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Unveiling safety profile of *Pereskia bleo* leaf extract: A comprehensive study on female toxicity and teratogenicity in Sprague Dawley rats

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KEYWORDS

Medicinal plant, *Pereskia bleo*, Teratogenicity, Herb(s) safety, Histopathology

Abstract

Pereskia bleo (*P. bleo*), renowned for its traditional medicinal use in various countries, is valued for its therapeutic potential. While medicinal plants hold immense promise, concerns regarding potential adverse effects on normal cells and teratogenicity remain. This study evaluated the influence of methanol extract of *Pereskia bleo* (MEPB) leaves on female toxicity and teratogenicity in Sprague Dawley rats. Female rats (n=40) were divided into four groups receiving MEPB doses (0, 250, 500, and 1000 mg/kg/day) during pre-mating, mating, and gestation periods. Gas Chromatography-Mass Spectrometry (GC-MS) analysis exhibited that MEPB leaves contain phenolic compounds, terpenoids, phytosterols, and fatty acids. Remarkably, all the animals exhibited regular oestrous cycles, maintaining body weight, and showed no signs of toxicity or abnormal behavior. Visceral organ weights and histological analysis revealed no significant alterations, attesting to the extract's safety. Pregnancy outcomes, including corpora lutea, implantation sites, percentages of pre- and post-implantation death, gravid uterine weight, number of live and dead fetuses, fetal body weight, and fetal sex ratio, remained unaffected. Furthermore, meticulous fetal examinations confirmed that MEPB did not impact foetal parameters or induce deformities. Importantly, daily MEPB consumption (250-1000 mg/kg) from pre-pregnancy to pregnancy did not compromise maternal or fetal well-being. These results underscore the lack of embryotoxic or teratogenic effects of MEPB, highlighting its potential for safe therapeutic applications.

Introduction

The profound connection between nature and medicine resonates across cultures and epochs, manifesting in the global utilization of plants for traditional and contemporary healing practices. Remarkably, nearly 80% of the world's population continues to place faith in the therapeutic embrace of medicinal plants, as endorsed by the World Health Organisation (WHO) [1]. This enduring reliance explains the legacy of nature's pharmacopeia and the compelling advantages inherent in these botanical elixirs. Medicinal plants confer a tapestry of benefits, amplifying their allure in the realm of healthcare. With accessibility and affordability at their core, these natural wonders offer an enticing alternative to pharmaceuticals, enriched by a minimized spectrum of adverse effects [2]. Such attributes amplify their appeal, particularly among pregnant women seeking natural remedies, driven by perceptions of reduced risk to both the mother and developing fetus [3]. This sentiment is future underscored by the classification of numerous modern medications as teratogens, igniting the quest for safer alternatives [4]. The surge in global medicinal plant consumption during pregnancy, as reported by Ahmed et al. (2018), charts a complex trend influenced by

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geography, ethnicity, culture, and economic status [5]. However, a disconcerting reality unfolds—a substantial lack of awareness among consumers regarding the potential perils woven into the fabric of seemingly benign natural medicines derived from these botanical reservoirs.

The embryonic phase, particularly the critical organogenesis window of the first trimester, emerges as a period of heightened vulnerability to teratogens [6]. Prior investigations have unveiled that active phytoconstituents from medicinal plants can breach the placental barrier, encroaching upon the delicate realm of the embryo and fetus. The repercussions are manifold, ranging from organ deformities, compromised growth, and impaired function to mutagenesis, carcinogenesis, growth stunting, and even the dire specter of fetal mortality or abortion [7].

Pereskia bleo (*P. bleo*) is a stalwart member of the Cactaceae family, and its roots are deeply intertwined with regions spanning the United States, Malaysia, India, Singapore, and Indonesia. This botanical luminary has been extolled for its versatile curative prowess, reputedly countering afflictions as diverse as headache, ulcers, hypertension, asthma, diabetes, bacterial infections, and even breast cancer, as illuminated by prior inquiries [8]. Its reputation transcends mere medicine; *P. bleo* has earned a place on the Malaysian culinary landscape, eaten raw as salad or brewed, often consumed as tea; a testament to its potent bioactive constituents elucidated through empirical investigations [9].

Yet, within the expanse of this botanical charisma, an intriguing void emerges—an uncharted realm awaiting inquiry. Despite its pivotal role in the Malaysian traditional herbal milieu, the potential teratogenic dimensions of *P. bleo* have remained an enigma, poised beyond the purview of scientific scrutiny. The present endeavor unfolds the quest to traverse this unexplored terrain, unraveling the ramifications inscribed within *P. bleo* leaves and dissecting their implications on female physiology and fetal development.

More attention has been paid to general plant toxicity than teratogenicity. Consequently, the current study unfolds its narrative, characterized by the quest for knowledge, the pursuit of safety, and the elevation of discourse. As the searchlight points at *P. bleo*, we embark on a journey bridging the gap between theory and practice, shaping a tapestry where tradition and science converge in botanical wisdom.

Materials and Methods

Sample collection

P. bleo leaves were collected from Melor, Kota Bharu, Kelantan, Malaysia and authenticated by a botanist from the Herbarium Unit, School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia. A voucher specimen numbered USM 11730 was deposited in the school's herbarium.

Preparation of sample

The collected *P. bleo* leaves were rinsed using tap water and dried in an oven at 50°C for three days. The dried leaves were ground into powder with an electric blender. Fifty grams (50 g) of the leaves powder was dissolved in 1500 mL of methanol and extracted using a Soxhlet apparatus for three days. The solvent in the produced extract was evaporated using a rotary evaporator. The plant extract was kept frozen at -20°C until used.

Phytochemical analysis of MEPB leaves

The MEPB leaves were subjected to the GC-MS method for phytochemical screening. A Hewlett-Packard 6890 gas chromatograph equipped with a 5973N mass detector and HP-5 fused silica capillary column was used for the GC-MS analysis (Agilent Technologies, USA). Helium was employed as the carrier gas at a 1.0 mL/min flow rate. The oven temperature was regulated from 50°C for 5 min to 300°C for 10 min at a rate of 25°C/min. The injector and interface temperatures were 280°C. One µL of the plant extract (MEPB leaves) was injected in split-less mode and subjected to MS full scan mode at 40-650 m/z. The ionization voltage was 70 eV. Chemsation software was used to collect the data and compared to ≥80%, matching to the National Institute of Standards and Technology (NIST02) and Wiley 275 libraries.

Dosage preparation

Three dosages of MEPB were prepared (250, 500, and 1000 mg/kg/day), representing low, medium, and high doses, respectively, while distilled water (DW) was used as vehicle control. Before oral dosing, the prescribed amount of MEPB was first weighed, reconstituted in DW, and stored in the refrigerator until used.

Ethical approval for animals

The research protocols for the in vivo study were approved by the USM Institutional Animal Care and Use

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Committee (USM IACUC), with an approval number USM/IACUC/2021/(130) (1154).

Animal husbandry and maintenance

Virgin female Sprague-Dawley rats (n=40), aged 8-10 weeks around 180-190 g and healthy males (n=20), aged 8-10 weeks, weighing between 200-250 g, were obtained from the Animal Research and Service Centre (ARASC), Universiti Sains Malaysia. The experimental animals were the females, while the males were only used for mating purpose. All animals were housed in separate polycarbonate cages at a temperature of 22-23°C in a well-ventilated environment with a humidity of 50±10% and artificial lighting with a 12-h light/dark cycle to eliminate the possibility of concomitant illnesses. They had unrestricted access to standard rat pellets and water ad libitum. The animals were allowed a 7days acclimatization period before the study began to facilitate adaptation.

