# Evaluation of the Antifertility Activity of Methanolic Extract of *Melia azedarach* and Their GC-MS Study

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## ABSTRACT

The present study was to evaluate the antifertility potential of *Melia azedarach* leaf extract (methanol) on female Wistar rats. The antifertility activity of the methanolic extract of the leaf was evaluated by two methods namely an anti-implantation activity study and an oestrous cycle study in female rats. Female rats were given methanolic extract for anti-implantation activity from the first to the seventh day of pregnancy. On the tenth day, laprotomization was done to determine how many number of implants, the number of resorption sites, and the number of corpora lutea. The extract was given for 15 days in the estrous cycle trial to cover three typical estrous cycles. Every morning, a vaginal smear of each animal was examined, and the length of each phase of the cycle was recorded. At doses of 100 mg and 200 mg/kg, respectively, the methanolic extract demonstrated substantial antiimplantation action of 56.25% and 67.86%. In comparison to the control group, the methanolic extract significantly increased the duration of the dioestrous stage and significantly decreased the duration of the meta oestrous phase. Due to the presence of phytoestrogens such as preg-4-en-3-one, 17.alpha.-hydroxy-17.beta.-cyano-, the methanolic extract of *Melia azedarach* leaf shows antifertility activity.

**Key Words:** *Antifertility activity, Oestrous cycle, Female rats, Steroids, Melia azedarach, GC-MS*

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## INTRODUCTION

Around the world, population management is a dangerous topic. One percent of pregnant women die from unexpected pregnancies or have abortions to prevent having unwanted children [1]. Although plants are a highly abundant source of phytoconstituents molecules, in the past 30 years most research efforts have been focused primarily on the development of antifertility drugs from the chemical source, with little emphasis given to the herbal source. New, efficient, and secure antifertility drugs can be discovered using a variety of herbal plants [2]. Synthetic medicines and steroidal contraceptives, which are used for fertility control, have several dangerous side effects, including hormonal imbalance, gastrointestinal distress, depression, hypertension, painful uterine contractions, and an increased risk of cancer [3]. As a result, we must use herbal contraceptive methods because they are affordable, secure, and have few adverse effects. *Melia azedarach* Linn. (*M. azedarach*) is one of the plants grown in different areas in India. *M. azedarach*, often known as mahaniba, belongs to the family Meliaceae. *M. azedarach*, also known as the chinaberry tree, bead tree. The whole plant or its parts (leaves, roots, and stem) are used for different medicinal purposes and have a long history of use by indigenous and tribal people of different parts of India. They have been used in this plant traditionally to treat dental pain, Piles, leprosy, itching, blood purifiers, asthma, and cough [4]. Different investigations have found that this

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plant possesses anticancer, antiviral, antimalarial, and antibacterial activity [5]. Folklore in West Bengal suggests that *M. azedarach* has antifertility properties. We, therefore, conducted this investigation to assess the estrous cycle study, antiimplantation action, and GC-MS analysis of the leaf of *M. azedarach* methanolic extract.

## MATERIALS AND METHODS

*Drugs/chemicals*

Ethinylestradiol (Infar India Ltd) was used standard drug for antifertility activity study. All other chemicals were obtained from Merck, Mumbai, India.

*Collection of plant materials*

The leaves of *M. azedarach* were taken in September 2021 from Jamalpur in the Purbabardhaman District of West Bengal, India, and identified by a taxonomist from the Botanical Survey of India in Howrah, West Bengal. The Department of Pharmacognosy, Bengal School of Technology (A College of Pharmacy), Chuchura, Hooghly and West Bengal has received a voucher specimen (CNH/Tech.II/2022/27 dated 23/05/2022) for deposit. India.

*Preparation of plant extract*

*M. azedarach* leaves (1.5 kg) were air dried, mechanically ground to a coarse powder, and then extracted for 48 hours in a Soxhlet extractor with 3 L of methanol. To produce a dark green solid mass (15% w/w), the extract was concentrated to dry in a Rota evaporator (Buchi type) at reduced pressure and moderate temperature (50-55 °C). A refrigerator was used to store the dry extract. For this investigation, a suspension of the dry extract made with Tween-80 (1% w/v) was distilled water.

*Phytochemical test*

The existence of several chemical components is qualitatively examined in the methanol extracts of *M. azedarach* (leaf). The extract was subjected to phytochemical screening utilizing the following reagents and substances, and corresponding analyses were made.

*Test for alkaloids*

*Hager's test:* A few ml of extract with 1 ml of Hager's reagents were added. The presence of a creamy white precipitate indicated the presence of Alkaloids.

*Wagner:* There were added 1 ml of extract and 1 ml of Wagner's reagent. Alkaloids were present as indicated by the formation of a reddish brown precipitate.

*Test for tannin*

*Lead acetate test:* 1 ml of extract and 3 drops of lead sub-acetate solution were added. A creamy gelatinous precipitate indicated the presence of tannins.

*Ferric chloride test:* There were added 2 ml of the extract together with 2 ml of the 5% ferric chloride. The existence of tannins was indicated by the color green.

*Test for glycosides*

*Borntrager's test:* A mixture of 2 mL filtrated hydrolysate and 3 mL chloroform was added. The chloroform layer was split after vigorous shaking. After adding a 10% ammonia solution, the existence of glycosides was detected by a pink-colored solution.

*Test for steroids*

*Salkowski:* A few ml of extract and a few drops of conc. $H_2SO_4$ was mixed. (Shake well and allow to stand). The presence of a red color (in the lower layer) indicated the presence of steroids.

*Test for flavonoids*

*Zinc hydrochloride test:* A few ml of extract was added with a pinch of zinc dust and after that conc. HCl along the side of the test tube was added. The appearance of a magenta color indicated the presence of flavonoids.

*Shinoda test:* 5mL of alcohol was used to dissolve plant extract. A few drops of concentrated HCl and pieces of magnesium ribbon were introduced. Flavonoids were present when the solution was pink to crimson.

