



Anti-fertility and abortifacient activity of hydroalcoholic fruit pulp extract of *Tamarindus indica*

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ABSTRACT

Background: Medicinal plants have been widely used for treatment and management of various diseases. There are many different types of commercialized synthetic contraceptives on the market today for controlling fertility, these cannot be utilized since they have serious side effects such weight gain, hormonal alterations, hypertension, and cancer. In India, indigenous women from Tripura & Maharashtra have historically utilized the fruit pulp of *Tamarindus Indicus* to induce abortion. However, there is currently no accessible scientific data supporting the use of this plant for fertility control.

Methods: The current study's objective was to evaluate the hydroalcoholic extract of *Tamarindus indica* fruit pulp's ability to induce abortion in female Wistar rats. In this experimental animal model, contraceptive activity of hydroalcoholic extract of *Tamarindus indica* (HAETI) was evaluated for anti-implantation, abortifacient, and estrogenic/antiestrogenic action. Female rats having ovariectomies were given HAETI extract (500 mg/kg) for 7 straight days to test to evaluate the estrogenic/anti-estrogenic action. All animals were killed on the eighth day, and the blood serum was then processed to determine the levels of several hormones, including LH, FSH, prolactin, estrogen, and others, as well as biochemical markers including cholesterol, total protein, glycogen, and alkaline phosphates. In this model, modifications to uterine histoarchitecture were also investigated. The extract was administered from the 1 to 7 day of pregnancy. On 10th day, laparotomy was performed to evaluate the no. of implants in female rats.

Results: Steroids, flavonoids, saponins, tannins, and alkaloids were detected during phytochemical analysis of the extract. the HAETI extract demonstrated significant anti-implantation and abortifacient effects. Additionally, the extract demonstrated significant estrogenic activity as evident by uterine histological parameters including increases in endometrium thickness and height, vaginal cornification. Further, the study found marked increases in glycogen, alkaline phosphate, estrogen, cholesterol levels when given alone or in combination with estradiol valerate. LH, FSH, and a little increase in prolactin levels were suppressed after HAETI administration.

Conclusions: Our study's findings showed that *Tamarindus indica* fruit pulp hydroalcoholic extract has a potent contraceptive effect, which may be explained by the phytoconstituents of the plant's capacity to inhibit implantation and have an estrogenic effect. Further investigation on *Tamarindus indica* as a potential natural anti-implantation agent allow for new abortive drug.

1. Introduction

According to WHO estimates, nearly 80 % of the population of developing nations relies on folkloric medicines, mostly those with plant origins, to help with their basic medical needs because they are unable to afford the costs of pharmaceutical treatments (Shah et al., 2009; Kaur

et al., 2011). There has been a thorough screening and study of Ayurvedic literature and folk medicine for anti-fertility effects in females worldwide. Although there are many different types of commercialized synthetic contraceptives on the market today for controlling fertility, these cannot be utilized since they have serious side effects such weight gain, hormonal alterations, hypertension, and cancer (Lopez et al.,

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2016). These negative effects drive pharmaceutical companies and modern science to create stronger herbal medications that are also self-administrable, less expensive, and entirely reversible, with few hazardous side effects. In recent years, there has typically been more emphasis placed on testing common herbal medications for their ability to work as a contraceptive. As a result, plant compounds with unalienable estrogenic and antiestrogen activities present themselves as a potent alternative source of contraception (Kumar et al., 2012) and according to previous literature macerate of the fruit have antifertility activity and increase the lactation (Joshi et al., 2022), thus we want to do this study.