Experimental protocol

General health and oestrous cycle

Following the acclimatization period, the animals were examined for any physical and behavioural abnormalities, body weights measured daily, any signs of toxicity, incidence of morbidity and mortality were documented.

Oestrous cyclicity was observed for 20 days, including 10 days during the pre-treatment. Those with regular oestrous cycles were randomly distributed and administered doses of 0 (control-DW), 250, 500, and 1000 mg/kg/day of the extracts. The oestrous cycle was continuously monitored during the 10 days treatment prior to mating.

The vaginal smear method described by McLean et al. (2012) was employed with slight modification [10]. Briefly, a smear pipette containing 0.9% sodium chloride solution was inserted into the vagina of each female rat. The fluid was then pipetted out 2-3 times, creating the vaginal lavage fluids. The fluids were examined under light microscope (10X and 40X) magnification. Leukocytes, epithelial, and cornified cells were identified. The cell proportions were analysed to determine the stages of the oestrous cycle. Additionally, the length of the oestrous cycle was calculated by counting the number of days from one oestrous cycle to the next [11].

Mating procedure

Following 10 days of treatment with MEPB leaves, female rats with regular oestrous cycles mate with the males. The females in the proestrous phase were housed overnight with the male rat in a 1:1 ratio. The next morning, between 8:00–10:00 am, vaginal plugs were collected from the cohabitated female and examined for the presence of sperm. A sperm-positive vaginal smear was referred to as "gestational day 1 (GD1)".

Examination of pregnant dam and foetus

Each female rat was housed in separate cage on confirmation of pregnancy. The pregnant rats were regularly monitored until the day of sacrifice to keep track premature birth. The body weights of pregnant female rats were also recorded daily.

Potential toxicity and teratogenicity on the pregnant dams and foetuses were analysed, and all pregnant rats of 20th GD delivered their pups via caesarean section. Briefly, the female rats were anaesthetised with 60 mg/kg of sodium pentobarbital (Nembutal®) by intraperitoneal (IP) injection prior to laparotomy, then an abdominal midsagittal skin incision was made to access the abdominal cavity. The dam's internal organs (uterus, ovaries, oviducts, adrenal glands, kidneys, and liver) were examined macroscopically for abnormalities before resection and weighing. The collected internal organs were histopathologically examined to assess toxicity.

The collected ovaries were used to tally the overall count of mature corpora lutea, facilitating the computation of the pre-implantation loss. Further, on the uterine horn of pregnant dams, implants were clearly visible as distinct swellings. The gravid uterine weights were recorded followed by gently opening and counting number of implantation sites on each horn, number of post-implantation death and the number of total foetuses. In contrast, uteri of apparently non-pregnant females were stained for 10 min in a 0.5% ammonium sulphide solution to determine the presence of any implantation sites. The resorptions were identified by the presence of dark residues. The number of resorption sites in each horn was recorded. For foetal parameters, all foetuses were thoroughly examined for the teratogenic parameters such as number of live or dead foetuses, body weight, sex ratio (male: female), and occurrence of external malformations. A macroscopically examination of each foetus for external abnormalities began with the head, moving down the neck to the nose, eyes, and external

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ears, ending with each arm, extremities, and the tail. Each foetus was examined macroscopically.

Histological preparation

The histopathological analysis for organs of the dams, such as the ovaries, adrenal glands, kidneys, and liver, was conducted. Briefly, the organ tissues were fixed in a 10% neutral buffered formaldehyde solution, exposed to concentrations (70%, 80%, 90%, and 100%) of alcohol for 60 min. For 30 min to 24 hours, the samples were cleared in a xylene solution at 56°C, followed by embedding in paraffin. The tissues were then cut into 5 µm thick slices and stained with hematoxylin-eosin. All the slides were microscopically analysed for structural defects at 40X magnification.

Statistical Analysis

GraphPad Prism version 8.0.1 (Graph Pad Software, USA) was used to analyse the data. The results were evaluated using a one-way analysis of variance (ANOVA), followed by Dunnett's post-hoc test to compare the control and treated groups. A statistically significant value was defined as a $p<0.05$. The data were reported as mean±SEM.

Results

Phytochemical screening

Phytochemical analysis of MEPB leaves by GC-MS revealed the presence of 36 compounds, including phytosterols, terpenoids, fatty acids, and phenolic compounds (Table 1).

Table 1: GC-MS analysis showing bioactive compounds in MEPB leaves.

No.	Components/Compound nature	Formula	Peak area (%)	Molecular weight (g/mol)	Retention time
1	(-)-Loliolide (Terpenoids)	$C_{11}H_{16}O_3$	2.39	196	11.308
2	4,8,12,16-Tetramethylheptadecan-4-olide (Terpenoids)	$C_{21}H_{40}O_2$	0.25	325	13.437
3	Neophytadiene (Terpenoids)	$C_{20}H_{38}$	1.22	279	11.512
4	Phytol (Terpenoids)	$C_{20}H_{40}O$	1.75	297	12.583
5	1-Hexadecanol (Fatty acid)	$C_{16}H_{34}O$	0.56	242	9.831
6	1-Octacosanol (Fatty acid)	$C_{28}H_{58}O$	0.38	411	15.699
7	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (Fatty acid)	$C_{19}H_{32}O_2$	2.13	282	12.541
8	Hexadecanoic acid, 2,3-dihydroxypropyl ester (Fatty acid)	$C_{19}H_{38}O_4$	3.52	331	13.941
9	Hexadecanoic acid, methyl ester (Fatty acid)	$C_{17}H_{34}O_2$	1.70	270	11.855
10	n-tetracosanol-1 (Fatty acid)	$C_{24}H_{50}O$	0.28	355	16.553
11	β-sitosterol (Sterols)	$C_{29}H_{50}O$	0.93	415	16.798
12	Campesterol (Sterols)	$C_{28}H_{48}O$	0.13	401	16.357
13	Stigmasterol (Sterols)	$C_{29}H_{48}O$	0.10	413	16.504

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14	2-Methoxy-4-vinylphenol (Phenol)	$C_9H_{10}O_2$	2.07	150	8.977
15	4-vinyl-phenol (Phenol)	C_8H_8O	2.18	120	8.403
16	2-Naphtalenamine (Aromatic amine)	$C_{10}H_9N$	0.54	143	10.251
17	Indole (Alkaloids)	C_8H_7N	0.18	117	8.865
18	Vitamin E	$C_{29}H_{50}O_2$	0.23	431	15.895
19	13-tetradecenal	$C_{14}H_{26}O$	1.44	210	14.501
20	1H-Pyrrole-2,5-dione,3-ethyl-4-methyl-	$C_7H_9NO_2$	1.16	139	8.473
21	2-Butanedioic acid (E)-,bis(2-ethylhexyl) ester	$C_{20}H_{36}O_4$	0.98	341	12.996
22	2-Pentadecanone,6,10,14-trimethyl-	$C_{18}H_{36}O$	0.64	268	11.533
23	2-Pyrrolidinone	C_4H_7NO	0.69	85	7.268
24	3-Methyl-4-phenylpyrrole	$C_{11}H_{11}N$	0.45	157	10.405
25	4-(1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	$C_{10}H_{12}O_3$	0.92	180	11.189
26	Bis(2-ethylhexyl) maleate	$C_{20}H_{36}O_4$	0.27	341	12.723
27	Butyrolactone	$C_4H_6O_2$	0.18	86	4.096
28	Cyclohexyl-15-crown-5	$C_{16}H_{30}O_5$	0.24	302	12.695
29	Cyclopropane, octyl-	$C_{11}H_{22}$	0.25	154	9.292
30	Cyclotetradecane	$C_{14}H_{28}$	0.87	196	12.415
31	Decyltetraglycol	$C_{18}H_{38}O_5$	0.84	335	12.786
32	Hexaethylene glycol monododecyl ether	$C_{24}H_{50}O_7$	0.89	451	15.062
33	Hexagol	$C_{12}H_{26}O_7$	0.91	282	13.724
34	Methyl 16-methyl-heptadecanoate	$C_{19}H_{38}O_2$	0.34	299	12.625
35	Methyl salicylate	$C_8H_8O_3$	5.66	152	8.186
36	Pyridine,2,4,6-trimethyl-	$C_8H_{11}N$	0.06	121	6.085