*Test for terpenoids*

*Liebermann-Burchard test:* 2 ml of the extract, 2 ml of acetic acid, 2 ml of chloroform, and 1 ml of concentrated H2SO4 were added. Terpenoids were present because of their blue-green appearance.

*Carbohydrate test*

*Molish test:* Molish reagent, 2 ml of extract, and 2 ml of concentrated H2SO4 were all added. The crimson ring was a sign that carbohydrates were present.

*Fehling's test:* Filtrate was combined with Fehling's solutions A and B in a volume of 1 ml each, and the mixture was then heated in a water bath before boiling. Carbohydrate presence was identified by a red precipitate.

*Amino acid test*

*Millon's test:* A few drops of Millon's reagent were added to 2 ml of extract. It did not produce a precipitate that was white and showed the presence of amino acids.

*Ninhydrin test:* I combined 2ml of the extract with 2 drops of the Ninhydrin solution. There was no purple-colored sol. of amino acids.



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*Fixed oil test*

*Saponification test:* When heated for two hours, an extract containing a few drops of 0.5 N alcoholic KOH and a drop of phenolphthalein caused the soap to develop, indicating the presence of fixed oil [6, 7].

*GC-MS study:* The samples were examined by the Institute for Analysis of Dairy, Food, and Cultures (IADFAC) Bangalore. For analysis, the chromatographic conditions listed below were used. The ion chamber temperature is 250 °C, the GC interface temperature is 250 °C, the front inlet temperature is 220 °C, the column is HP 5 Ms, the carrier gas is highly pure helium, the flow rate is 1 ml/min, the mass analyzer is the quadruple double-focusing mass analyzer, the analyzer is photon multiplier tube and electron impact ionization is 70 eV.

*Determine the components:* GC-MS mass spectrum interpretation was done with the help of the National Institute of Standards and Technology (NIST). The mass spectra of the unidentified chemical substance and those of its recognized constituents that are housed in the NIST database were compared.

*Acute toxicity study*

The acute toxicity investigation of the methanol extracts of *M. azedarach* adhered to OECD guideline 420 (leaf) [8].

For this investigation, female, non-gestational rats weighing 150–200 g were employed. All animals were monitored for toxicities such as diarrhea, decreased appetite, hair erection and damage, lacrimation, convulsion, salivation, lethargy, paralysis, and mortality continuously for the first hour, intermittently for the following three hours, continuously for the following 24 hours, and periodically for 14 days.

Wister breed, colony-bred virgin female albino rats weighing 150–200 g were employed in our antifertility study. In the animal house, rodent food and unlimited water were available to all the animals. The humidity was 55.5% and the temperature was 22.2 °C. The Institutional Animal Ethical Committee of Bengal School of Technology (A College of Pharmacy), Chuchura, Hooghly, West Bengal, accepted all animal experimentation protocols (Registration No.: 1726/CPCSEA/IAEC/2022-002).

All the experiments were performed and followed by the Committee for the Control and Supervision of Experimentation on Animals (CPCSEA).

*Pharmacological activity study*

- *Estrous cycle study*

Estrous cycle research used vaginal smears and the length of several estrous cycle phases. For the estrous cycle investigation, albino female rats from colonies were chosen because they had regular estrous cycles. Rats typically go through an estrous cycle every 4-5 days. Phases of the estrous cycle are (i) estrous (cornified epithelial cells), (ii) metaestrous (cornified cells plus leucocytes), (iii) diestrous(leucocytes), and (iv) proestrous (epithelial cells). The above-mentioned animals were split into three groups, each with six animals, to evaluate the impact of methanol extracts on the estrous cycle. Only a vehicle was given to group (I) (Tween-80, 1%). The methanolic extract was given to groups II and III at doses of 100 mg and 200 mg/kg body weight, respectively. Three typical estrous cycles were covered during the 15-day treatment period. Every morning, a vaginal smear from the experimental animals was examined, and the length of each phase of the cycle was recorded [9, 10].

- *Anti-implantation activity studies*

For antiimplantation action, virgin female albino rats from a colony were employed (150–200 g). Each day in the morning, the vaginal smears from each female rat were examined. For the experiment, only the rats with a healthy estrous cycle were chosen. The antifertility activity was measured following Khanna and Chowdhuary's descriptions [11]. In the evening of proestrus, the female rats were housed with male rats that were fertile in a 2:1 ratio, and the next day, the copulation evidence was reviewed. Rats with dense aggregates of spermatozoa or the copulation plug in their vaginal smears were separated, and the day was declared to be day 1 of pregnancy. These rats were then divided into 4 groups, each with six rats. The group (I) serves as the control and only got the vehicle (between 80, 1%). Groups III and IV got dosages of 100 mg and 200 mg b.w. of methanol extract, respectively, whereas group (II) received ethinylestradiol as the conventional treatment at a dose of 0.45 mg/kg b.w. All therapies were administered orally. From day 1 through day 7 of pregnancy, the aforementioned therapies were administered, and on day 10, laparotomies were carried out under mild ether anesthesia while maintaining sterility. The number of implantation sites, corpora lutea in the ovary, and resorption sites in the uteri were all counted. The rats were allowed to mature after the abdominal incision was closed with sterile sutures under aseptic conditions. Calculations were made for early and anti-implant activity rates. The combined early abortifacient and anti-implantation activity of the studied materials results in % of antifertility activity. Below are the computation formulae [12].

% of abortifacient activity = (No of resorption/No of corpora luteum) × 100

% of anti-implantation activity = 100 - (No of implantation/No of corpora luteum) × 100

% of total antifertility activity = % of anti-implantation activity + % of abortifacient activity

*Histology study*

Ovarian tissue samples from both control and treatment animals were first fixed in Bouins solution for 24 hours, then dried with alcohol before being embedded in paraffin. For the histological analysis of the ovary, the paraffin block slices were cut at a 6 m thickness and stained with hematoxylin-eosin [13].