Tamarindus indica L. (Caesalpinaceae), also known as (Imli) in Hindi and (Amarind) in English, is a tropical African evergreen multifunctional tree with an incredibly gorgeous crown, alternate and pinnate leaves, and fragrant flowers (Badoriaya et al., 2011). Historically, the plant's many parts have been used to treat a variety of ailments, including measles, asthma, diabetes, leprosy, inflammation, cough, diarrhea, and snake and insect bites (Havinga et al., 2010). Nearly every component of the plant, including the leaves, bark, seeds, fruits, etc., has some benefit. It is also rich in nutrients and is crucial for human nutrition (Mohamed et al., 1992). According to numerous studies, *Tamarindus indica* exhibits a wide range of important pharmacological properties, including antiemetic (Khan et al., 2005), cytotoxic (Fatimi et al., 2007), anti-microbial (Doughari, 2006), antioxidant (Siddhuraja, 2007), anti-diabetic (Maitiet et al., 2004), spasmolytic. Phytoconstituents that were reported in previous findings as in *Tamarindus indica* pulp catechin, epicatechin, taxifolin, eriodictyol, apigenin, luteolin etc. (Kuru, 2014) and in leaves tartaric acid, oxalic acid, malic acid, citric acid, vitamin C, α -carotene, Lupeol, lupanone, palmitic acid oleic acid, ferulic acid, caffeic acid, m geraniol, limonene, pipelicolic acid, orientin, isoorientin and naringenin (Katsayal et al., 2019). It includes organic acids like tartaric acid, citric acid, and malic acid as well as vitamins. In India, indigenous women from Tripura & Maharashtra have historically utilized the fruit pulp of *Tamarindus indica* to induce abortion (Patil, 2016; Das et al., 2014). However, there is currently no accessible scientific data supporting the use of this plant for fertility control. The objective of the proposed work was to assess the contraceptive potential of *Tamarindus indica* hydroalcoholic fruit pulp extract on female rats. Further, the estrogenic/anti-estrogenic effects, and other biochemical parameters were also assessed in ovariectomized female rats.

2. Material and methods

2.1. Collection of plant and extraction

Tamarindus indica fruit pods were purchased from a local market of Kurukshetra, Haryana. Plant material was authenticated from CSIR-NISCAPR, New Delhi. Where specimen has been secured (NISCAIR/RHMD/Consult/2017/3014-41) for future use. Fruit pulp was removed from the seeds, and using a cold maceration technique, an alcoholic hydro extract of the fruit pulp was created. Fruit pulp weighing about 1000 g was broken up into little pieces and steeped in 4 l of hydro alcohol for 10 to 15 days (Ethanol: Water; 90:10). 15 days later, the extract was filtered. Using the rotary evaporator (Heidolph 4011, USA), extract was further concentrated and converted into a semi-solid state before being lyophilized. The extract yield was found to be 15.5 % w/w. Thereafter, the extract was kept at 4 °C in a refrigerator.

2.2. Phytochemical screening

Tamarindus indica hydroalcoholic fruit pulp extract (HAETI) underwent preliminary phytochemical screening to identify the phytoconstituents based on reported chemical tests (Khandelwal, 2007).

2.3. Animals

In the current investigation, female Wistar rats (180–200 g) were procured from the Lala Lajpat Rai University of Veterinary and Animal Sciences at Hisar, Haryana India. Female rats with mature ovariectomies were used to assess the estrogenic activity, and Wistar strain healthy, fertile male and female rats were chosen to assess the abortifacient effect. In preparation for the exploratory work, the animals were housed in polypropylene cages enclosed with stainless steel gauze, bedding with rice husk that will not attach to the fur, and acclimated for two weeks. The entire research project was carried out in an animal house and Ad libitum water and standard laboratory feed were (Normal pellet diet) provided, which is available in Haryana and their composition is for 1 kg, the following ingredients are used: 60 % cornflour for carbohydrate, 20 % fishmeal for protein, 10 % wheat flour or bran flour for fibres, 7 % oil seed cake, 2 % bone meal, and 1 % salt. The Institutional Animal Ethical Committee approved the animal study protocol and procedures under Protocol No. IPS/IAEC/2017/294, and the investigation studies were conducted as per the standards established by CCSEA, i.e., the Committee for the Control and Supervision of Experiments on Animals. All experimental methods were conducted in full adherence to ethical standards.

2.4. Sample & dose preparation

Dose of the extract was selected on the bases of previous studies as 500 mg/kg (Ouedraogo et al., 2023). Before use, the needed amount of HAETI was newly suspended in 1 % CMC to reconstitute the extract dose, which was then taken orally (p.o.). Groups in the vehicle control got the same amount of CMC (1 % v/v in saline) orally as 10 ml/kg. The standard group received subcutaneous injections of estradiol valerate (1 g/rat each day).

2.5. Antifertility studies

The estrogenic, anti-estrogenic, abortifacient, anti-implantation studies along with the hormonal analyses, were performed to assess the antifertility activity of plant extracts (Vuyyala et al., 2021).

2.5.1. Anti-implantation investigation

Wistar albino female rats were housed overnight in a cage with a male (2:1) which have proven fertility. From day 1 to day 7 of gestation, the extract was administered (p.o.) in female rats in different groups at dose of 500 mg/kg. The vehicle was given to the control group (CMC 1 %). On the 10th day of the pregnancy, the female rats were laparotomized using a high concentration of sodium thiopentone, and the total number of implantation sites were counted in the uterine horns (El-Ishaq et al., 2019).