Assessment of oestrous cycle

The microscopic examination of vaginal smears of virgin rats is presented in Figure 1. In all experimental groups, proestrous tissue has a proliferation of round-nucleated cells interspersed with a few nucleated cornified epithelial cells. The oestrous phase is made up of nucleated cornified epithelial cells. Metoestrous comprised of numerous leucocytes and a few nucleated cornified epithelial cells. Finally, leukocytes, as well as a few epithelial and cornified cells, are observed in dioestrous (Figure 1). The mean duration length of two oestrous cycles in rats treated with MEPB leaves at 250,

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500, and 1000 mg/kg/day did not differ significantly ($p>0.05$) when compared with the control group (Figure 2A). Additionally, Figure 2B shows the mean duration of one oestrous cycle (days) in all experimental groups. The results showed no statistically significant difference ($p>0.05$) in the average length (hours) of each cycle phase in the treated groups compared with the control group (Figure 2C). The finding indicated that MEPB leaf is non-toxic to the ovarian system.

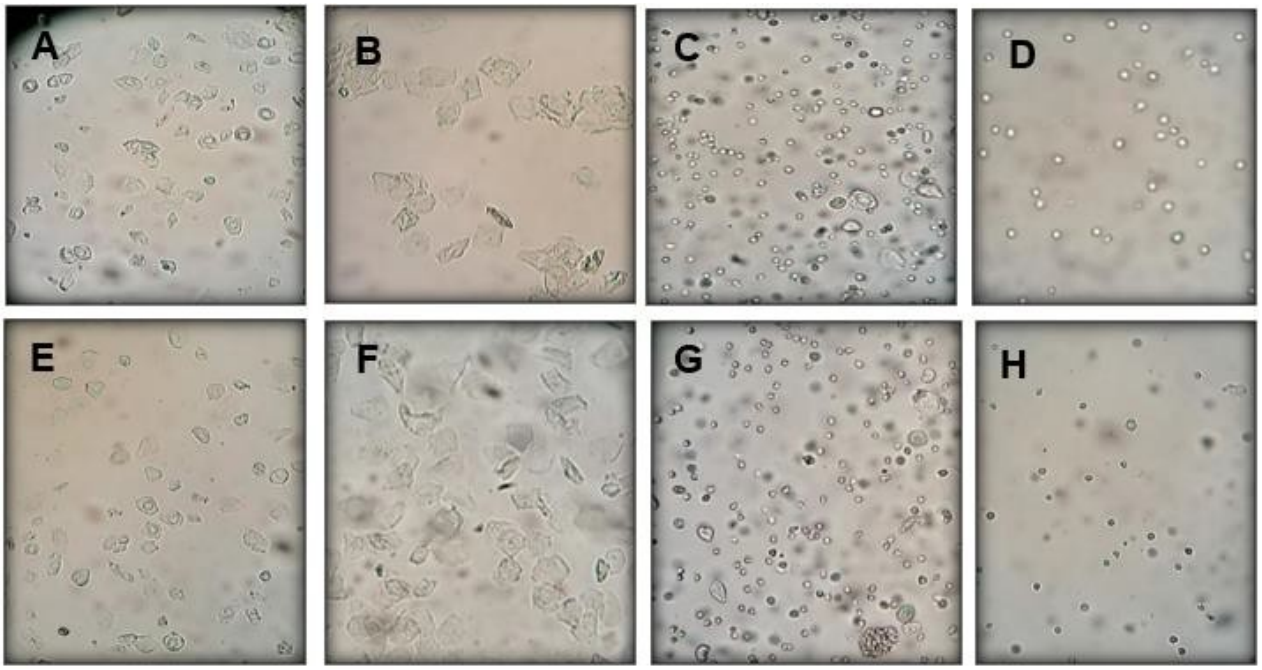

Figure 1: Vaginal smear for virgin female rats. A–D refer to the control group (DW), while E–H refer to the treated groups at 250, 500, and 1000 mg/kg/day of MEPB leaves. The light microscopic examination (10X magnification) indicated normal morphology in all the experimental groups. A and E: Refer to proestrous, B and F: Refer to oestrous, C and G: Refer to metoestrous; and D and H: Refer to dioestrous, n=10.

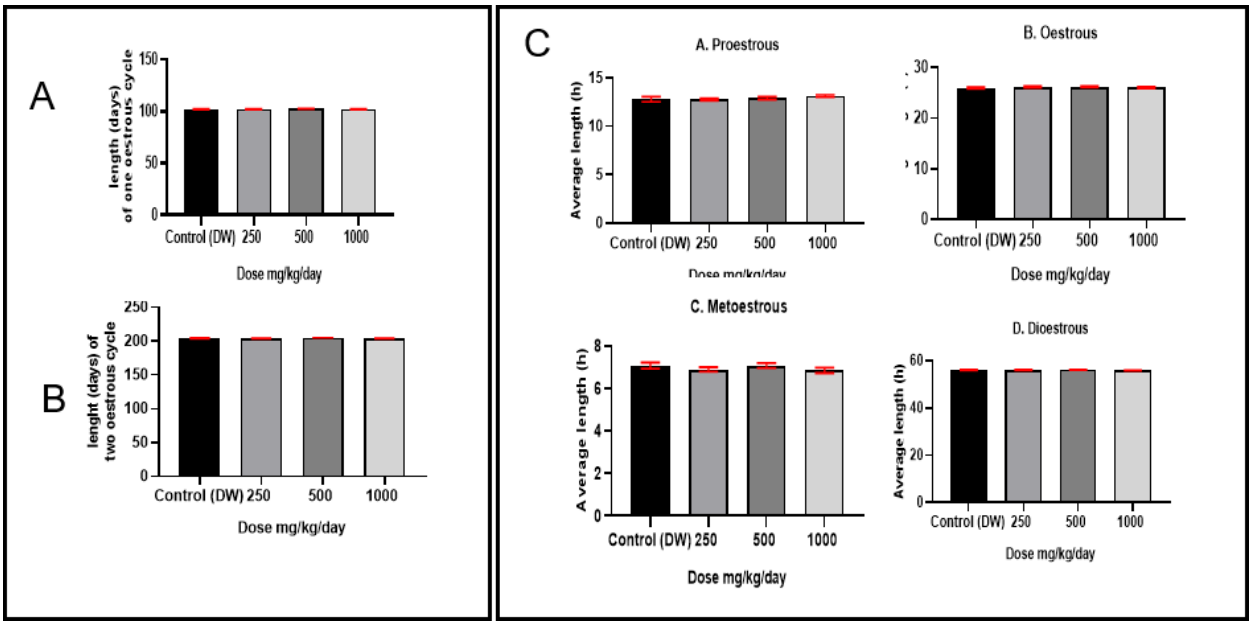

Figure 2: A: Duration of two oestrous cycles (days) for 10 days. B: Average length of the one oestrous cycle (days) for 10 days. C: Average length (h) of each oestrous cycle for 10 days. The data are reported as mean±SEM. There were no

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significant (p>0.05) differences in cycle phase duration for all treated groups with MEPB leaves at 250, 500, and 1000 mg/kg/day compared with the control group, n=10.