*Analysis of statistics*

One-way analysis of variance (ANOVA) and Dunnett's t-test were used to statistically examine the group differences. Statistics were deemed significant at P = 0.05. The mean value SD is used to express all data.

## RESULTS AND DISCUSSION

*GC-MS analysis*

*M. azedarach* methanolic extract's GC-MS chromatogram mentioned in **Figure 1**, revealed forty peaks corresponding to the presence of forty bioactive chemicals. By contrasting the mass spectra of the unidentified compounds with known spectra kept in the NIST-11 collection, the unidentified compounds were described and identified in **Table 1**, the discovered compounds were displayed together with their retention time (RT), weight, molecular formula, molecular weight, peak area (%), and type of the compounds.

**Table 1.** Quantitative compounds identified from methanol extract of *M. azedarach* (leaf) by GC-MS analysis.

| Sl No. | Chemical compound's name | Retention time (RT) | Molecular formula | Molecular weight | Peak area (%) | Nature of compound |
|---|---|---|---|---|---|---|
| 1 | 1-Bromo-1-chloroethane | 2.315 | $C_2H_4BrCl$ | 286.82 | 4.74 | Haloalkane |
| 2 | 2-Amino-1,3-propanediol | 6.98 | $C_3H_9NO_2$ | 91.11 | 0.61 | Amino diol |
| 3 | Silane, trimethyl- | 8.111 | $C_3H_{10}Si$ | 74.20 | 0.78 | Silane, trimethyl- |
| 4 | Silacyclopentane | 8.542 | $C_4H_{10}Si$ | 86.21 | 0.51 | organosilicon compound |
| 5 | 2-Methylbut-2-enoic acid | 9.107 | $C_5H_8O_2$ | 100.12 | 0.81 | Monounsaturated fatty acid |
| 6 | 2-(Ethylamino)ethanol | 9.336 | $C_4H_{11}NO$ | 89.08 | 6.03 | Alkanolamines |
| 7 | 3-Hexen-2-one, 5-methyl- | 9.496 | $C_7H_{12}O$ | 112.17 | 2.23 | α,β-Unsaturated carbonyl compound |
| 8 | 1,2,3,4-Butanetetrol, [S-(R*,R*)]- | 9.595 | $C_4H_{10}O_4$ | 112.12 | 1.50 | Triol |
| 9 | 2-Nitrosobenzaldehyde | 9.766 | $C_7H_5NO_2$ | 135.12 | 0.59 | Aromatic aldehyde |
| 10 | Ethanone, 1-(1-cyclohexen-1-yl)- | 9.87 | $C_8H_{12}0$ | 124.18 | 2.36 | α,β-Unsaturated carbonyl compound |
| 11 | 1,4-Dimethoxy-2-butyne | 9.994 | $C_6H_{10}O_2$ | 114.14 | 0.59 | Acetylene derivatives |
| 12 | 3,4-Dimethylpent-3-en-2-one | 10.135 | $C_7H_{12}O$ | 112.17 | 8.14 | Aldehyde |
| 13 | 2,7-Dimethyloctane | 10.321 | $C_{10}H_{22}$ | 142.29 | 0.59 | Hydrocarbon |
| 14 | 1-Butyl-1-methyl-2-propylcyclopropane | 10.394 | C11H22 | 154.30 | 1.38 | Cycloalkanederivatives |
| 15 | 3-Ethyl-4-methyl-1H-pyrrole-2,5-dione | 10.752 | $C_7H_9NO_2$ | 139.15 | 1.83 | Heterocyclic ketones |
| 16 | 1H-Inden-1-one, octahydro- | 10.892 | $C_9H_{14}O$ | 138.21 | 0.71 | Indanederivatives |
| 17 | 3-Methyl-1-(pyrrolidin-3-yl)but-2-en-1-one | 10.96 | $C_{19}H_{37}NO$ | 295.51 | 0.92 | pyrrolidine derivatives |
| 18 | 2-Methoxy-4-vinylphenol | 11.219 | $C_9H_{10}O_2$ | 150.18 | 2.94 | Aromatic phenolic compound |
| 19 | Phenol, 2,6-dimethoxy- | 11.489 | $C_8H_{10}O_3$ | 154.17 | 2.57 | Phenolic compound |
| 20 | Methyl 3-phenylprop-2-enoate | 11.66 | $C_{10}H_{10}0_2$ | 162.19 | 1.97 | Ester of cinnamic acid |
| 21 | 1-Pyrrolidinamine, N-ethylidene- | 11.925 | $C_6H_{12}N_2$ | 112.18 | 5.46 | Heterocyclic compound |
| 22 | Undecanoic acid, 10-methyl-, methyl ester | 12.381 | $C_{13}H_{26}O_2$ | 214.35 | 0.93 | Methyl-branched fatty acid |

