetized, dissected, and blood samples were collected immediately. Serum was obtained through cardiac puncture and centrifuged at 3500 rpm for 15 minutes. The serum was then stored for subsequent hormone evaluation.

Serum FSH was quantified using the Roche Elecsys FSH assay (Ref. 11775863122, Roche, Indianapolis, IN, Germany), serum LH levels were determined using the Roche Elecsys LH assay

(Ref. 11732234122, Roche, Germany), serum progesterone levels were measured with the Snibe Maglumi Progesterone assay (Ref. 014210311, Snibe, Germany), and serum estradiol levels were assessed using the Snibe Estradiol Maglumi assay (Ref. 015220111, Snibe, Germany).
These hormone levels were evaluated using automated chemiluminescence immunology analyzers: Roche COBAS E411 (Roche Diagnostics, Basel, Switzerland) for FSH and LH, and Snibe Maglumi 800 (Snibe, Germany) for progesterone and estradiol. All chemicals used were of standard grade.

Histopathological Examination

Ovaries were promptly fixed in 10% buffered formalin for 24 to 48 hours for subsequent histological analysis. After fixation, each ovarian tissue sample underwent routine processing and embedding in paraffin. Thin 5-µm sections were obtained from paraffin-embedded tissue samples. These sections were stained with hematoxylin-eosin, and light photomicroscopy was employed for examination and documentation.

Assessment of Estrous Cycle Cyclicity

Estrous cycle assessment involved daily vaginal smears taken between 09:00 and 10:00 AM. Smears were collected and analyzed following the methodology outlined by Mayasari et al. for the examination of three cell types and dominant cells. Each rat was restrained gently but firmly, and a sterile cotton swab moistened with 0.9% NaCl was utilized for smear collection. Giemsa stain was used for smear staining. Slides were examined using ×10 and ×40 objective lenses of a light microscope. Estrous phases were characterized based on cytological appearance[18]:

- Proestrous: Predominantly composed of nucleated epithelial cells.
- Estrous: Predominantly composed of anucleated cornified cells.
- Metestrous: Predominantly composed of equal parts of nucleated epithelial cells, leukocytes, and anucleated cornified cells.
- Diestrus: Predominantly composed of prominent polymorphonuclear leukocytes and a few epithelial and cornified cells.

Estrous cycle patterns were classified as:

- Regular estrous cycle: Estrous phase detected at least twice during a 4 to 5-day sampling period.
- Irregular estrous cycle: Reduced frequency of estrus and proestrus phases, accompanied by an increased frequency of metestrus and diestrus phases.

Toxicity Evaluation of P. guajava L. Extract

Acute Toxicity Assessment

Acute toxicity studies were conducted following OECD guidelines 425 and were carried out in three phases:
Phase 1: Rats were divided into four groups of two rats each. Graded doses (500, 1000, 1500, 2000 mg/kg) of the plant extract were administered orally. Rats were observed over the next 24 hours (0.25, 0.5, 1, 2, 4, 12 hours) for behavioral changes (dullness, restlessness, sedation, agitation), signs of toxicity, and mortality.
Phase 2: Based on Phase 1 observations, three groups of rats (n = 2) received higher doses (2500, 3000, 3500 mg/kg) of the plant extract orally. Animals were continuously monitored for 24 hours for general behavioral changes, toxicity symptoms, and mortality—at intervals of 15 minutes, 30 minutes, 1 hour, 2 hours, 4 hours, and 12 hours.
Phase 3: Building on Phase 2 findings, two groups of rats (n = 2) were given even higher doses (4000, 5000 mg/kg) of the plant extract orally. Observations for behavioral changes, signs of toxicity, and mortality were continued for 24 hours [19].

Sub-acute Toxicity Evaluation

Sub-acute toxicity study adhered to OECD guidelines 407, with slight adaptations (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]. A control group (n = 6) received only the vehicle during the same period. All animals were observed for mortality, abnormal clinical signs, and body weight changes during the 14-day treatment. On the 14th day, rat body weight was measured. Rats were anesthetized, dissected, and blood samples were collected—some with EDTA for hematological studies and some without EDTA for biochemical studies.

Phytochemical Analysis of P. guajava L. Extract

The aim of phytochemical analysis was to identify the constituents responsible for the plant's pharmacological activities.

GC–MS analysis was carried out on plant extracts using Gas Chromatography equipment coupled with MS (5977B). Separation utilized a 0.25 μm film DB-5 fused-silica capillary column (J&W Scientific, Folsom, CA). The mass spectrum was scanned (at 1.5 scans/s) from m/z 50 to 650, and compound identification relied on the Mass Hunter library, in conjunction with retention time.

Statistical Analysis

Results were presented as mean ± S.E.M., and statistical analysis utilized one-way ANOVA followed by post hoc Tukey test.

RESULTS

Effect of P. gujava L. extract/fractions on the estrus cycle irregularity caused by BPA.

The influence of P. guajava L. extract/fractions on estrous cycle irregularities caused by BPA exposure was examined. Rats treated with 500mg/kg and 250mg/kg of P. guajavaL. extract displayed a significant improvement ($p < 0.05$) in the frequency of all estrous cycle phases compared to the disease control (DC) group. Rats exposed to BPA exhibited an elevated occurrence of metestrus and diestrus phases and a reduction in proestrus and estrus phases compared to the placebo control (PC) group.

However, co-administration of P. guajava L. extract at doses of 250mg/kg and 500mg/kg (PG and PG2) led to a decrease in the frequency of metestrus and diestrus phases and an increase in proestrus and estrus phases.