2.5.2. Abortifacient study

Female Wistar albino rats were separated into two groups on the first day of gestation, with six animals in each group. On the tenth day of pregnancy, the animals underwent laparotomies in sanitary conditions while being lightly sedated with ether. The rats underwent surgery and were allowed to develop to full term (Vuyyala et al., 2021).

Group 1 Control: 1 % CMC was delivered; Group 2 received suspensions of hydroalcoholic extract (500 mg/kg dissolved in 1 % CMC solution). Throughout the trial, the female rats were assessed for vaginal hemorrhage. Thereafter, they were laparotomized on the day 21 of gestation, and then studied for no. of litters and compared for resorption percentage with the early no. of implantation assessed on the 10th day of gestation (Dabe et al., 2020).

2.5.3. Estrogenic and antiestrogenic activity

One week prior to use, mature female albino wistar rats, weighing 100–120 g, had their bilateral ovaries removed under a mild anesthetic

and under sanitary conditions. Ovaries were removed, and the uterine horns were externalized through a laparotomy incision (Krishna et al., 2022).

Group 1: Control – were administered with 1 % CMC.

Group 2: Standard- Estradiol Valerate (1 µg/rat/day) injected subcutaneously suspended in Arachis oil.

Group 3: Test- received 500 mg/kg HAETI

Group 4: Test + Standard (500+1 µg/rat/day)

For seven days straight, all doses and the vehicle were given orally. Animals were slain at predetermined intervals, such as the eighth day, and the uterine horns were immediately dissected out and freed from clinging fat, blotted, and quickly weighed on a delicate balance. The tissues were dried in alcohol, stored in formalin solution. Thereafter, tissues were embedded in paraffin, sectioned and then stained with a hematoxylin-eosin solution for the examination of histological parameters (H & E Stain). Using a calibrated ocular micrometer, the stained slides were examined under a microscope for the uterus' diameter, endometrium's thickness, and endometrial epithelium's height in 10 randomly chosen sections.

2.6. Hormonal analysis

For hormone estimation, the Merck Kit technique (Merck cat # 15891), or Merck apparatus (Autoanalyser), was utilized. Approximately 2 ml of blood was extracted and then immediately transferred into an EDTA-coated traveler. The materials were properly combined, allowed to sit at ambient temperature for 30–40 min, and then centrifuged for 15 min at 3000 rpm. FSH, LH, 17-estradiol, and prolactin were measured in serum using the enzyme-linked immunoassay (EIA) technique after serum had been isolated from blood (Kokate, 1999).

2.7. Biochemical estimation

Frozen uterine tissue samples from mature rats that had undergone ovariectomies were analyzed for the biochemical parameters: Cholesterol, Glycogen and Alkaline phosphate (Lowry et al., 1951); cholesterol (Oser, 1965); alkaline phosphate; & sialic acid (Warren, 1959).

2.8. Statistical analysis

All information is presented as the mean $\pm$ SEM of six animals from each group. Using the trial version of GraphPad Prism, the means were statistically assessed using one-way analysis of variance (ANOVA) followed by Dunnett's test. The P value < 0.05 was regarded as statistically significant.

3. Results

3.1. Phytochemical investigation

It has been demonstrated that many phytoconstituents from natural sources are effective at preventing pregnancy. Many scientific studies on the antifertility effects of flavonoids, glycosides, alkaloids, and steroids (Hiremath et al., 2000) have been conducted. The study results found that *Tamarindus indica* fruit pulp extract contains alkaloids, steroids, flavonoid glycosides, tannins, saponins, and carbohydrates.

3.2. Acute oral toxicity study

This study was performed on rats to determine toxicity and the minimum lethal dose (LD) of the extract. No negative clinical symptoms were observed in the animals during and after the treatment period. No mortality, inclusive of any sign of clinical toxicity has been found at the dose of 2000 mg/kg. Hence, LD50 cut-off was considered to be 2000

mg/kg. Thereafter, 1/4th of the LD50 i.e. 500 mg/kg was selected to evaluate the contraceptive action of the extract.