| | | | | | | |
|---|---|---|---|---|---|---|
| 23 | 2H-Pyrazole-3-carboxylic acid, 2-methyl- | 13.061 | C13H13N3O3 | 259.27 | 0.66 | Phenylcarbamoylmethyl esterderivativs |
| 24 | 1-Naphthalenol, decahydro, (1.alpha.,4a.alpha.,8a.alpha.) | 13.211 | C10H18O | 154.25 | 0.81 | Juniper camphor |
| 25 | Benzoic acid, 3-hydroxy-, methyl ester | 13.357 | C8H8O3 | 152.15 | 2.22 | Ester |
| 26 | Ursane-3,16-diol, (3.beta.,16.alpha.,18.alpha.,19.alpha.,20.beta.)- | 13.808 | C30H1502 | 443.74 | 0.56 | Terpenoids |
| 27 | Guaiacol, 4-butyl- | 14.016 | C11H16O2 | 180.25 | 1.75 | Phenolic compounds |
| 28 | Preg-4-en-3-one, 17.alpha.-hydroxy-17.beta.-cyano- | 14.991 | C20H29N02 | 315.46 | 1.35 | Phytosterol |
| 29 | Tetradecanoic acid, 12-methyl-, methyl ester, (S)- | 15.344 | C16H3202 | 256.43 | 2.33 | Fatty acid methyl esters |
| 30 | 1,13-Tetradecadiene | 15.598 | C14H26 | 194.36 | 0.74 | Unsaturated hydrocarbon |
| 31 | 8-Methylnonanoic acid, methyl ester | 17.009 | C11H22O2 | 186.30 | 0.75 | Medium-chain fatty acid ester |
| 32 | Tetradecanoic acid | 17.186 | C14H28O2 | 228.31 | 0.90 | Saturated fatty acid |
| 33 | Benzene-1,2,3-triamine | 17.466 | C6H9N3 | 123.16 | 0.53 | Aromatic amines |
| 34 | Methanamine, N-[2-methyl-1-(1-methylethyl)butylidene]- | 18.763 | C9H19N | 141.26 | 4.15 | Secondary Amines |
| 35 | 2-[2-(2-Methyl-1-propenyl)cyclopropyl]-2-propanol, trans | 18.945 | C10H180 | 154.25 | 3.60 | Primary alcohol |
| 36 | Tridecanoic acid, methyl ester | 19.651 | C14H28O2 | 228.38 | 2.37 | Fatty acid ester |
| 37 | 1-Acetyl-4-hydroxy-pyrrolidin-2-one | 20.553 | C6H9NO3 | 143.14 | 0.76 | Heterocyclic derivatives |
| 38 | Hexadecanoic acid, methyl ester | 20.932 | C17H3402 | 270.46 | 19.64 | Ester of fatty acid |
| 39 | n-Hexadecanoic acid | 24.502 | C16H3202 | 256.43 | 4.88 | Fatty acid |
| 40 | 7-Oxabicyclo[4.1.0]heptane | 25.41 | C6H10O | 98.15 | 3.83 | Cycloaliphatic epoxide |



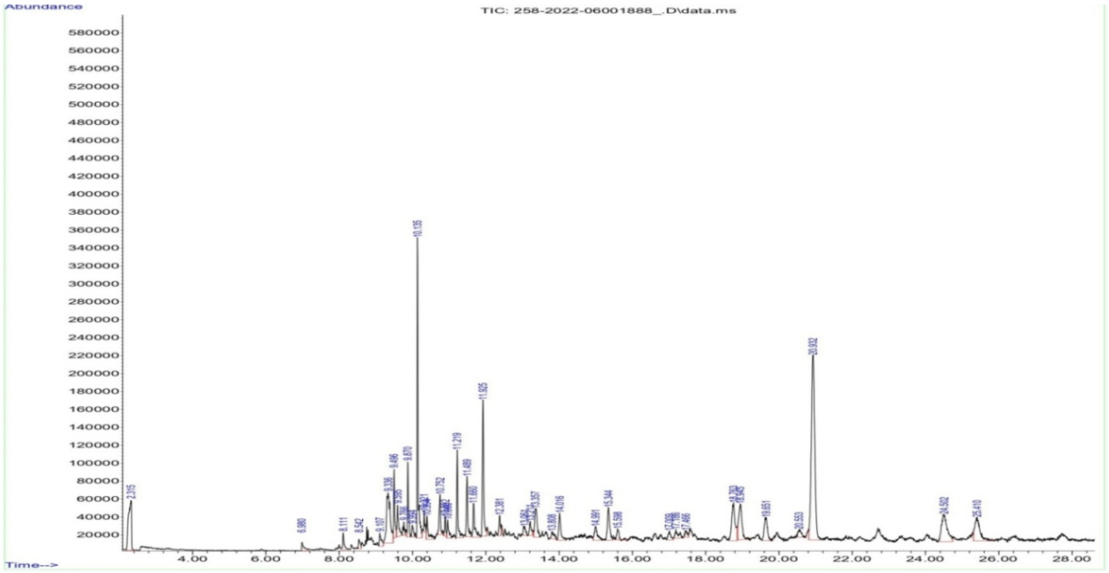



**Figure 1.** Chromatogram (GC/MS) of the methanolic extract of the leaf of *M. azedarach*

The results showed that preg-4-en-3-one, 17.alpha.-hydroxy-17.beta.-cyano- (1.35%), hexadecanoic acid, methyl ester (19.64%), Undecanoic acid, 10-methyl-, methyl ester (0.93%), Tetradecanoic acid (0.90%), Tetradecanoic acid, 12-methyl-, methyl ester (2.33%), and n-Hexadecanoic acid (4.88%) were found as major compounds in the methanolic extract of *M. azedarach.*