The estrous cycle cytology was observed and categorized into different phases: proestrus, estrus, metestrus, and diestrus. Representative photomicrographs in Figure 1 illustrate the cytological features of each phase.

Table 1 provides a summary of the average duration of each estrous cycle phase in different treatment groups. Rats treated with P. guajavaL. extract alone (PG, PG2) exhibited estrous cycle cytology significant difference ($p < 0.05$) to the disease control rats. However, co-treatment of BPA with P. guajava dichloromethane or aqueous fraction (PGDCM, PGAQ) did not restore estrous cycle cytology significant difference ($p < 0.05$)to the levels of the disease control.

Figure No.1: Representative photomicrographs displaying features of estrous cycle cytology at x10 magnification. The The proestrus phase was primarily composed of nucleated epithelial cells (a-d); The estrus phase primarily featured anucleated cornified cells (e-h); The metestrus phase included similar proportions of nucleated epithelial cells, anucleated cornified cells, and leukocytes (i-l); The diestrus phase predominantly consisted of leukocytes (m-p).

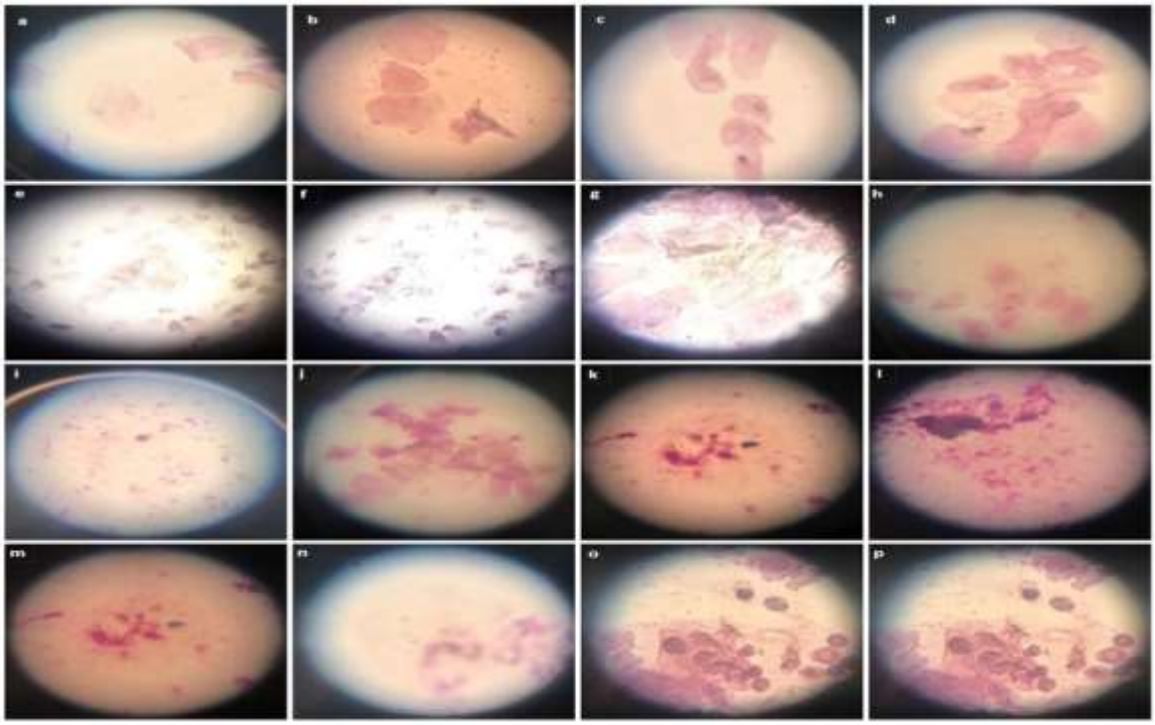


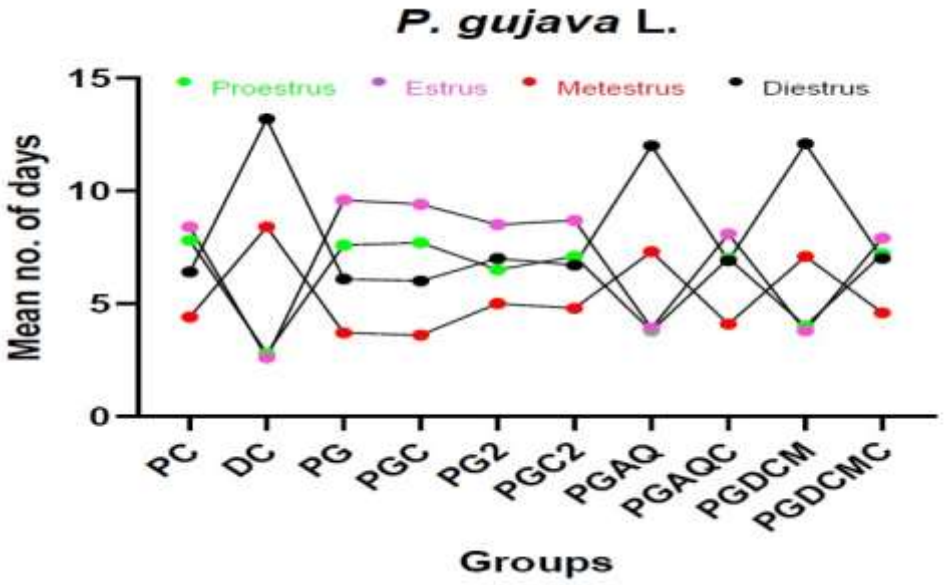
Table 1: Mean number of days each phase of estrus cycle lasted per treatment group of P. gujava L. compared to disease control.