3.3. Antifertility study

3.3.1. Anti-implantation and abortifacient activities

The 500 mg/kg dose of HAETI was shown to be effective ($p < 0.01$) than control and the percentage inhibition of implantations and the number of litters born in Wistar rats were found to be 49.16 and 96.42 %, respectively, when compared to control. After the pregnancy was over, all of the animals in the reversible effect research group displayed a normal estrous cycle, and the number of implantations on the tenth day of pregnancy was found to be normal when compared to controls. Hence, Fruit pulp extract from HAETI was discovered to be reversibly efficient.

The hydroalcoholic extract showed maximal inhibition of implants at a dosage level of 500 mg/kg. Table 1 displays the findings of the anti-implantation investigation, and Table 2 displays the percentage resorption index. These findings demonstrate the extract's abortifacient properties. A rise in the extract's percentage resorption index indicates that the embryo's development has failed (Dhanwad et al., 2005). The extract treatment may alter the way the uterine lining functions or increase maternal toxicity, which could lead to more early resorption and late foetal demise. The extracts' ability to reduce implantation sites may be a result of their estrogenic or anti-estrogenic activity. Hence, the current results showed that the extract may be effective in before as well as after the implantation condition.

3.3.2. Uterine weight and vaginal opening

Table 3 shows the effects of the hydroalcoholic extract of *Tamarindus indica* fruit pulp's (HAETI) estrogenic action on the adult ovariectomized rat uterus. Adult rats' uterine weight increased significantly after 500 mg/kg of the hydroalcoholic extract treatment ($p < 0.01$) in comparison to control group. Thee uterotrophic alterations such as width of the uterus ($p < 0.01$), thickness of the endometrium ($p < 0.01$), and luminal epithelium's height were more pronounced. Animals in the test group had fluid-filled, inflated uteri that resembled the proestrous/estrous phase. Open vaginas were visible in the treated rats. The extract treated group animals showed nucleated and cornified epithelial vaginal cells. In comparison to controls, simultaneous treatment of estradiol valerate and hydroalcoholic extract significantly increased uterine weight, diameter, height, and thickness ($p < 0.01$) (Table 4) as well as cholesterol levels. As a result, it demonstrated significant estrogenic action in addition to the standard (Estradiol valerate). Consequently, the estrogen-induced uterotrophic effect was supported.

A decrease in estrogen level, and consequently a reduction in uterine mass, was observed after ovariectomy in mature female rats. Estradiol valerate (EDV) was administered subcutaneously to ovariectomized rats, and this resulted in marked rise in uterine weight. Further, hyperemia and hypertrophy of the uterine tissue and increase of uterine luminal fluid was observed.

Table 1
Effect of HAETI extract on anti-Implantation activity.

Treatment (Dose)	Anti-Implantation activity		
	No. of rats showing no implantation sites on day ten	No. of Implants mean $\pm$ SEM	Mean % inhibition of implantation
Control (1 % CMC)	Nil	10.33 $\pm$ 0.42	Nil
HAETI (500 mg/kg)	Nil	1.16 $\pm$ 0.30 ^b	88.7 %

Values are expressed as mean $\pm$ SEM. P values $a = P < 0.05$, $b = P < 0.01$, $c = P < 0.001$ when compared with normal control.

Table 2

Effect of HAETI extract on abortifacient activity.

Treatment (Dose)	Abortifacient activity		
	No. of Implantation	No. of litters	% Resorptions
Control	8.00 ± 0.71	7.98 ± 0.50	NIL
HAETI (500 mg/kg)	7.01 ± 0.57	0.16 ± 0.03 ^c	96.42 %

Values are expressed as mean ± SEM. P values $a = P < 0.05$, $b = P < 0.01$, $c = P < 0.001$ when compared with normal control.

Table 3

Effect of HAETI extract and HAETI + standard on uterine weight and vaginal cornification.

Treatment	Dose	Uterine weight	Vaginal Cornification
Control	1 % CMC	0.078 ± 0.006	NIL
Standard	Estradiol valerate (1 µg/rat per day)	0.133 ± 0.009 ^a	+++
HAETI	500 mg/kg	0.210 ± 0.01 ^b	+ to ++
HAETI + Standard	500 mg/kg + 1 µg/rat per day	0.312 ± 0.006 ^b	+++

Values are expressed as mean ± SEM. P values $a = P < 0.05$, $b = P < 0.01$, $c = P < 0.001$ when compared with normal control. Whereas, +: Nucleated Epithelial Cells, ++: Nucleated Epithelial Cells and Cornified Cells, +++: Cornified cells.

Table 4

Effect of HAETI extract and HAETI + Standard on diameter of uterus, thickness of endometrium and height of endometrial epithelium.