Assessment of body weight in female rats

Average body weight for virgin and pregnant rats-treated groups with MEPB leaves (250, 500, and 1000 mg/kg/day) and the control group is shown in Figure 3A and B. Data from this study revealed that oral administration of MEPB leaves in all dosages had no significant (p>0.05) impact on the average body weight of virgin and pregnant rats. All experimental groups' body weights were recorded from the day of mating up to the day of sacrifice (day 20).

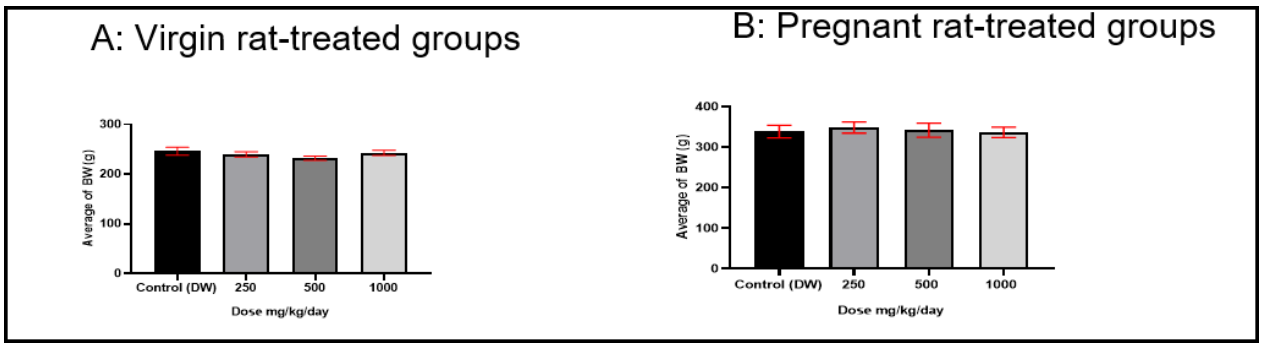

Figure 3: Body weights (BW) data of rat experimental groups. The data are reported as mean±SEM. Non-significant (p>0.05) differences between the MEPB-treated and control groups, n=10.

Assessment of clinical observation

No clinically relevant symptoms of toxicity, including salivation, lacrimation, rhinorrhea chewing jaw motion, convulsions, writhing, loss of hair, tremors, squints, yellowing of fur, stool changes, morbidity, and mortality were observed in any of the animals throughout the study.

Assessment of maternal absolute organ weights

Table 2 displays the mean weights of visceral organs for all experimental groups. The current findings revealed no significant difference (p>0.05) in the mean weights of the ovaries, oviducts, adrenal glands, kidneys, liver, and uterus in all MEPB-treated groups when compared to the control group.

Table 2: Absolute organ weights of experimental groups.

Organs	Doses (mg/kg/day)			
	Control (DW)	250	500	1000
Ovaries (mg)	41.16±1.249	42.43±1.13	41.20±1.23	41.42±1.27
Oviducts (mg)	14.88±0.57	13.48±0.58	13.96±0.80	13.85±0.73
Adrenal glands (mg)	26.29±0.82	26.63±0.74	25.75±0.77	26.86±0.6996
Kidneys (g)	0.88±0.010	0.91±0.008	0.89±0.012	0.89±0.011
Liver (g)	14.58±0.47	14.82±0.27	14.19±0.35	15.05±0.42
Uterus (g)	4.14±0.3	4.23±0.2	4.14±0.2	4.29±0.16

The values are expressed as mean±SEM. P>0.05, non-significant differences in all MEPB-treated groups, compared with the control group, n=10.

Pregnancy and maternal outcomes

The mean of corpus lutea in all MEPB-treated groups was within normal limits, when compared to the control

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group. No significant differences ($p>0.05$) were recorded between various dosages of the treated and the control groups (Table 3). Additionally, post-implantation survival rate was 100%. There were no effects on the pregnancy index and total number of implantation sites in all treated groups compared to the control group. There were no adverse effects of MEPB leaves on the pregnancy or the ensuing termination (Table 3).

There was no evidence of post-implantation loss (0%) and pre-implantation loss (resorption) (0%) in all MEPB-treated groups, when compared to the control group. The current data showed that all foetuses were alive and there were no dead foetuses in all rats treated with MEPB at 250, 500, and 1000 mg/kg/day, when compared to the control group. There was no effect ($p>0.05$) on the mean of the total number of foetuses, and foetuses sex ratio in all MEPB-treated groups compared to the control group (Table 3).

There were no significant differences ($p>0.05$) in the mean of weight of gravid uterus, weight of foetuses with placenta and amniotic fluid, and body weight of foetuses only, in all rats' administration with distilled water (control group) and those given MEPB leaves at doses of 250, 500, and 1000 mg/kg/day (treated group) (Table 3). 4). In addition, all rat-treatment did not significantly ($p>0.05$) differ from the control in crown-rump length (CRL), abdominal circumference, eye and tongue measurements (length and width), and limbs length (upper and lower) in both sides (Table 5).

Assessment of external abnormalities in the foetus

Microscopical examination and visual inspection showed none of the foetuses from the dams treated with any of MEPB dosages displayed any exterior abnormalities as compared to the control group (Table

Table 3: Summary of the pregnancy and maternal outcome data.

Parameters	Doses (mg/kg/day)			
	Control	250	500	1000
Percentage of resorption (%)	0	0	0	0
Post-implantation survival (%)	100	100	100	100
Pregnant index (%)	100	100	100	100
Post-implantation loss (resorption) (%)	0	0	0	0
Total number of implantation sites	116	114	117	114
Pre-implantation loss (%)	12.2±0.2	12.1±0.08	13.2±0.09	12.2±0.09
Number of corpora lutea	14.5±0.7	14.1±0.4	14.2±0.6	14.1±0.8
Live foetus	116	114	117	114
Dead foetus	0	0	0	0
Number of foetus	10.5± 0.8	10.0± 0.5	10.2± 0.8	10.8± 0.4
Sex ratio (male:female)	1:1.6	1:1.2	1:1.4	1:1.3
Weight of gravid uterus (g)	59.16±3.4	58.73±2.2	58.62±2.7	61.14±2.7
Body weight of foetus with placenta and amniotic fluid (g)	5.29±0.03	5.17±0.05	5.22±0.04	5.17±0.04

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Body weight of foetus only (g)	3.64±0.03	3.57±0.04	3.63±0.05	3.63±0.05

Statistically not significant ($p>0.05$), n=10.

Table 4: Teratogenic data of MEPB-treated and control dosages.