*Acute oral toxicity test*

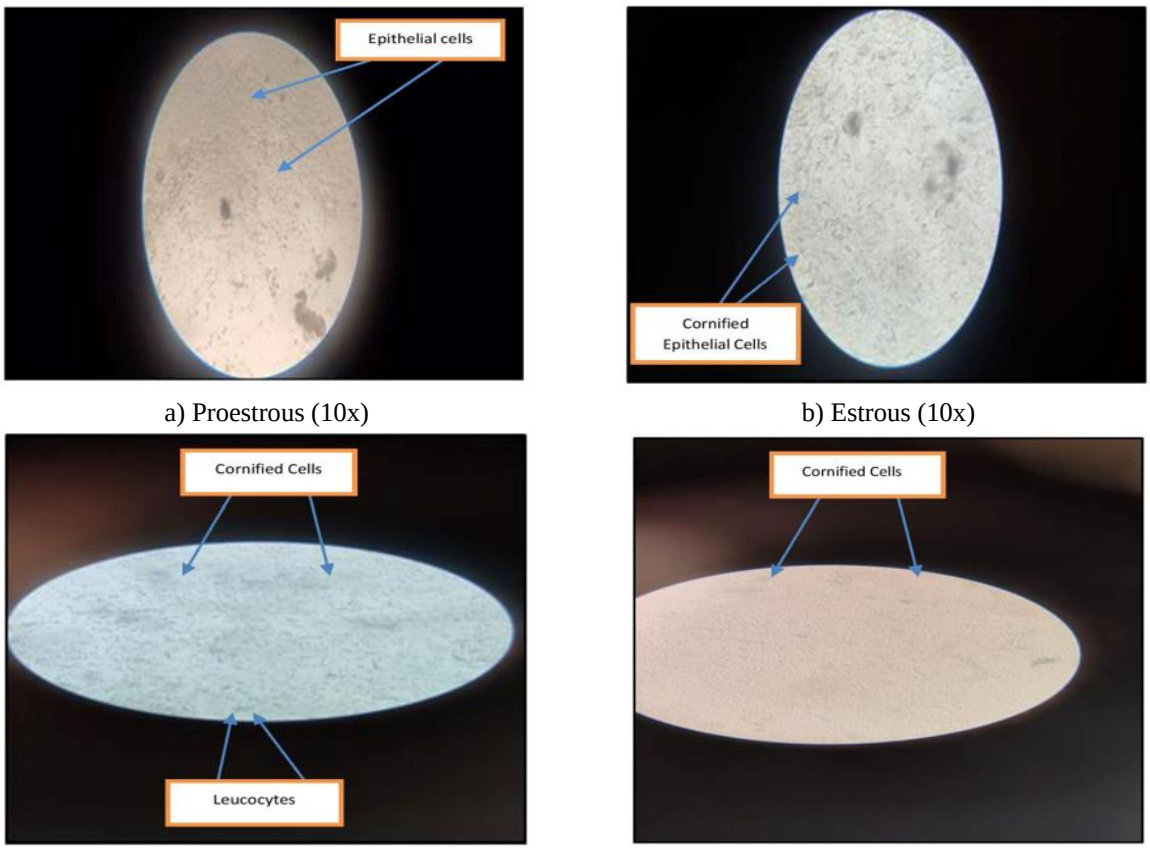
Animals did not exhibit any changes in their motor activity, diarrhea, lacrimation, convulsions, or state of coma following a single oral administration of the maximum dose (2000 mg/kg). There were no observable alterations in the body, behavior, or nervous system. Additionally, no deaths were noted for the course of the observation period's full 14 days. Animals treated with methanolic extract tolerated doses up to 2000 mg/kg body weight without experiencing any ill effects. For our current investigation, we employ the extract at dosage levels of 100 mg and 200 mg/kg body weight. Acute oral toxicity is mentioned in **Table 2**.

**Table 2.** Effect of methanolic extract of *M. azedarach* (leaf) duration of different phases of the estrous cycle (Mean duration ± SD in days)

| Group | Treatment | Dose (mg/kg) | Duration of estrous cycle (days) | Mean days of metestrous | Mean days of pro-estrous | Mean days of estrous | Mean days of diestrous |
|---|---|---|---|---|---|---|---|
| (i) | Control | Tween 80 (1 ml/kg) | 14.82 | 2.33 ± 0.51 | 1.83 ± 0.40 | 1.33 ± 0.50 | 9.33 ± 0.81 |
| (ii) | Methanolic extract | 100 mg/kg | 14.66 | 2.00 ± 0.63* | 1.50 ± 0.54 | 1.50 ± 0.54 | 9.66 ± 0.75 |
| (iii) | Methanolic extract | 200 mg/kg | 14.98 | 1.33 ± 0.51* | 1.30 ± 0.51 | 1.66 ± 0.51 | 10.66 ± 0.81* |

N = 6, One-way ANOVA followed by Dunnett's test. *P < 0.05 when compared to the control group

The result of the methanolic extract of *M. azedarach* (leaf) on the estrous cycle is shown in **Table 2**. Rats treated (100 mg and 200 mg/kg body weight) with extract showed a significant increase (P < 0.05) in the duration of the diestrous phase and a significant decrease (P < 0.05) in the duration of the metaestrous phase as compared to control. Different phases of estrous cycles are shown in **Figure 2**.




a) Proestrous (10x)

b) Estrous (10x)

c) Metaestrous (10x)

d) Diestrous (10x)