Treatment (Dose)	Diameter of uterus (µm ± SEM)	Thickness of Endometrium (µm ± SEM)	height of endometrial epithelium (µm ± SEM)
Control	335.07 ± 6.95	51.82 ± 3.58	9.37 ± 0.65
Estradiol valerate	601.43 ± 4.35 ^b	126.54 ± 1.75 ^b	13.15 ± 2.40 ^a
HAETI (500 mg/kg)	388.22 ± 4.85 ^b	66.04 ± 3.89 ^b	21.20 ± 2.79 ^b
HAETI (500 mg/kg) + Standard (1 µg/rat per day)	624.6 ± 5.75 ^c	204.44 ± 1.03 ^b	32.37 ± 2.58 ^c

Values are expressed as mean ± SEM. P values $a = P < 0.05$, $b = P < 0.01$, $c = P < 0.001$ when compared with normal control.

3.3.3. Uterine histological findings

According to histological examination, In Group 1 (Control) the uteri of the animals shared many of the same traits, including an infantile look, a limited lumen, a compact stroma with thin and atrophic endometrium, and a sparse number of small and inactive endometrial glands. Simple cuboidal epithelial cells and, in some spots, squamous epithelium coated the endometrium. Many cells with rounded, noticeably pigmented basal nuclei and little cytoplasm were present in the lamina propria. Consequently, the well-known effects of ovariectomy, or estrogen deficiency, were confirmed (Sud and Setty, 1974). In Group 2 (standard) animals (given with estradiol valerate) exhibited endometrial epithelial hypertrophy and hyperplasia, as well as an expanded or completely absent lumen lined with stratified columnar epithelium. Expanded/stimulated uterine glands with edematous, loose, and stromal tissue. Endometrium thickness and luminal epithelium height both increased. In Group 3, the uterus was stimulated, uterine lumen size increased along with rise in the no. of uterine glands with improved glandular secretion, and the increase in the height of the luminal epithelium were found after the oral administration of HAETI (500 mg/kg) in comparison to the control group. These results revealed the

estrogenic nature of the plant extract. Further, we found cytoplasmic or localized vacuolization. Cells in the lamina propria had conspicuous nucleoli that were big, clear, and centered. There detected a great number of eosinophil cells in the endometrial stroma.

Group 4 received HAETI (500 mg/kg) along with estradiol valerate, and they experienced larger uteri and clear viscous fluid than the other groups. Both stratified columnar epithelial cells and non-keratinized stratified squamous epithelium were seen in some regions. The lamina propria included a sizable number of eosinophils, some of which invaded the superficial or glandular epithelium. Huge cuboidal cells that make up dilated endometrial glands. Indicating the extract's potent estrogenic properties (Figs. 1, 2, 3 and 4). Similar estrogenic effects on uterine histology have been noted in numerous cases following oral administration of different plant extracts.

3.3.4. Hormonal analysis

It has been observed that alteration of sex hormones may lead to pregnancy failure (Yakubu and Bukoye, 2009). Therefore, sex hormones were assessed in terms of their roles in maintaining pregnancy. The cessation of the estrus cycle and ovulation is implied by the drop in FSH concentration (Ganguly et al., 2007). The significant decrease in serum LH levels may be linked with luteolysis process that occur prior to parturition (Yakubu and Bukoye, 2009) as shown in Table 5. LH is related to the maintenance and consistent operation of the corpora lutea. In the current investigation, the luteal phase was not sustained due to the lower LH level, which resulted in an increase in prolactin levels. These findings were corroborated with a previous study (Ganguly et al., 2007). They found that increased prolactin level along with suppression of LH secretion resulted due to the rise in estrus cycle period. Abortifacient and anti-implantation activity of the HAETI extract attributed to luteolysis and alleviation in the progesterone levels in the blood. Further, it has been found that disturbances in estrogen/progesterone equilibrium could lead to anti-implantation activity (Dhanwad et al., 2005).