Organs	Teratogenic parameter	Doses (mg/kg/day)			
		Control (DW)	250	500	1000
Tongue	Aglossia, Ankyloglossia,Tie Tongue, Macroglossia, Microglossia, and Bifid Tongue	None	None	None	None
Upper and lower Limbs	Meromelia, Amelia, Micromelia, Phocomelia Syndactyly, Polydactyly, Synpolydactyly, Cleft hand and foot	None	None	None	None
External ear defect	Anotia, Microtia, and Preauricular pit or sinus	None	None	None	None
Eyes	Synophthalmia, Microphthalmia, and Anophthalmia	None	None	None	None
Mouth	Incomplete cleft lip, Bilateral cleft lip, Cleft lip, jaw, and palate, Isolated cleft palate, Oblique facial cleft, and Midline cleft lip	None	None	None	None

The data are recorded upon microscopic examination and visual inspection, n=10.

Table 5: The findings showed no foetal abnormalities across all doses of MEBP leaves when compared to the control group.

Parameters	Doses (mg/kg/day)			
	Control	250	500	1000
CRL (cm)	3.4±0.02	3.3±0.03	3.3±0.03	3.3±0.03
Abdominal circumference (cm)	2.6±0.03	2.6±0.03	2.6±0.03	2.5±0.04
Eye length (mm)	4.77±0.08	4.61±0.072	4.56±0.067	4.71±0.007
Eye width (mm)	2.05±0.006	2.045±0.005	2.034±0.012	2.056±0.006
Tongue length (mm)	5.41±0.05	5.62±0.04	5.34±0.07	5.31±0.04
Tongue width (mm)	2.02±0.004	2.03±0.005	2.04±0.011	2.04±0.005

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Upper limb length (cm)	1.13±0.006	1.13±0.006	1.13±0.006	1.12±0.006
Lower limb length (cm)	1.09±0.005	1.09±0.006	1.08±0.006	1.07±0.005

The values are expressed as mean±SEM. Statistically not significant ($p>0.05$), n=10

Histopathology of maternal visceral organs

Examination of the ovaries, adrenal glands, liver, and kidney sections under a light microscope revealed normal histology and the lack of damage, atrophy, or hypertrophy in both the control and treated groups (Figure 4).

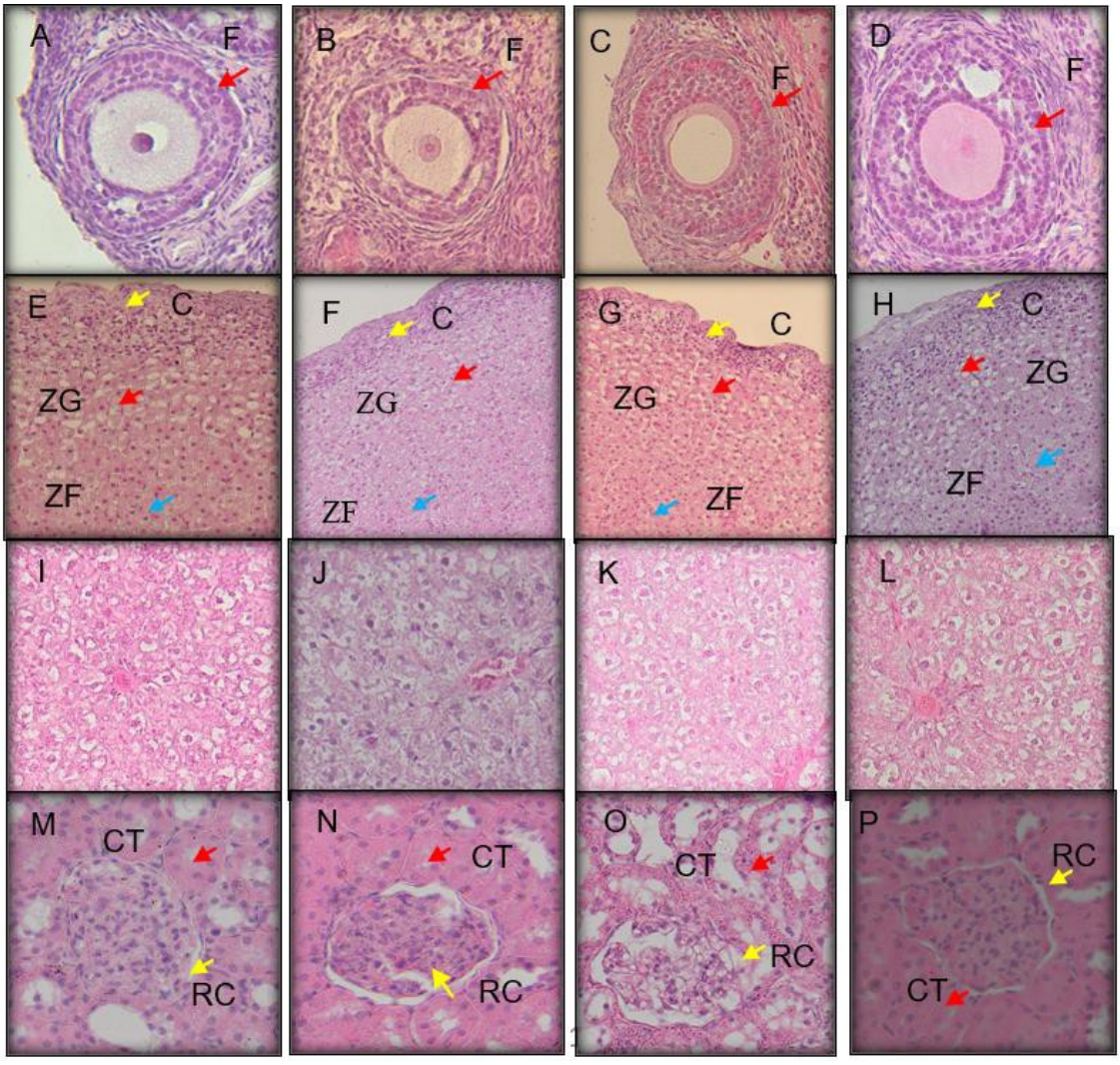

Figure 4: In-depth pathological assessment of the visceral organ tissues from female rats for 29 days of treatment (10 days before pregnancy and 19 days during pregnancy), (haematoxylin and eosin; magnification 40X). (A, E, I, and M), are for the control group (DW). Whereas the treated groups were divided into 3 groups with various dosages (B, F, J, and N) are for 250 mg dose-group; (C, G, K, and O) are for 500 mg dose-group; and (D, H, L, and P) are for 1000 mg dose-group. Microscopical examination of ovaries section (B-D), adrenal glands section (F-H), livers section (J-L), and kidneys section (N-P) in all MEPB-treated groups showed a normal morphology when compared with the control group. Follicle (F), Capsule (C), Zona glomeruloza (ZG), Zona Fasciculata (ZF), Convoluted tubule (CT), and Renal corpuscle (RC).

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Discussion

Humans have always relied on nature for their existence and lived in close harmony with it. Medicinal plants have played an important role in the history of human healthcare [12]. Despite their pivotal roles in various aspects, there are concerns regarding the use of medicinal herbs during pregnancy. Most research on medicinal plants concentrate on active ingredients and pharmacological activities rather than toxicity towards pregnancy [5]. Plant-derived bioactive compounds have garnered attention for their potential health benefits, with MEPB leaves being no exception. Our GC-MS analysis uncovered a rich array of bioactive constituents in the extracts, including phytosterols, terpenoids, fatty acids, and phenolic compounds. These compounds offer diverse bioactivities, from the antimicrobial [13] and anti-cancer properties [9] of phenolic compounds; to the neuroprotective effect of phytosterols [14]. Terpenoids, as evidenced by Kobayashi and colleagues demonstrated a potential in mitigating effects of allergies [15]. This highlights their role in allergy management. Moreover, the presence of fatty acid classes hints at their promise in promoting heart health by reducing cholesterol levels and offering cardio-protective benefits [16]. These revelations provide a strong foundation for future research, unlocking the therapeutic potential of MEPB leaves and their significance in natural remedies and preventive health strategies.