Group	Proestrus	Estrus	Metestru	Diestrus
Placebo control	7.8 ± 1.3*	8.4 ± 1.4*	4.4 ± 0.3*	6.4 ± 1.1*
Disease control	2.8 ± 1.5	2.6 ± 1.8	8.4 ± 1.8	13.2 ± 0.7
PG	7.6 ± 1.4*	9.6 ± 1.5*	3.7 ± 0.4*	6.1 ± 0.9*
PGC	7.7 ± 1.5*	9.4 ± 2.1*	3.6 ± 1.5*	6.0 ± 1.1*
PG2	6.5 ± 1.7*	8.5 ± 1.3*	5.0 ± 0.4*	7.0 ± 1.2*
PGC2	7.1 ± 1.7*	8.7± 1.5*	4.8 ± 0.5*	6.7 ± 0.5*
PGAQ	3.8 ± 1.1 n.s.	3.9 ± 1.1 n.s.	7.3 ± 0.8 n.s.	12.0 ± 0.8 n.s.
PGAQC	7.0 ± 1.9*	8.1 ± 0.9*	4.1 ± 0.4*	6.9 ± 0.4*
PGDCM	4.0 ± 1.1 n.s.	3.8 ± 1.3 n.s.	7.1 ± 1.9 n.s.	12.1 ± 0.3 n.s.
PGDCMC	7.2 ± 1.8*	7.9 ± 1.4*	4.6 ± 0.4*	7.0 ± 1.0*

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

Figure 2 illustrates a graphical presentation comparing the mean duration of each estrous cycle phase for various P. guajavaL.extract/fractions treatment groups in comparison to placebo and positive control.

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



Effects of P. guajavaL. Extract/Fractions on Reproductive Hormone Levels

Treatment with P. guajava L. extract resulted in a significant increase in blood levels of FSH, LH, 17β-estradiol, and progesterone in a dose-dependent manner ($p < 0.05$). However, co-treatment of BPA with P. guajavaL. dichloromethane or aqueous fraction did not lead to a significant enhancement in blood hormone levels compared to the disease control. Table 2 presents the levels of reproductive hormones in different P. guajavaL. treatment groups compared to the disease and placebo control groups. Both PG and PG2 effectively reversed the BPA-induced decline in hormone levels, while PGDCM and PGAQ did not show the same effect.

Table 2 Levels of reproductive hormones in all P. guajava L. treatment groups (n = 8).

Group	FSH (mIU/ml)	LH (mIU/ml)	17β-Estradiol (pg/ml)	Progesterone (ng/ml)
Placebo control	11.08 ± 1.22*	16.01 ± 1.03*	353.8 ± 35.3*	14.43 ± 1.11*
Disease control	5.65 ± 1.60	8.32 ± 1.28	233.0 ± 11.8	6.51 ± 2.57
PG	9.4 ± 1.5*	13.3 ± 1.1*	321.3 ± 32.4*	14.3 ± 0.8*
PGC	10.5 ± 1.6*	15.2 ± 2.1*	368.9 ± 22.5*	15.7 ± 1.1*
PG2	8.6 ± 1.8*	13.2 ± 1.1*	310.1 ± 24.4*	12.0 ± 1.1*
PGC2	10.4 ± 1.8*	15.4 ± 1.6*	357.1 ± 27.5*	15.5 ± 0.7*
PGDCM	5.7 ± 1.3n.s.	9.2 ± 1.0n.s.	239.4 ± 24.8n.s.	6.9 ± 0.7n.s.
PGDCMC	10.3 ± 1.7*	14.9 ± 0.8*	343.1 ± 40.4*	14.7 ± 0.6*
PGAQ	5.6 ± 1.2n.s.	9.5 ± 1.2n.s.	238.8 ± 41.9n.s.	6.8 ± 0.6n.s.
PGAQC	10.2 ± 1.7*	15.0 ± 1.1*	353.8 ± 33.4*	15.7 ± 1.4*

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

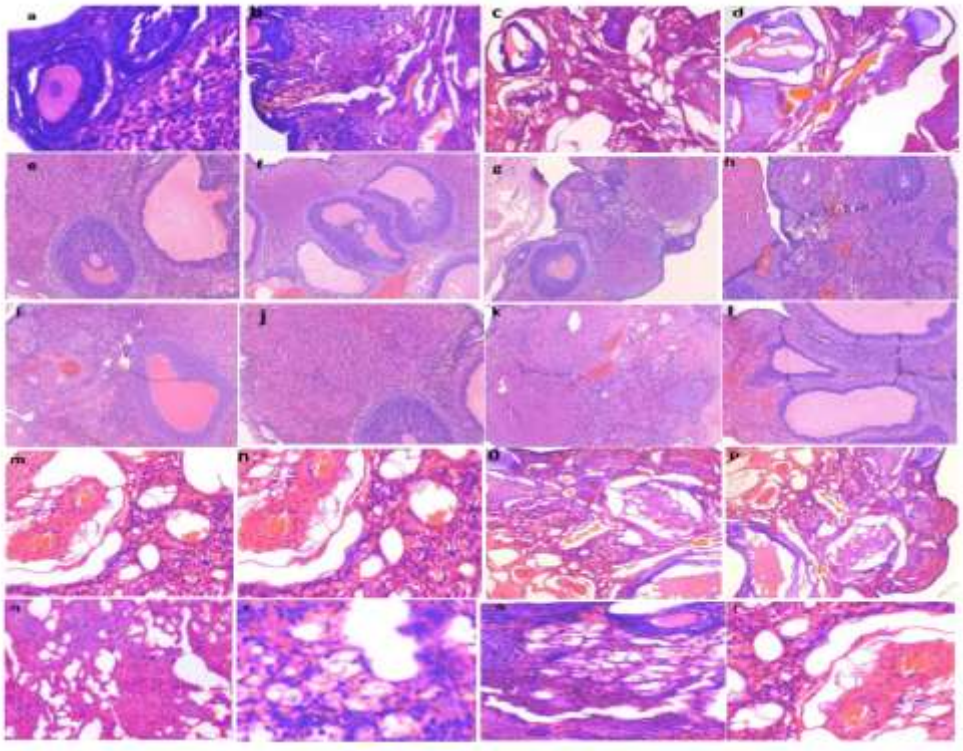
Effects of P. gujava L. Extracts and Fractions on BPA-Induced Follicular Disruption and Ovarian Histopathology in Rats