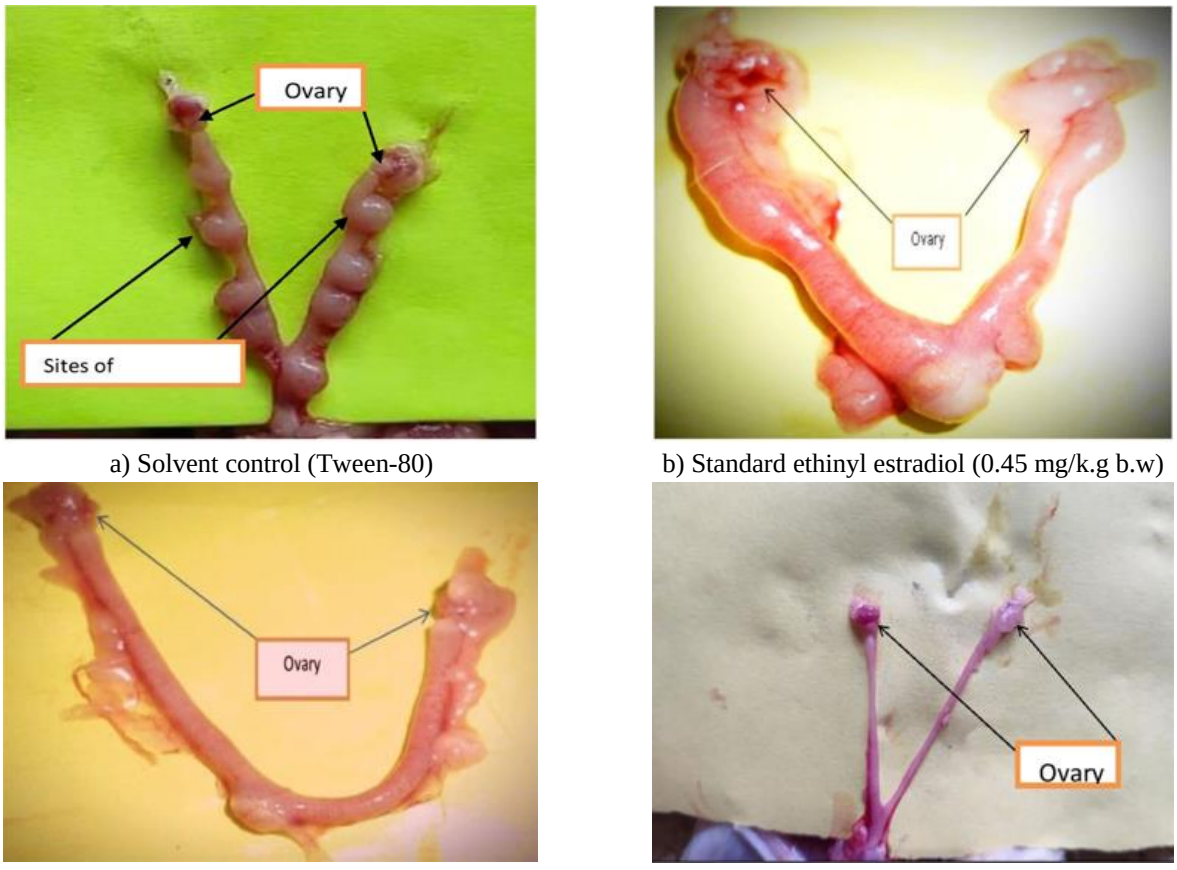
**Figure 2.** Different stages of estrous cycles

**Table 3.** Postcoital anti-implantation activity of methanolic extract of *M. azedarach* leaves (Mean ± SD)

| Group | Treatment | Dose (mg/kg) | The quantity of implantation sites | Number of corpus leuteum | Number of resorption | % of Anti-implantation activity | % of Abortifacient activity | % of total Anti-fertility activity |
|---|---|---|---|---|---|---|---|---|
| I | Control solvent (Tween 80,1%) | 1 ml/kgb.w | 9.50 ± 2.16 | 10.66 ± 1.21 | 0 ± 0.00 | 10.88% | 0% | 10.88% |
| II | Standard (Ethinylestradiol) | 0.45 mg/kgb.w | 0.66 ± 0.51* | 8.83 ± 1.94 | 0.50 ± 0.54 | 92.52% | 5.66% | 98.21% |
| III | Methanolic extract I | 100 mg/kgb.w | 3.50 ± 2.73* | 8.00 ± 1.41* | 2.16 ± 1.6 9* | 56.25% | 27% | 83.25% |
| IV | Methanolic extract II | 200 mg/kgb.w | 2.14 ± 1.47* | 6.66 ± 3.14* | 1.50 ± 0.83* | 67.86% | 22.5% | 90.36% |

N = 6, One-way ANOVA followed by Dunnet's test. *P < 0.05 when compared to control

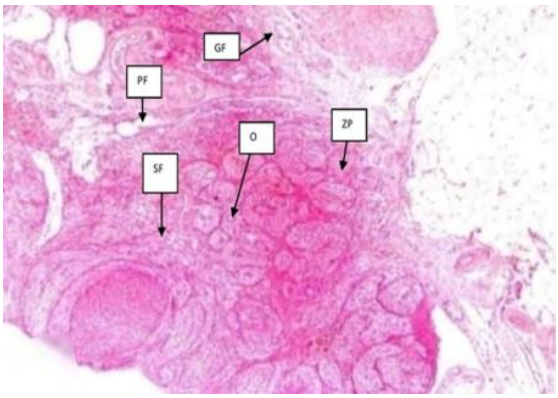
**Table 3** displays the impact of the *M. azedarach* (leaf) methanolic extract on antiimplantation action. According to the research, methanolic extract demonstrated substantial (P < 0.05) antiimplantation action at doses of 100 mg and 200 mg/kg body weight. The outcomes demonstrated that the extract has an antifertility impact that is dosage-dependent. Data revealed that extract-produced antifertility activity has dose-related responses that are more potent than those in the control group. In the rats treated with 100 mg and 200 mg/kg body weight of extract, respectively, the percentage of overall antifertility action was 83.25% and 90.36%. The antiimplantation activity of ethinylestradiol, which was used as the standard, was 98.18%. **Figure 3** depicts a picture demonstrating antiimplantation activity.



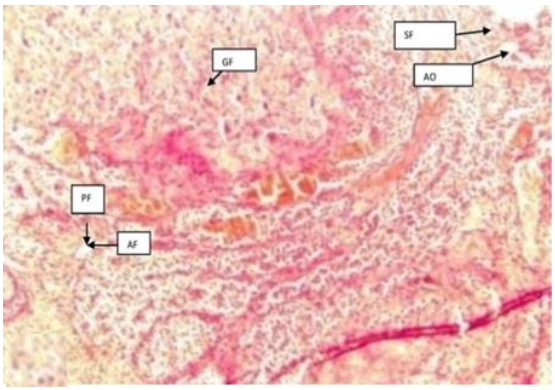

a) Solvent control (Tween-80)
b) Standard ethinyl estradiol (0.45 mg/k.g b.w)
c) Methanolic extract (100 mg/k. g. b. w.)
d) Methanolic extract (200 mg/k. g. b. w.)