Toxicity experiments on medicinal plants must be conducted to assess their potential health risks before their development as modern medicinal products. Findings from toxicity studies are important to provide a more accurate picture of the effects of medicinal plant extracts on human health [17]. Evaluation of the oestrous cycle is a crucial stage in ensuring that females have a healthy reproductive system [18]. As a preliminary step, the current study assessed the effects of MEPB leaves with various dosages on the oestrous cyclicity of virgin female rats. Preclinical studies have shown that many medicinal plants affect the oestrous cycle in a rat model as some phytochemicals can profoundly affect the adult female reproductive system [19]. *P. bleo* leaves contain various bioactive compounds, including alkaloids, fatty acids, flavonoids, phytosterols, and others [20]. It was reported that certain alkaloids caused an oestrous cycle abnormality in Wistar rats model [21]. It is well-established that the histology of a vaginal smear is the primary predictor of healthy ovarian physiology [22]. Oestrous cycle phases in healthy rodents are often identified as oestrous, metoestrous, dioestrous, and proestrous [23]. These cycle phases are determined based on the cell types and proportions observed in the vaginal smear. Analysis of oestrous cycle in rats provides insight into the health of the reproductive hypothalamic-pituitary-ovarian axis [24]. The gonadotropin-releasing hormone (GnRH) plays a pivotal role in mammalian reproduction. Gonadotrophins, including follicle-stimulating hormone (FSH) and luteinising hormone (LH), are released by the pituitary gland in response to GnRH, which is produced by the hypothalamus. FSH and LH are important for cycle maintenance. The vaginal changes that occur at regular intervals during an oestrous cycle are a sign of normal function of both the neuroendocrine-reproductive system and ovarian function [25]. Based on the microscopical observations in the current study, the histology of the vaginal smear for all MEPB-treated rats demonstrated comparable findings with the normal oestrous cycle shown by the control group. The data found that all female rats treated with herbal extract had a normal progression of the stages of their oestrous cycles with a normal transition of the cytological components of their vaginal smear and a similar length of each phase of the cycle. These findings proved that MEPB leaves are safe for the hypothalamic-pituitary-ovarian axis of the rat model. Apart from the regular oestrous cyclicity, the current investigation demonstrated that all female rats had no significant physical and behavioural abnormalities, apparent clinical signs of toxicity, or cases of morbidity and mortality. The treatment group's results were consistent with the control group's findings. Previous research found that 14 days of therapy with 2500 mg/kg of crude *P. bleo* extract in mice did not result in any clinical indicators of toxicity or death [26]. Besides, Abdul-Wahab and colleagues also reported that a single dose of 500 mg/kg/day of *P. bleo* leaves extract did not result in any abnormal behavior [27]. As there is alteration in behaviours; body weight, and organ weight are frequently seen as early clinical signs and indicators of toxicity [28]. No clinical symptoms of toxicity were observed in previous investigations of several medicinal plant extracts at doses of 200, 400, 800, and 3200 mg/kg [29]. Acute toxicity

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was not shown in clinical trials when the plant extract was given at doses ranging from 10 to 5000 mg/kg/day [30]. The body weight parameter of the current study showed that both the treated and control groups exhibited a steady and typical increase in their mean body weights during the pre-treatment period. Likewise, pregnant rats in all MEPB-treated groups also exhibited a comparable rise in body weight when compared to the control group. It appears that the MEPB leaves are non-toxic and safe for consumption. Previous animal studies documented that several medicinal plants did not reflect obvious effects on body weight [31]. Many medicinal plants have the potential to disrupt the physiological processes of internal organs, resulting in organ damage and altering organ enzymes [32]. In animal models, studies reported that the liver, kidneys [33], ovaries [34], and adrenal glands [35] are typically damaged by toxic chemicals in medicinal plants. Alterations in organ weight, such as organ swelling, atrophy, and hypertrophy, are referred to as organ toxicity [36]. As for this study, there was no statistically significant change in the visceral organ weights of all the herbal-treated females as compared to the control group. Moreover, histopathological analysis of the current investigation revealed that all internal organs in all female rats had normal morphology which was consistent with those of control animals. Histological analysis is the gold standard for diagnosing pathological alterations in organs as histo-morphological change is the hallmark of toxicity in organs [37]. Based on previous investigations, it was revealed that the presence of sperm in the vaginal opening of the female is an indicator of successful mating [38]. The current data obtained from sperm-positive vaginal smears demonstrated that all rat groups were 100% successful in mating. The number of corpora lutea, gravid uterus weight, implantation sites, pre-and post-implantation loss, early and late resorptions, number of live and dead foetuses, and foetal sexes ratio, are all measured to ascertain whether MEPB is toxic or non-toxic to the maintenance of pregnancy. The embryo had two options after implantation: normal development or absorption. Most medicinal plant extracts may prevent an embryo from successfully implanting, leading to a slowed developmental rate or even causing absorption [39]. Most medicinal plants contain secondary bioactive compounds, such as alkaloids that can influence embryonic development [40]. The number of implantations and intrauterine mortality may be estimated using the number of corpora lutea. Previous investigation reported that the typical range for corpora lutea in healthy rats is 8-16, while the findings of a previous study showed that the number of implantations is proportional to the total number of live foetuses and resorptions [41]. It is well acknowledged that healthy rodents produce a sizable number of offspring, approximately 6-15 foetuses [42]. The current findings showed that all herbal-treated animals exhibited a normal range for the number of foetuses (live/dead), number of corpora lutea, percentages of implantation loss (pre-and post), and number of implantation sites when compared with the control group. The current data found no evidence of resorption (early or late) across all treatment groups. The foetal sex ratio of all the treated dams also fell within the established normative values for rats [43]. There were no prenatal abortions in any of the treatment groups animals. Several medicinal plants, such as Chamaemelum nobile, Salvia officinalis, and Trigonella foenum-graecum [44] cause miscarriage as they cause earlier uterine contractions by disrupting hormone levels [45]. The embryo-foetus can be affected by toxic substances via direct or indirect pathways. In the direct pathway, numerous medicinal plants can pass through the placenta and reach the foetus, which might result in foetal death or birth defects, due to damage to developing tissue. Through indirect pathways, the toxicant can cause damage to the placenta tissue and impair placental function, resulting in the prevention of the normal growth of embryos and fetuses [46]. In a rodent model, several studies documented that medicinal plants utilised during pregnancy have caused foetal mortality, retarded growth, and teratogenicity, decreased the gestational index, live birth index, litter size, body weight, and external abnormalities [47]. The development and growth of foetuses were measured using the morphological score, litter weight, and crown-rump length (CRL) [48]. Changes in body weight and CRL are considered classic indicators of fetal toxicity [49]. As observed in this current experiment, there were no variations in the morphological score, litter weight, and CRL of rat foetuses after their dams were exposed to MEPB leaves at various doses. No treatment-related or otherwise noticeable structural abnormalities were found in the exterior morphological examinations of rat foetuses. Additionally, no obvious external abnormalities were

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seen in the head, eyes, ears, mouths, tongues, nose, fore- and hindlimbs, digits, and tail of the rat foetuses.