3.3.5. Biochemical estimation

In most instances, uterine protein levels rise throughout the implantation period in mammalian characteristics. Increased protein concentration begins as a result of the decrease in water content and extended consistency brought on by progesterone. Moreover, the endometrial secretion proteins and blood plasma proteins found in luminal uterine are unrestricted (Beier et al., 1991). In the current study, it was shown that the control group of bilaterally ovariectomized adult rats had very low protein content in their uteri. After administering EDV subcutaneously, a marked ($p < 0.05$) rise in protein content of uterus was observed. Moreover, when HAETI fed alone, marked ($p < 0.05$) rise in protein content of uterus was noted (Table 6). Though, as compared to EDV alone treated rats, administering the HAETI+EDV showed a

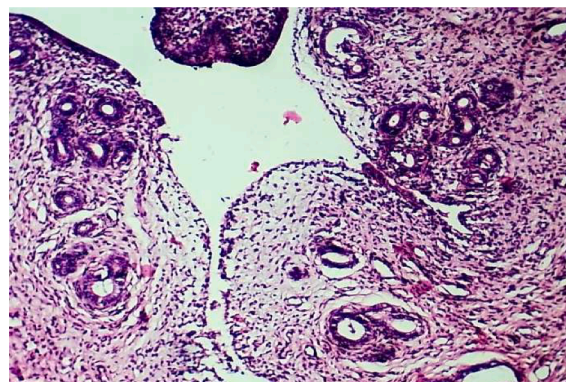


Fig. 1. Photomicrograph showing a transverse section of the uterus of control rats (Surface epithelium with no secretory activity).

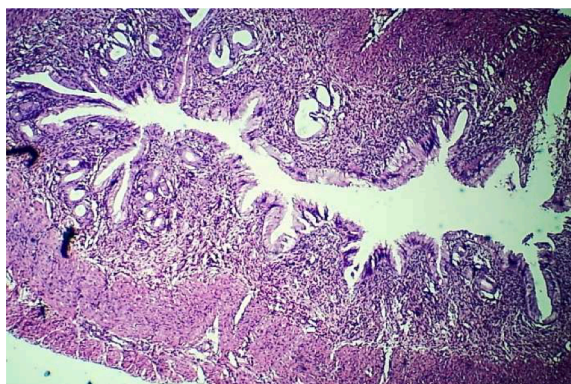


Fig. 2. Photomicrograph of a transverse section of the uterus of Estradiol Valerate treated rats, indicating proliferation stage i.e. obliterated lumen and loose stroma and increasing height of luminal epithelium.

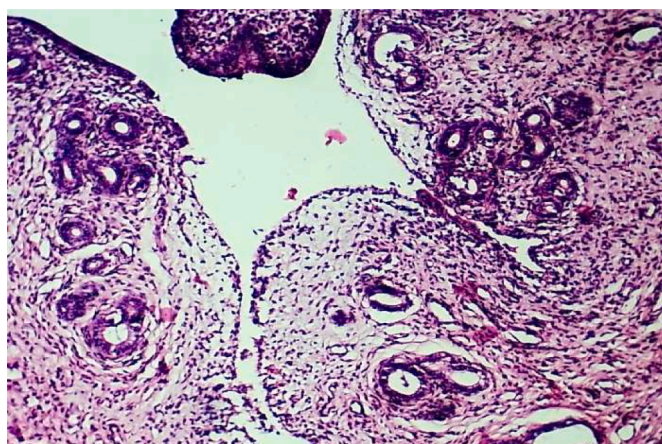


Fig. 3. Photomicrograph showing a transverse section of the uterus of HAETI, 500 mg/kg p.o. treated rats, indicating an increase in the height of endometrium and loose and edematous stroma with stimulated uterine glands.

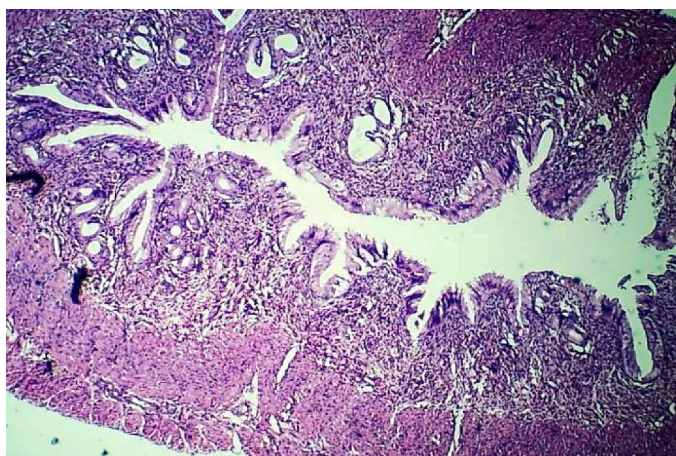


Fig. 4. photomicrograph of a transverse section of the uterus (HAETI+ Standard) treated rats, showing stratified columnar cells with prominent nuclei and stimulated uterine glands.

profound ($p < 0.05$) rise in the uterine protein content of ovariectomized rats. In mature ovariectomized female rats, a synergistic ($p < 0.05$) increase in protein content was found when treated with HAETI extract and EDV. This shows that HAETI has a potent estrogenic effect.