The extent of abnormalities was more apparent in the ovaries of rats administered with BPA alone (DC) compared to rats administered with BPA concomitantly treated with P. gujava L. (PG, PG2 group).

The results revealed adverse morphological and histopathological changes in the ovaries of rats in BPA-administered groups. A notable decrease was observed in corpus luteum and antral follicles, accompanied by an increase in cystic and atretic follicles in the ovaries of rats exposed to BPA. Histopathological assessments were conducted on all ovaries from the experimental groups to ascertain the extent of these ovarian histopathological abnormalities (Fig. 3.8).

Consistently, the histological changes in ovaries were evident across all experimental groups. The control group (PC) and rats treated only with P. gujava L. (PGC, PGC2) exhibited significantly different ovarian morphology compared to the histological results of the ovaries in the disease control (DC) group. Similarly, the control group (PC) and rats treated alone with P. gujava L. dichloromethane fraction and P. gujava L. aqueous fraction (PGDCMC, PGAQC) displayed significantly different ovarian morphology compared to the histological results of the disease control (DC) ovaries. However, the control group (PC) and rats concomitantly treated with BPA using P. gujava L. dichloromethane fraction and P. gujava L. aqueous fraction (PGDCM, PGAQ) did not exhibit significantly different ovarian morphology compared to the histological results of the disease control (DC).

Figure 3: Histopathological evaluations of ovaries from rats treated withP. guajava 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 futures of ovary with the present of secondary and primary follicle in the ovarian cortex of BPA-exposed rats concomitantly treated with P. gujava L. (PG, PG2 groups) (e-h). In rats treated with P. gujava L. alone (PGC, PGC2, PGDCMC, PGaqC 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 P. gujava L. dichloromethane fraction and aqueous fraction PGDCM, PGAQ not significantly different to DC (q-t).



SAFETY PROFILE AND PHYTOCHEMICAL ANALYSIS

In acute toxicity studies, P. gujava L. ethanol aqueous (70:30) extract displayed no signs of toxicity during the observation period. Additionally, sub-acute toxicity tests revealed no significant differences in body weights, glucose levels and liver enzymes (ALT, AST, and ALK Phosphatase) among treated groups compared to the placebo control (Table 3 and 4). However, there was a significant decrease ($p < 0.05$) in serum levels of totalcholesterol and triglycerides (Table 4).

Phytochemical analysis identified several beneficial compounds in P. guajavaL. extract includinggallic acid, chlorogenic acid (CGA), caffeic acid, catechin, epicatechin, epigallocatechin, rutin(Figure 4, Table 4).

Table 3 Effect of P. gujava L. on weight of different organs in sub-acute toxicity studies

Parameter	Control	PG 500 mg/kg	PG 250 mg/kg
Body weight (g)	232.88 ± 7.30	226.00 ± 6.53 n.s.	219.63 ± 6.63 n.s.
Heart weight (g)	1.42 ± 0.03	1.46 ± 0.05 n.s.	1.44 ± 0.04 n.s.
Kidney weight (g)	2.84 ± 0.12	2.73 ± 0.05 n.s.	2.68 ± 0.07 n.s.
Liver weight (g)	12.55 ± 0.40	11.45 ± 0.44 n.s.	11.10 ± 0.43 n.s.

Table 4 Effect of P. gujava L. on biochemical parameters in sub-acute toxicity studies.

Parameter	Control	PG 500 mg/kg	PG 250 mg/kg
Glucose (mmol/L)	7.35 ± 0.40	6.31 ± 0.46 n.s.	6.23 ± 0.55 n.s.
Total cholesterol (mg/dl)	63.75 ± 3.87	47.13 ± 5.47 *	62.25 ± 3.90 n.s.
Triglycerides (mg/dl)	91.88 ± 5.59	72.50 ± 4.91 *	86.88 ± 4.81 n.s.
SGPT(ALT) U/L	62.50 ± 4.01	58.13 ± 3.89 n.s.	54.38 ± 3.59 n.s.
SGOT(AST) U/L	135.00 ± 8.18	133.13 ± 6.33 n.s.	127.50 ± 8.81 n.s.
ALK Phosphatase U/L	146.25 ± 9.44	140.00 ± 11.18 n.s.	138.75 ± 9.44 n.s.