Conclusion

In conclusion, the comprehensive evaluation of the methanol extract of MEPB leaf on female Sprague Dawley rats, encompassing pre-mating, mating, and gestation periods, has provided robust evidence of its safety for potential therapeutic applications. The absence of adverse effects on oestrous cycles, body weight, and behaviour, coupled with unchanged visceral organ weights and histological profiles, collectively confirm the extract's non-toxic nature. Furthermore, the unaltered pregnancy outcomes, foetal parameters, and absence of deformities underscore the lack of embryotoxic, or teratogenic consequences associated with MEPB consumption. These findings not only validate the traditional medicinal use of *P. bleo* but also highlight its promise as a safe option for therapeutic interventions. As future research continues to unveil the intricate mechanisms underlying these observations, the foundation for exploiting MEPB's therapeutic potential within the realm of safe and effective treatments remains solid.

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Conflict of interest

All the authors declared that they did not have any competing interest.

References

1. Afzal, M.; Khan, A.S.; Zeshan, B.; Riaz, M.; Ejaz, U.; Saleem, A.; Zaineb, R.; Sindhu, H.A.; Yean, C.Y.; Ahmed, N. Characterization of bioactive compounds and novel proteins derived from promising source citrullus colocynthis along with in-vitro and in-vivo activities. Molecules 2023, 28, 1743.
2. Illamola S. M., Amaeze O. U., Krepkova L. V., Birnbaum A. K., Karanam A., Job K. M., Bortnikova V. V., Sherwin C. M., Enioutina E. Y., 2020. Use of herbal medicine by pregnant women: What physicians need to know. Front Pharmacol. 10, 1483.
3. Ahmed, N.; Karobari, M.I.; Yousaf, A.; Mohamed, R.N.; Arshad, S.; Basheer, S.N.; Peeran, S.W.; Noorani, T.Y.; Assiry, A.A.; Alharbi, A.S. The antimicrobial efficacy against selective oral microbes, antioxidant activity and preliminary phytochemical screening of Zingiber officinale. Infection and Drug Resistance 2022, 2773-2785.
4. Amin, Z.S.; Afzal, M.; Ahmad, J.; Ahmed, N.; Zeshan, B.; Hashim, N.H.H.N.; Yean, C.Y. Synthesis, Characterization and Biological Activities of Zinc Oxide Nanoparticles Derived from Secondary Metabolites of Lentinula edodes. Molecules 2023, 28, 3532.
5. Ahmed M., Hwang J. H., Hasan M. A., Han D., 2018. Herbal medicine use by pregnant women in Bangladesh: a cross-sectional study. BMC Complement Altern Med. 18(1), 1-9.
6. Assiry, A.A.; Ahmed, N.; Almuaddi, A.; Saif, A.; Alshahrani, M.A.; Mohamed, R.N.; Karobari, M.I. The antioxidant activity, preliminary phytochemical screening of Zingiber zerumbet and antimicrobial efficacy against selective endodontic bacteria. Food Science & Nutrition 2023.
7. Ejaz, U.; Afzal, M.; Mazhar, M.; Riaz, M.; Ahmed, N.; Rizg, W.Y.; Alahmadi, A.A.; Badr, M.Y.; Mushtaq, R.Y.; Yean, C.Y. Characterization, Synthesis, and Biological Activities of Silver Nanoparticles Produced via Green Synthesis Method Using Thymus Vulgaris Aqueous Extract. International Journal of Nanomedicine 2024, 453-469.
8. Zareisedehizadeh S., Tan C. H., Koh H. L., 2014. A review of botanical characteristics, traditional usage, chemical components, pharmacological activities, and safety of *Pereskia bleo* (Kunth) DC. Evid Based Complement Alternat Med. 2014.
9. Mohd-Salleh S. F., Wan-Ibrahim W. S., Ismail N., 2020. *Pereskia bleo* Leaves Extract Induces Cell Death via Cell Cycle Arrest and Apoptosis in Cervical Cancer Cells HeLa. Nutr Cancer. 72(5), 826-834.
10. McLean A. C., Valenzuela N., Fai S., Bennett S. A., 2012. Performing vaginal lavage, crystal violet staining, and vaginal cytological evaluation for

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 740-755 | ISSN:2251-6727

mouse estrous cycle staging identification. J Vis Exp. 67, e4389.

11. Fuad W. E. M., Jaafar H., Sulaiman S. A. 2017. The effects of Malaysian herb, Labisia pumila var. alata on oestrous cyclicity and reproductive parameters of nulliparous rats. Sains Malaysiana. 46 (10), 1721-1726.
12. Ramzan, M.; Karobari, M.I.; Heboyan, A.; Mohamed, R.N.; Mustafa, M.; Basheer, S.N.; Desai, V.; Batool, S.; Ahmed, N.; Zeshan, B. Synthesis of silver nanoparticles from extracts of wild ginger (Zingiber zerumbet) with antibacterial activity against selective multidrug resistant oral bacteria. Molecules 2022, 27, 2007.
13. Karunakaran T., Ismail I. S., Ee G. C. L., Nor S. M. M., Palachandran K., Santhanam, R. K., 2018. Nitric oxide inhibitory and anti-Bacillus activity of phenolic compounds and plant extracts from Mesua species. Rev Brasil Farmacogn. 28, 231-234.
14. Alvarez-Sala A., López-García G., Attanzio A., Tesoriere L., Cilla A., Barberá R., Alegría, A., 2018. Effects of plant sterols or β-cryptoxanthin at physiological serum concentrations on suicidal erythrocyte death. J Agric Food Chem. 66(5), 1157-1166.
15. Kobayashi Y., Sato H., Yorita M., Nakayama H., Miyazato H., Sugimoto K., Jippo T., 2016. Inhibitory effects of geranium essential oil and its major component, citronellol, on degranulation and cytokine production by mast cells. Biosci Biotechnol Biochem. 80(6), 1172-1178.
16. De Souza R. J., Mente A., Maroleanu A., Cozma A. I., Ha V., Kishibe T, Uleryk E., Budylowski P., Schünemann H., Beyene J., Anand S. S., 2015. Intake of saturated and trans unsaturated fatty acids and risk of all cause mortality, cardiovascular disease, and type 2 diabetes: systematic review and meta-analysis of observational studies. BMJ. 2015, 1-16.
17. Pour B. M., Latha L. Y., Sasidharan S., 2011. Cytotoxicity and oral acute toxicity studies of Lantana camara leaf extract. Molecules.16(5), 3663-3674.
18. Cora M. C., Kooistra L., Travlos G., 2015. Vaginal cytology of the laboratory rat and mouse: review and criteria for the staging of the estrous cycle using stained vaginal smears. Toxicol Pathol. 43(6), 776-793.
19. Paula V. G., Cruz L. L., Sene L. B., Gratão T. B., Soares T. S., Moraes-Souza R. Q., Damasceno D. C., Volpato G. T., 2020. Maternal-fetal repercussions of Phyllanthus niruri L. treatment during rat pregnancy. J Ethnopharmacol. 254, 112728.
20. Tan M. L., Sulaiman S. F., Najimuddin N., Samian M. R., Muhammad T. T., 2005. Methanolic extract of *Pereskia bleo* (Kunth) DC.(Cactaceae) induces apoptosis in breast carcinoma, T47-D cell line. J Ethnopharmacol. 96(1-2), 287-294.
21. Fatima U., Shahid S., 2018. Pharmacological Activities of Carica papaya Linn. J Basic Appl Sci. 14, 210-216.
22. Dos Santos A. C., Viana D. C., Oliveira G. B., Silva R. S., Oliveira M. F., Assis-Neto A. C., 2017. Follicular development and morphological changes in the vaginal epithelium during the estrous cycle of Galea spixii. Microsc. Res Tech. 80(2), 167-176.
23. Ajayi A. F., Akhigbe R. E., 2020. Staging of the estrous cycle and induction of estrus in experimental rodents: an update. Fertil. Res Pract. 6(1), 1-15.
24. Auta T., Hassan A. T., 2016. Alteration in oestrus cycle and implantation in Mus musculus administered aqueous wood ash extract of Azadirachta indica (neem). Asian Pacific J Reprod. 5(3), 188-192.
25. Bashir H., Upadhyay A., Mittal D. K., 2020. ANALYSIS OF CROCUS SATIVUS (SAFFRON) ON ESTROUS CYCLE AND INDUCED REVERSIBLE STERILITY IN RAT. World J Pharmc Pharmaceut Sci. 9(5), 1760-1765.
26. Sim K. S., Nurestri A. S., Sinniah S. K., Kim K. H., Norhanom A. W., 2010. Acute oral toxicity of *Pereskia bleo* and Pereskia grandifolia in mice. Pharmacogn. Mag. 6(21), 67-70.
27. Abdul-Wahab I. R., Guilhon C. C., Fernandes P. D., Boylan, F., 2012. Anti-nociceptive activity of *Pereskia bleo* Kunth. (Cactaceae) leaves extracts. J Ethnopharmacol. 144(3), 741-746.
28. Liu X., Zheng L., Zhang R., Liu G., Xiao S., Qiao X., Wu Y., Gong Z., 2016. Toxicological evaluation of advanced glycation end product Nε-(carboxymethyl) lysine: Acute and subacute oral