**Figure 3.** Post-coital anti-implantation activity of methanolic extract of leaf of *M. azedarach.*

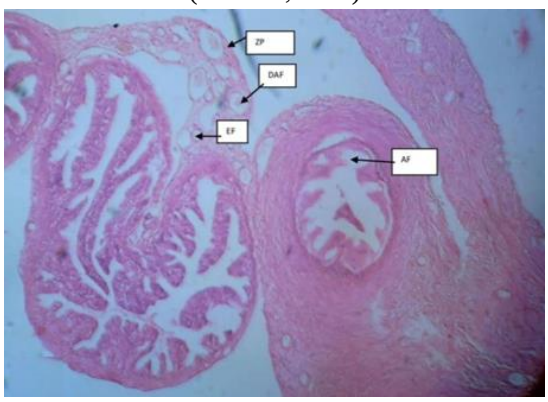
The photograph of ovary control animals and treated animals' activity is shown in **Figure 4**.



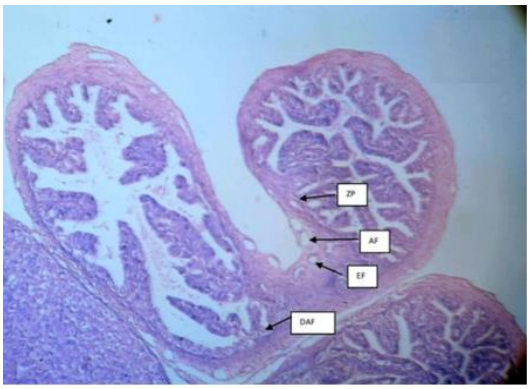

a) Section of rat ovary treated with Tween 80, 1% as solvent control showing normal conditions of ovary (ZP-zona pellucida, AF-Antral follicle, GF-Graffian follicle, PF-Primary follicle, SF-Secondary follicle, O-Oocyte) (H and E, 100x)




b) Histopathological changes in the ovary of rats treated with Tween 0.45 mg per kg Ethinylestradiol as a standard drug (AF-Antral follicle, AO-Absence of the oocyte, GF-Graffian follicle, PF-Primary follicle, SF-Secondary follicle) (H and E, 100x)




c) Histopathological changes in the ovary of rats treated with methanolic extract 100 mg per kg showing (ZP-zona pellucida, AO-Absence of oocyte, DAF-Disintegrated antral follicle, EF-Empty follicle) (H and E, 100x)




d) Histopathological changes in the ovary of rats treated with methanolic extract 200 mg per kg showing (ZP-zona pellucida, AO-Absence of oocyte, DAF-Disintegrated antral follicle, EF-Empty follicle) (H and E, 100x)

**Figure 4.** The photograph of ovary control animals and treated animals' activity

Histological examination of the ovary of control rats showed normal features including various stages of follicles with graffian follicles (primary and secondary follicles). The primary and secondary follicles appeared with distinct nuclei. All the follicles showed normal features like antral follicles, intact oocytes, and zonal pellucida. Treated rats with the standard drug Ethinyl estradiol at a dose of 0.45 mg per kg/ body weight and plant extract dose at 100 mg and 200 mg per kg/ body weight showed that the entire ovary was shrunken and primary and secondary follicles did not appear distinct with the nucleus. The antral follicle was highly specific without oocyte. These histology changes may be due to the inhibitory effect of the extract. The all-histological examination of the rat ovary is shown in **Figure 4**.

In female albino rats, the methanolic leaf extract of *M. azedarach* was tested for its antifertility effects using the following criteria: estrous cycle analysis and anti-implantation activity analysis. In reproductive biology, implantation is a fairly complicated process. Various hormonal and metabolic changes occur during uterine implantation [14]. In our experimental work, the methanolic extract decreased the number of implantation sites on both sides of the uterus in mice at doses of 100 mg and 200 mg/kg b.wt. Antiimplantation or antizygotic activity may be the cause of the decrease in the number of implantation sites in the uterus [15]. For implantation and pregnancy maintenance, a balance between progesterone and estrogen, two female sex hormones, is necessary. Any hormonal imbalance involving progesterone and estrogen might lead to infertility or anti-implantation [16, 17]. As a result, an estrogenic activity that causes ova to exit the fallopian tube may be the cause of the antiimplantation action [18]. Preg-4-en-3-one, 17.alpha.-hydroxy-17.beta.-

cyano- substance was detected in the GC-MS study of the methanolic extract of *M. azedarach*. Due to this phytosterol's affinity for estrogenic receptors, which causes infertility in animals, it has been stated that it exhibits estrogenic action [19]. In pregnant rats, it was discovered that methanolic extract had a dose-dependent anti-implantation action. The reduction in abortifacient activity shows how the methanolic extract also affects the implantation phase. Additionally, the methanolic extract showed the most anti-fertility action at a dosage level of 200 mg. kg, b.w. This is demonstrated by the reduction in the number of implantation sites and abortifacient activity [20]. The results of this study demonstrated that the histological slide of the ovary underwent various alterations as a result of the methanolic leaf extract of *M. azedarach*. Ovarian deterioration was evident, and granulosa cells were broken. The extract treatment revealed a reduction in the quantity of mature-sized follicles. According to Prakash and Jonathan, the administration of Ferula extract to the ovary causes alterations in the histological architecture of degeneration [21]. Changes in Ovary architecture may be due to estrogenic or antiestrogen agents [22]. Changes in estrogen or progesterone balance can interfere with the coordination between fertilized eggs. Such type of activity attributed due phytosterol present in the methanolic leaf extract of *M. azedarach*. The length of the diestrous phase has significantly increased, according to the current investigation on the impact of the methanolic extract on the estrous cycle of female rats. However, compared to control animals, the treated groups showed a statistically significant reduction in the length of the metaestrous phase. By interfering with the release of the female sex hormones estrogen and progesterone, which are necessary for uterine receptivity to the embryo, the lengthening of the diestrous phase and reduction in the metaestrous phase may reduce the likelihood of conception [23]. We got a new phytoestrogen like Preg-4-en-3-one, 17.alpha.-hydroxy-17.beta.-cyano- from our GCMS study. The interruption of the estrous cycle is caused by phytoestrogens such as Preg-4-en-3-one, 17.alpha.-hydroxy-17.beta.-cyano-, may be the cause of this effect. Preliminary phytochemical studies indicated the presence of steroids, glycosides, flavonoids, and triterpenoids in the methanolic extract of the leaf of *M. azedarach*. According to the literature, steroids, flavonoids, and glycosides are known to exhibit antifertility activity [24, 25].