Table 5

Effect of HAETI extract and HAETI + Standard on LH, FSH, Prolactin, and Estradiol level in the blood.

Treatment (Dose)	LH (pg/ml)	FSH (pg/ml)	Prolactin (pg/ml)	Estradiol (pg/ml)
Control	7.10 ± 0.50	8.32 ± 0.58	40.73 ± 0.76	0.27 ± 0.07
Estradiol valerate	3.84 ± 0.16 ^b	4.81 ± 0.29 ^b	41.86 ± 0.37 ^a	8.73 ± 0.09 ^b
HAETI	5.81 ± 0.09 ^a	6.59 ± 0.44 ^a	40.80 ± 0.54	3.10 ± 0.13 ^a
HAETI	1.90 ± 0.32 ^c	2.82 ± 0.25 ^c	41.95 ± 0.16 ^b	11.21 ± 0.29 ^c
(500 mg/kg) + Estradiol valerate				
(1 µg/rat/day)				

Values are expressed as mean ± SEM. P values $a = P < 0.05$, $b = P < 0.01$, $c = P < 0.001$ when compared with normal control.

Table 6

Effect of HAETI extract and HAETI + Standard on protein, cholesterol, glycogen, alkaline phosphate levels.

Treatment	Protein (mg/kg)	Cholesterol (mg/kg)	Glycogen (mg/kg)	Alkaline phosphate
Control	2.19 ± 0.04	2.97 ± 0.05	2.75 ± 0.06	0.43 ± 0.026
Estradiol Valerate (EDV)	4.25 ± 0.16 ^b	5.12 ± 0.17	4.53 ± 0.16 ^b	0.82 ± 0.024
HAETI	3.01 ± 0.36 ^a	4.25 ± 0.36 ^b	3.65 ± 0.27 ^a	0.59 ± 0.025
HAETI + EDV	5.58 ± 0.12 ^c	6.16 ± 0.29 ^c	5.41 ± 0.28 ^b	0.78 ± 0.025

Values are expressed as mean ± SEM. P values $a = P < 0.05$, $b = P < 0.01$, $c = P < 0.001$ when compared with normal control.

Moreover, the uterine weight increased as a result of the uterus's protein content rising. The above mentioned findings are consistent with a number of earlier reports (Wadhwa, 1982; Ahirwar et al., 2007). When estradiol valerate was administered, there was an increase in the levels of alkaline phosphate. Moreover, when HAETI was administered alone, there was a substantial increase in the amount of alkaline phosphate in the uterus ($p < 0.05$). Its combination treatment with EDV, however, has a synergistic effect. These findings show/unveil that *Tamarindus indica* hydroalcoholic extract increased the activity of alkaline phosphatase, possibly as a result of its estrogenic influence.

It has been observed that cholesterol content in the uterus of intact and ovariectomized rats increased by plant extracts with estrogenic properties, and decreased by those with antiestrogenic properties (Wadhwa, 1982). Cholesterol acts as a substrate in the ovary steroidogenesis. We found that treatment of the extract alone and in combination with EDV significantly increased ($P < 0.05$) the level of cholesterol in the uterus of ovariectomized rats, thereby demonstrating the estrogenic character of extract.

4. Discussion

Despite the extensive advantages of natural remedies, very little experimental studies have been conducted to reveal the potential mechanism of action and effectiveness of the traditional medicines (Yuan et al., 2016). *Tamarindus indica* is used as gelling agent, thickener and stabilizer in food industry. Tribal women in the Indian states of Tripura and Maharashtra utilize the fruit pulp of the *Tamarindus indica* as an abortifacient drug. 0.3 Million tons of *Tamarindus indica* produced per year in India. *Tamarindus indica* seeds are used for both residential and commercial purposes. In the present study the groups were treated with the most remarkable dose of plant extract stipulated fantastic

results as those of the standard medication, which was further justified or supported by histopathological parameters and hormonal analysis of the pregnancy-interrupted activity on female Wistar rats.