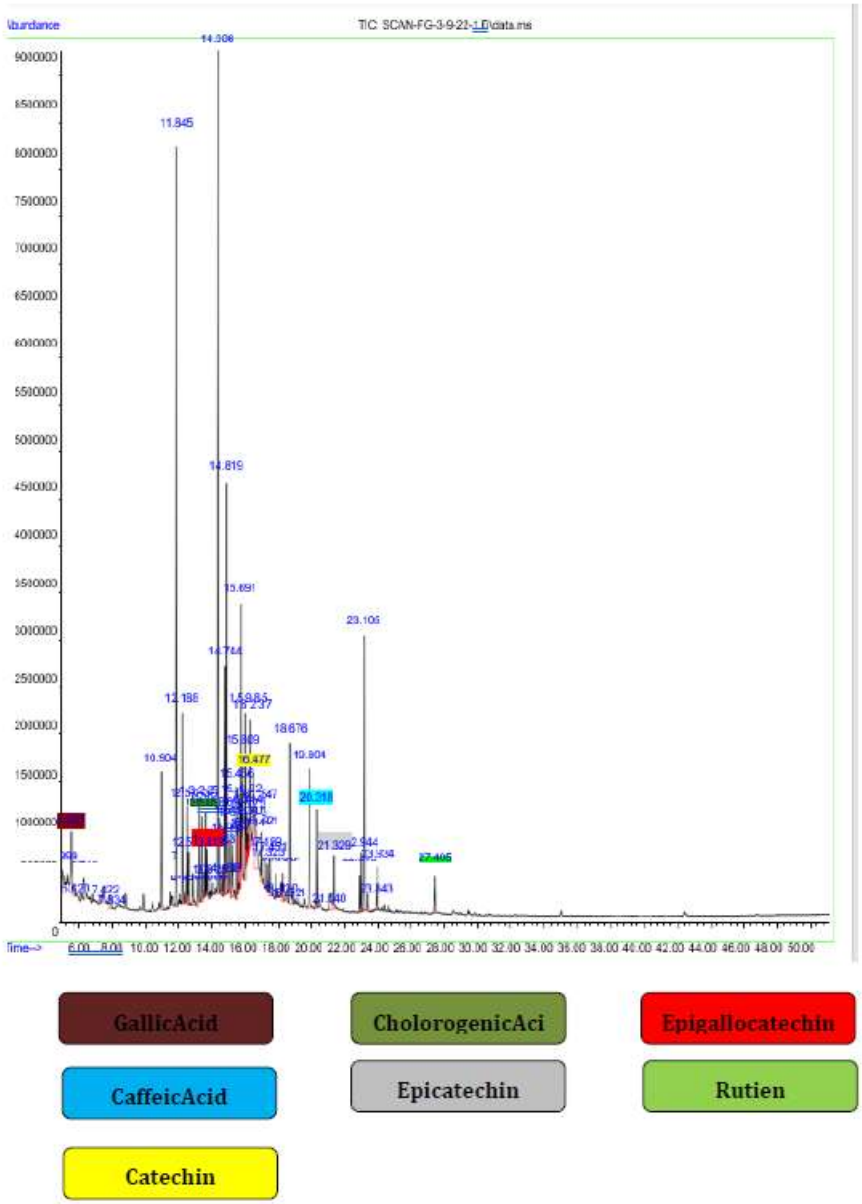
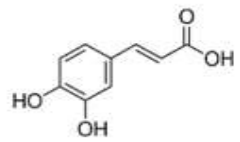
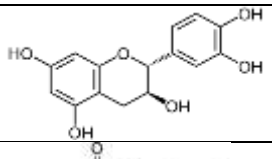
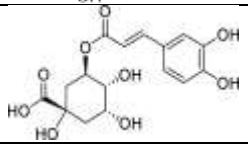
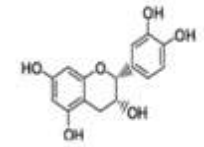
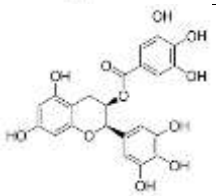
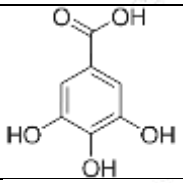
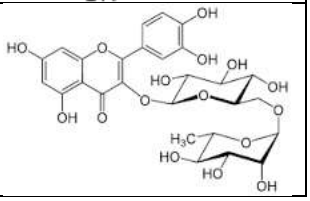


Figure 4: GC-MS profile of P. guajava L. extract

Table 4 GC-MS analysis of P. guajava L. extract

Sr. No	Chemical Name	R.T	Mol. Formula	Mol. Mass (g/mol)	Structure
1	Caffeic acid	20.318	$C_9H_8O_4$	180.16	
2	Catechin	16.477	$C_{15}H_{14}O_6$	290	
3	Chlorogenic acid	13.313	$C_{16}H_{18}O_9$	354	

4	Epicatechin	21.329	$C_{15}H_{14}O_6$	290	
5	Epigallactocatechin	13.819	$C_{22}H_{18}O_{11}$	458	
6	Gallic acid	5.491	$C_7H_6O_5$	170.12	
7	Rutin	27.405	$C_{27}H_{30}O_{16}$	610	