## CONCLUSION

Forty phytoconstituents were found in a methanolic extract of *M. azedarach* leaf after GC-MS analysis. The results of the current study suggest that the methanolic extract of the *M. azedarach* leaf has an antifertility effect and is safe when administered at the study's effective antifertility levels. The presence of phytoestrogens such as Preg-4-en-3-one, 17.alpha.-hydroxy-17.beta.-cyano-, may be the cause of this antifertility action. Future research is required to link the biological features of additional particular chemicals.

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## REFERENCES

[1] Glasier A, Gulmezoglu MA, Schmid GP, Moreno CG, Vanlook PF. Sexual and reproduction health: a matter of life and death. Lancet. 2006;368(9547):1595-607.
[2] Kumar D, Kumar A, Prakash O. Potential antifertility agents from plants: a comprehensive review. J Ethnopharmacol. 2012;140(1):1-32.
[3] Connell K O, Davis AR, Kerns J. Oral contraception: side effects and depression in adolescent girls. Contraception. 2007;75(4):299-304.
[4] Hossain MDS, Saed A, Karmakar UK, Hossain MDA; Assessment of phytochemical, analgesic and antioxidant profile of *Melia azedarach* L. [Leaves] (Family- Meliaceae). Pharm Innov J. 2013;2(7):64-9.
[5] Shekhawat KK, Rao DV, Batra A. Phyto-morphological overview of medicinal plant: *Melia azedarach* Linn. J Funct Environ Botany. 2014;6(2):318-27.
[6] Kokate CK, Purohit AP, Gokhale SB. Practical pharmacognosy. 3rd ed., Vallabh Prakashan, New Delhi; 1994.
[7] Harborne JB. Photochemical method: a guide to modern techniques of plant analysis. 2nd ed. Chapman and Hall Publishers, London. 2013;1:1224-6.
[8] OECD-Guidance Document on Acute Oral Toxicity. Environment health and safety monograph series on testing and assessment no-24: guideline no 420; 2000.
[9] Vogel HG, Vogel WH. Drug discovery and evaluation. Biomed Pharmacother. 1998;52(1):47-9.
[10] Ojah A, Borthakur MK, Kalıta JC. Effect of methanolic extract of Persicaria hydropiper root on

estrous cycle and reproductive hormones in female albino mice. Uttar Pradesh J Zool. 2021;42(13):94-102.

[11] Agarwal A, Allan JJ. Antifertility effects of herbs: need for responsible reporting. J Ayurveda Integr Med. 2010;1(2):129-31.

[12] Thanamool C, Thaeomor A, Chanlun S, Papirom P, Kupittayanant S. Evaluating the anti-fertility activity of *Talinum paniculatum* (Jacq.) Gaertn in female wistar rats. Afr J Pharm Pharmacol. 2020;7(26):1802-7.

[13] Stadtländer Ch. Di Fiore's atlas of histology with functional correlations victor P. Eroschenko. Microsc Microanal. 2005;11:472.

[14] Laloraya M. Fluidity of the phospholipid bilayer of the endometrium at the time of implantation of the blastocyst--a spin label study. Biochem Biophys Res Commun. 1990;167(2):561-7.

[15] Hafez ESE. Reproduction and breeding techniques for laboratory animals. Lea and Febiger. Philadelphia, PA. 1970.

[16] Psychoyos A, Prapas I. Inhibition of egg development and implantation in rats after post-coital administration of the progesterone antagonist RU 486. J Reprod Fertil. 1987;80(2):487-91.

[17] Vasudeva N, Sharma SK. Post-coital antifertility activity of *Hibiscus rosa-sinensis* Linn. roots. Evid Based Complement Alternat Med. 2008;5(1):91-4.

[18] Jain S, Choudhary GP, Jain DK. Pharmacological evaluation and antifertility activity of *Jatropha gossypifolia* in rats. Biomed Res Int. 2013;2013:125-980.

[19] Dane P, Patil S. Evaluation of saponin from *Trigonella foenum* arcicum seed for the antifertility activity. Asian J Pharm Clin Res. 2021;5(3):154-7.

[20] Elmazar MM, Nau H. Potentiation of the teratogenic effects induced by coadministration of retinoic acid or phytanic acid/phytol with synthetic retinoid receptor ligands. Arch Toxicol. 2004;78(11):660-8.

[21] Pathak S, Prakash AO. Effect of hexane extract of *Ferula jaeschkeana* on the uterus of adult ovariectomised rats. Anc Sci Life. 1992;12(1-2):231-41.

[22] Thompson SA, Rowley DR, Heidger PM Jr. Fine structural studies of rat seminal vesicle in castrated and intact animals following estrogen treatment. Am J Anat. 1979;154(4):525-44.

[23] Sandeep G, Dheeraj A, Sharma NK, Jhade D, Bharti A. Effect of plumbagin free alcohol extract of *Plumbago zeylanica* Linn. root on reproductive system of female Wistar rats. Asian Pac J Trop Med. 2011;4(12):978-84.

[24] Sheeja E, Joshi SB, Jain DC. Antiovulatory and estrogenic activity of *Plumbago rosea* leaves in female albino rats. Indian J Pharmacol. 2009;41(6):273-7.

[25] Vaidya P, Padmashali S, Vagdevi HM, Sathyanarayana ND. Antifertility effect of the plant *Balanites roxburghii* (Balanitaceae) in female rats. Indian J Pharm Sci. 2006;3:347-51.



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