toxicity studies. Regul Toxicol Pharmacol. 77, 65-74.
29. Shamaki B. U., Sandabe U. K., Abdulrahaman F. I., Ogbe A. O., Hassan Z. I., Yusuf I. L., Bitrus W., Zongoma Y., Isikhuemhen O. S., 2017. Toxicity studies and body weights changes in Wistar rats following oral administration of methanol extract from indigenous Ganoderma sp. MOJ Biol Med. 1(5), 138-141.
30. Sule F. A., Anosike J. C., Nweje-Anyalowu P. C., Momoh T. B., Atojoko M. A., Elah S. O., Idakwoji P. A., 2022. Phytochemical Composition and Anti-Obesity Effect of the Methanol Extract of Ziziphous Mucronata Leaves in Wistar Rats. Amer J Biomed Sci Res. 6(5).
31. Ikpeazu V. O., Ugbogu E. A., Emmanuel O., Uche-Ikonne C., Okoro B., Nnaemeka J., 2018. Evaluation of the safety of oral intake of aqueous extract of Stigma maydis (corn silk) in rats. Acta Sci Pol Technol Aliment. 17(4), 387-397.
32. Michael B., Yano B., Sellers R. S., Perry R., Morton D., Roome N., Johnson J. K., Schafer K., 2007. Evaluation of organ weights for rodent and non-rodent toxicity studies: a review of regulatory guidelines and a survey of current practices. Toxicol Pathol. 35(5), 742-750.
33. Loha M., Mulu A., Abay S. M., Ergete W., Geleta B., 2019. Acute and subacute toxicity of methanol extract of Syzygium guineense leaves on the histology of the liver and kidney and biochemical compositions of blood in rats. Evid Based Complement Alternat Med. 2019.
34. Theise N. D., 2007. Liver biopsy assessment in chronic viral hepatitis: a personal, practical approach. Mod Pathol. 20(1), S3-S14.
35. de Carvalho C. A., Thomazini, J. A., 2013. Evaluación morfométrica y anatómica del corazón de ratas Wistar. Int J Morphol. 31(2), 724-728.
36. Lazic S. E., Semenova E., Williams D. P., 2020. Determining organ weight toxicity with Bayesian causal models: Improving on the analysis of relative organ weights. Sci Rep. 10(1), 1-12.
37. Yuet Ping K., Darah I., Chen Y., Sreeramanan S., Sasidharan S., 2013. Acute and subchronic toxicity study of Euphorbia hirta L. methanol extract in rats. BioMed Res Int. 1-14.
38. da Rocha C. F., Lima Y. D. M. S., Carvalho H. O., Pinto R. C., Ferreira I. M., Castro A. N., Lima C. S., Carvalho J. C. T., 2018. Action of the hydroethanolic extract of the flowers of Acmella oleracea (L.) RK Jansen on the reproductive performance of Wistar females rats: a popular female aphrodisiac from the Amazon. J Ethnopharmacol. 214, 301-308.
39. Leite S. P., de Medeiros P. L., da Silva E. C., de Souza Maia M. B., de Menezes Lima V. L., Saul D. E., 2004. Embryotoxicity in vitro with extract of Indigofera suffruticosa leaves. Reprod Toxicol. 18(5), 701-705.
40. Poole R. K., Poole D. H., 2019. Impact of ergot alkaloids on female reproduction in domestic livestock species. Toxins.11(6), 364.
41. Akbar B., 2010. Tumbuhan Dengan Senyawa Aktif Yang Berpotensi Sebagai Bahan Antifertilitas, Jakarta: Adabia Press. Halaman. 2010, 4-6.
42. Murray S. A., Morgan J. L., Kane C., Sharma Y., Heffner C. S., Lake J., Donahue L.R., 2010. Mouse gestation length is genetically determined. PloS One. 5(8), e12418.
43. Ema M., Endoh K., Fukushima R., Fujii S., Hara H., Hirata-Koizumi M., Hirose A., Hojo H., Horimoto M., Hoshino N., Hosokawa Y., 2014. Historical control data on developmental toxicity studies in rodents. Congenit Anom. 54(3), 150-161.
44. Abdul M. I. M., Siddique S., Rahman S. A. U., Lateef D., Dan S., Mandal P., Bose A., 2018. A critical insight of modern herbal drugs therapy under the purview of toxicity and authenticity. Biomed Res. 29(16), 3255–3260.
45. Sjöström L., Narbro K., Sjöström C. D., Karason K., Larsson B., Wedel H., Lystig T., Sullivan M., Bouchard C., Carlsson B., Bengtsson C., 2007. Effects of bariatric surgery on mortality in Swedish obese subjects. N Engl J Med. 357(8), 741-752.
46. Dugershaw B. B., Aengenheister L., Hansen S. S. K., Hougaard K. S., Buerki-Thurnherr T., 2020. Recent insights on indirect mechanisms in developmental toxicity of nanomaterials. Part. Fibre Toxicol. 17, 1-22.
47. Teshome D., Tiruneh C., Berhanu L., Berihun G., Belete Z. W., 2021. Developmental Toxicity of Ethanolic Extracts of Leaves of Achyranthes

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 740-755 | ISSN:2251-6727

aspera, Amaranthaceae in Rat Embryos and Fetuses. J Exp Pharmacol.13, 555-563.

48. Seyoum G., 2016. Influence of methionine supplementation on nicotine teratogenicity in the rat. Ethiop Pharmaceut J. 32(1), 37-54.
49. Mayer C., Joseph K. S., 2013. Fetal growth: a review of terms, concepts and issues relevant to obstetrics. Ultrasound Obstet Gynecol. 41(2), 136-145.