DISCUSSION

Recent correlations have been drawn between adult reproductive physiology and elevated body burdens of BPA. For example, a recent study reports that females with high BPA burdens are potentially more susceptible to miscarriage [8 Sugiura]. In a study, serum BPA levels were detected (limit of assay detection: 0.5 ng/mL) in 41.8% of infertile women and 23.3% of fertile women [9]. Exposure to BPA in everyday life is practically unavoidable. BPA is an estrogenic endocrine disrupting chemical, and the adverse effects on different body organs are a cause of concern. Therefore, there is a need to curb exposure to BPA in both adults and pregnant women. Even though potential molecular mechanisms underlying BPA-induced toxicity have been investigated, there is currently no specific targeted treatment for BPA-induced toxicity in humans. Hence, there is a need to develope a therapeutic drug that antagonizes BPA-induced toxicity. In the past, NPs have significantly contributed to drug discovery to treat diseases, particularly cancer and infectious diseases[21,22]. An example is a fungus-derived fingolimod drug, which the FDA approved for the treatment of multiplesclerosis[23,24,25]. One of the major advantages of NPs in drug discovery is that they confer multiple ―targets‖ by targeting more than one signaling pathway [26]. The plant extracts, including P. integerrima, green tea, soy-rich diet, Gb, KRG, and ginseng, seem to be the most promising in alleviating BPA-induced toxicity. However, the active compounds in these extracts need to be explored. On the other hand, natural compounds, such as RSV, luteolin, lycopene, AS IV, genistein, and curcumin are found to be most promising in mitigating BPA toxicity. In the future, more research should be conducted to explore the complex network of molecular mechanisms to precisely understand the roles of NPs. The main challenges of NP-based drug development are attributed to its poor bioavailability and determining the optimal dose. Hence, further pharmacokinetic studies in clinical settings are warranted.

As reported in several studies [27, 28, 29, 30, 31, 32, 33], estrous cyclicity was disrupted by BPA in rodent models. It was observed that the estrous cycles became persistent diestrus, persistent estrous or ultimately to acyclicity. Interestingly, we observed an improvement in the percentage of normal estrous cycle in BPA-exposed rats treated with P. guajava L. Disruption of normal estrous cycle is an indicator to alteration in the function of the hypothalamic-pituitary axis in BPA-exposed female rats [29] by interfering with the normal production of gonadotrophin releasing hormone (GnRH) and thereby decreasing the secretion of FSH and LH.

In this study, it was found that treatment with P. guajava L could hinder the disruption in normal estrous cycle via the reversal of FSH and LH hormones to their normal levels, which is reflected in the normalization of GnRH production in the brain. These results are also in line with the improvement of morphological findings in the ovarian follicles. All these improvements could be explained by the fact that P. guajava L contains alkaloids, terpenes, and flavonoids (phenolic compounds). Our findings are consistent with previous research indicating that these compounds possess potential anti-infertility effects due to their radical scavenging and antioxidant properties [34, 35]. Notably, published studies have reported the protective effects of specific flavonoids on reproductive disorders. Rajan et al. (2017) demonstrated the beneficial impact of soy flavonoids in a rat model of PCOS [36]. Similarly, Jahan et al. (2016) described the protective effects of rutin on PCOS, highlighting its ability to modulate various biochemical parameters [37]. Furthermore, gallic acid, a significant component of our extract, has been shown to inhibit the deleterious effects of CdCl2 on ovarian histopathological structure, antioxidant enzymes, and oxidative stress [38]. Kaempferol is a flavonoid. Emma Mendonca, 2013 reported that in vitro, it plays its role [39]. These findings show the potential usage of P. guajava L. for effective use in the management of reproductive system diseases.These findings collectively support the potential usage of naturally-occurring compounds, such as those identified in P. guajava L, in the management of reproductive system diseases. Further research is warranted to elucidate the underlying mechanisms of action and to explore the clinical applications of these compounds in reproductive health interventions.

When BPA-exposed rats are treated with P. guajava L, there was a marked improvement in the morphological abnormalities in ovarian follicules. In addition to the reduced number of atretic follicles, there was also improvement in the percentage of animals with normal estrous cycle compared to BPA-exposed rats without P. guajava L treatment. These results illustrated the ability of P. guajava L as a potential agent that is able to reduce ovarian toxicity, hence demonstrating its contribution to the protective mechanism against the genotoxic effects induced by BPA.

CONCLUSIONS

In conclusion, the current study underscores the potential of P. guajava Lextractin restoring BPA-induced female reproductive system irregularities. The presence of compounds like gallic acid, caffeic acid, kaempferol, vanillin, and ferulic acid highlights the promising role of these natural products in mitigating the adverse effects of BPA. Further research is needed to find out the exact mechanisms underlying their actions and explore their clinical applications in reproductive health interventions.

REFERENCES

1. Jain, M., & Singh, M. (2022). Environmental toxins and infertility. StatPearls [Internet]. Treasure Island, FL: StatPearls Publishing. Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK576379/ (Birnbaum)
2. Pivonello, C., Muscogiuri, G., Nardone, A., Garifalos, F., Provvisiero, D. P., Verde, N., ... & Colao, A. (2020). Bisphenol A: An emerging threat to female fertility. Reproductive Biology and Endocrinology, 18, 22. doi: 10.1186/s12958-019-0558-8.
3. La Rocca, C., Tait, S., Guerranti, C., Busani, L., Ciardo, F., Bergamasco, B., ... & Lucente, C. (2014). Exposure to endocrine disrupters and nuclear receptor gene expression in infertile and fertile women from different Italian areas. International Journal of Environmental Research and Public Health, 11, 10146-10164. doi: 10.3390/ijerph111010146.
4. Tachibana, T., Wakimoto, Y., Nakamuta, N., Phichitraslip, T., Wakitani, S., Kusakabe, K., ... & Hondo, E. (2007). Effects of bisphenol A (BPA) on placentation and survival of the neonates in mice. Journal of Reproduction and Development, 53, 509-514. doi: 10.1262/jrd.18171.
5. Honma, S., Suzuki, A., Buchanan, D. L., Katsu, Y., Watanabe, H., & Iguchi, T. (2002). Low dose effect of in utero exposure to bisphenol A and diethylstilbestrol on female mouse reproduction. Reproductive Toxicology, 16, 117-122. doi: 10.1016/S0890-6238(02)00006-0.

6. Lawson, C., Gieske, M., Murdoch, B., Ye, P., Li, Y., Hassold, T., & Hunt, P. A. (2011). Gene expression in the fetal mouse ovary is altered by exposure to low doses of bisphenol A. Biology of Reproduction, 84, 79-86. doi: 10.1095/biolreprod.110.084814.
7. Rodríguez, H. A., Santambrosio, N., Santamaría, C. G., Muñoz-de-Toro, M., & Luque, E. H. (2010). Neonatal exposure to bisphenol A reduces the pool of primordial follicles in the rat ovary. Reproductive Toxicology, 30, 550-557. doi: 10.1016/j.reprotox.2010.07.008.
8. Sugiura-Ogasawara, M., Ozaki, Y., Sonta, S., Makino, T., & Suzumori, K. (2005). Exposure to bisphenol A is associated with recurrent miscarriage. Human Reproduction, 20, 2325-2329. doi: 10.1093/humrep/deh888.
9. Mínguez-Alarcón, L., Gaskins, A. J., Chiu, Y. H., Williams, P. L., Ehrlich, S., Chavarro, J. E., ... & Hauser, R. (2015). Urinary bisphenol A concentrations and association with in vitro fertilization outcomes among women from a fertility clinic. Human Reproduction, 30, 2120-2128. doi: 10.1093/humrep/dev183.
10. Vahedi, M., Saeedi, A., Poorbaghi, S. L., Sepehrimanesh, M., & Fattahi, M. (2016). Metabolic and endocrine effects of bisphenol A exposure in market seller women with polycystic ovary syndrome. Environmental Science and Pollution Research International, 23, 23546-23550. doi: 10.1007/s11356-016-7573-5.
11. Shen, Y., Zheng, Y., Jiang, J., Liu, Y., Luo, X., Shen, Z., ... & Sun, Z. (2015). Higher urinary bisphenol A concentration is associated with unexplained recurrent miscarriage risk: Evidence from a case-control study in eastern China. PLoS ONE, 10(6), e0127886. doi: 10.1371/journal.pone.0127886.
12. Flores, G., Wu, S. B., Negrin, A., Kennelly, E. J. (2015). Chemical composition and antioxidant activity of seven cultivars of guava (Psidium guajava) fruits. Food Chemistry, 170, 327-335. doi: 10.1016/j.foodchem.2014.08.063.
13. Naseer, S., Hussain, S., Naeem, N., Pervaiz, M., & Rahman, M. (2018). The phytochemistry and medicinal value of Psidium guajava (guava). Clinical Phytoscience, 4, 32. doi: 10.1186/s40816-018-0094-7.
14. Nantitanon, W., Yotsawimonwat, S., & Okonogi, S. (2010). Factors influencing antioxidant activities and total phenolic content of guava leaf extract. LWT - Food Science and Technology, 43, 1095-1103. doi: 10.1016/j.lwt.2010.03.002.
15. Gutiérrez, R. M. P., Mitchell, S., & Solis, R. V. (2008). Psidium guajava: A review of its traditional uses, phytochemistry, and pharmacology. Journal of Ethnopharmacology, 117, 1-27. doi: 10.1016/j.jep.2008.01.025.
16. Tariq, A., Adnan, M., Iqbal, A., Sadia, S., Fan, Y., Nazar, A., ... & Khan, A. L. (2018). Ethnopharmacology and toxicology of Pakistani medicinal plants used to treat gynecological complaints and sexually transmitted infections. South African Journal of Botany, 114, 132-149. doi: 10.1016/j.sajb.2017.09.012.
17. Alamgeer, Chabert, P., Akhtar, M. S., Jabeen, Q., Delecolle, J., Heintz, D., Garo, E. and Oak, M. H. (2016). Endothelium-independent vasorelaxant effect of a Berberis orthobotrys root extract via inhibition of phosphodiesterases in the porcine coronary artery. Phytomedicine., 23(8): 793-799.
18. Mayasari, A., Suryawan, A., Christita, M., Simamora, A. T. J., Abinawanto, A., Suryanda, A., & Bowolaksono, A. (2018). Vaginal swab cytology aplication to determine the estrus cycle of lowland anoa (bubalus depressicornis, smith, 1927) in captivity. MATEC Web of Conferences, 197, 06008.
19. Akhila JS, Shyamjith D, Alwar M. Acute toxicity studies and determination of median lethal dose. Current science 2007:917
20. Roy, C. K., Kamath, J. V., & Asad, M. (2006). Hepatoprotective activity of Psidium guajava Linn. leaf extract. Indian Journal of Experimental Biology, 44(4), 305-311. PMID: 16629373.

21. Atanasov, A. G. et al. Discovery and resupply of pharmacologically active plant-derived natural products: a review. Biotechnol. Adv. 33, 1582–1614 (2015). Article CAS PubMed PubMed Central Google Scholar
22. Harvey, A. L., Edrada-Ebel, R. & Quinn, R. J. The re-emergence of natural products for drug discovery in the genomics era. Nat. Rev. Drug Discov. 14, 111–129 (2015).
23. Newman, D. J. & Cragg, G. M. Natural products as sources of new drugs from 1981 to 2014. J. Nat. Prod. 79, 629–661 (2016).ArticleCASPubMedGoogle Scholar
24. Waltenberger, B., Mocan, A., Šmejkal, K., Heiss, E. H. E. H. & Atanasov, A. A. G. A. G. Natural products to counteract the epidemic of cardiovascular and metabolic disorders. Molecules 21, 807 (2016).ArticlePubMed CentralCASGoogle Scholar
25. Tintore, M., Vidal-Jordana, A. & Sastre-Garriga, J. Treatment of multiple sclerosis — success from bench to bedside. Nat. Rev. Neurol. 15, 53–58 (2019).ArticleCASPubMedGoogle Scholar
26. Yang, Y., Wei, S., Zhang, B., & Li, W. (2021). Recent progress in environmental toxins-induced cardiotoxicity and protective potential of natural products. Frontiers in Pharmacology, 12, 699193. doi: 10.3389/fphar.2021.699193. [PMC free article] [PubMed] [CrossRef]
27. Adewale, H. B., Jefferson, W. N., Newbold, R. R., & Patisaul, H. B. (2009). Neonatal bisphenol-A exposure alters rat reproductive development and ovarian morphology without impairing activation of gonadotropin-releasing hormone neurons. Biology of Reproduction, 81(4), 690-699. doi: 10.1095/biolreprod.109.078261. [PMC free article] [PubMed] [CrossRef]
28. Markey, C. M., Coombs, M. A., Sonnenschein, C., & Soto, A. M. (2003). Mammalian development in a changing environment: Exposure to endocrine disruptors reveals the developmental plasticity of steroid-hormone target organs. Evolution and Development, 5(1), 67-75. doi: 10.1046/j.1525-142X.2003.03011.x. [PubMed] [CrossRef]
29. Rubin, B. S., Murray, M. K., Damassa, D. A., King, J. C., & Soto, A. M. (2001). Perinatal exposure to low doses of bisphenol A affects body weight, patterns of estrous cyclicity, and plasma LH levels. Environmental Health Perspectives, 109(7), 675-680. doi: 10.1289/ehp.01109675. [PMC free article] [PubMed] [CrossRef]
30. Howdeshell, K. L., Hotchkiss, A. K., Thayer, K. A., Vandenbergh, J. G., vom Saal, F. S., & Vandenbergh, J. G. (1999). Exposure to bisphenol A advances puberty. Nature, 401(6755), 763-764. doi: 10.1038/44517. [PubMed] [CrossRef]
31. Mendoza-Rodriguez, C. A., Garcia-Guzman, M., Baranda-Avila, N., Morimoto, S., Perrot-Applanat, M., & Cerbon, M. (2011). Administration of bisphenol A to dams during the perinatal period modifies molecular and morphological reproductive parameters of the offspring. Reproductive Toxicology, 31(2), 177-183. doi: 10.1016/j.reprotox.2010.10.013. [PubMed] [CrossRef]
32. Fernandez, M., Bianchi, M., Lux-Lantos, V., & Libertun, C. (2009). Neonatal exposure to bisphenol A alters reproductive parameters and gonadotropin-releasing hormone signaling in female rats. Environmental Health Perspectives, 117(5), 757-762. doi: 10.1289/ehp.0800267. [PMC free article] [PubMed] [CrossRef]
33. Nikaido Y, Yoshizawa K, Danbara N, Tsujita-Kyutoku M, Yuri T, Uehara N, Tsubura A. Effects of maternal xenoestrogen exposure on development of the reproductive tract and mammary gland in female CD-1 mouse offspring. Reprod Toxicol. 2004;18(6):803–811. doi: 10.1016/j.reprotox.2004.05.002. [PubMed] [CrossRef] [Google Scholar]
34. Oke, J. M., & Hamburger, O. (2002). Screening of some Nigerian medicinal plants for antoxidant activity using 2, 2, Diphenyl-Picryl-Hydrazyl radical, 5, 77–79.
35. Oladimeji, O. S., Lawal, O. A., & Ogundimu, E. O. (2014). Anti-oxidants levels in female rats administered pro-fertility ethanolic leave extract of byrsocarpus coccineus. European Scientific Journal, 10(15), 349–363.
36. Rajan, R. K., M, S. S. K., & Balaji, B. (2017). Soy isoflavones exert beneficial effects on letrozole-induced rat polycystic ovary syndrome (DCOS) model through anti-androgenic mechanism. Pharmaceutical Biology, 55(1), 242–251.

37. Jahan, S., Munir, F., Razak, S., Mehboob, A., Ain, Q. U., Ullah, H., Afsar, T., Shaheen, G., & Almajwal, A. (2016). Ameliorative effects of rutin against metabolic, biochemical and hormonal disturbances in polycystic ovary syndrome in rats. Journal of Ovarian Research, 9(1).
38. Rotimi, D., Ojo, O. A., Emmanuel, B. A., Ojo, A. B., Elebiyo, T. C., Nwonuma, C. O., & Oluba, O. M. (2021). Protective impacts of gallic acid against cadmium-induced oxidative toxicity in the ovary of rats. Comparative Clinical Pathology, 30(3), 453–460.
39. Mendonca, E. M. F. T. (2013). Kaempferol Exhibits Progestogenic Effects in Ovariectomized Rats. Journal of Steroids & Hormonal Science, 05(03).