The hormonal investigation further validated the contraceptive effect of *Tamarindus indica* fruit pulp. To increase lactation, eat Kunu (a kind of porridge) prepared with fruit of *Tamarindus indica* and *Ximenia americana* or drink a macerate of *Tamarindus indica* fruits in water (Joshi et al., 2022). Under the influence of the ovarian hormone estrogen, the uterus and the female reproductive system go through numerous physiologic and biochemical changes (Prakash and Mathur, 1979). It is also known that ovarian hormones can change the biochemical components of the female reproductive system. Although the estrogen is known to perform a more pronounced activity in the ovary. The administration of estrogen or progesterone in ovariectomized rats caused significant rise in the weight of vagina, cervix and uterus (Mirabolghasemi et al., 2017). The estrogenic and antiestrogenic properties of numerous contraceptive drugs have been evaluated from/by this inherent virtue. The amount of estrogen that leads to the uterus and reproductive tract atrophy is reduced when ovariectomized female rats are used in estrogenic and anti-estrogenic activities. Exogenic administration of estrogenic substances to ovariectomized rats induces uterotropic effects, such as the accretion of watery fluid known to promote ballooning of the uterus, vaginal cornification and opening, keratinization, an increase in uterine glycogen content, and pro (Williamson and Okpako, 1996). Thus, unfavorable conditions are created, and the uterine milieu is altered. The increased height of the luminal epithelium with loose stroma and the proliferation of uterine glands with columnar cells were evidence of the estrogenic impact on the uterus in the HAETI-treated rat. Moreover, reproductive hormones like LH, FSH, Estrogen, and Progesterone are necessary for fertilization. Exact equilibration of progesterone and estrogen secretion is required for the implantation and maintenance of pregnancy. Any hormonal imbalance could prevent implantation or lead to an abortion (Psychoyos, 1996). Typically, hormonally valuable substances disrupt the uterine hormonal environment and have a contraceptive effect (Mishra et al., 2011). As estrogen has the potential to initiate and enhance uterine contractility to evacuate fertilized egg along with abortifacient and post-coital anti-implantation effects, the current results of the HAETI extract may be mediated through estrogenic action (Shibeshi et al., 2006). In current study we were used the dose of *Tamarindus indica* as 500 mg/kg of Hydro alcoholic extract, which were reported safer and not shows any cytotoxic effects but showing pharmacological activity at this dose (Komutarin et al., 2003; Ouédraogo et al., 2023). The findings of this study were supported by histological analyses of the antifertility action on albino female rats, suggested that the group treated with the most significant concentration of plant showed great results as those of the conventional medicine. The findings of this study clearly demonstrate that the percentage inhibition of implantations in wistar rats, at the doses 500 mg/kg of Hydro alcoholic extract of *Tamarindus indica* were found to be significant that is 88.7 % compared to control showing anti-implantation activity and Hydro alcoholic extract of *Tamarindus indica* at the dose of 500 mg/kg reported the percentage resorptions in wistar rats 96.42 % compared to control showing abortifacient activity along with this the HAETI show significantly increase in uterine weight of wistar rats. The extract also showed the presence of cornified and nucleated epithelial cells in the vaginal smear of the rats. Hence show plant is estrogenic in nature. Oral administration of HAETI at 500 mg/kg. Caused a noteworthy increase in the uterine weight versus the control group. The uterotrophic changes, like the uterus diameter, height and thickness of endometrium were significantly increased when compared with the control group animals. Simultaneous administration of standard i.e. estradiol valerate and hydro alcoholic extract caused noteworthy rise in the uterine diameter and weight, along with cornification of cells in the vaginal smear (versus the control; Synergistic reaction along with Estradiol valerate, further corroborated its estrogenic activity. In histological findings, HAETI at the dose 500 mg/kg

showed a marked rise in height of endometrial epithelium, endometrium thickness, and diameter of uterus when comparison with control group, therefore showing estrogenic nature. The present biochemical findings also supported the estrogenic nature of the HAETI as it increased the protein, cholesterol, glycogen and alkaline phosphate levels in the serum of ovariectomized rats along with this it significantly reduced the level of LH, FSH and significantly increased the estradiol and prolactin hormone. Decline in the concentration of FSH and LH are responsible for the interruption in the estrus cycle and the regular working of the corpora lutea. So, these results were also supported the pregnancy interruption activity of the plant.

5. Conclusion

The current investigation was successful in examining the supporting data for conventional use of *Tamarindus indica* hydro alcoholic extract's putative antifertility and abortifacient characteristics. The presence of flavonoids, phytosterols, and terpenoids in the extracts may contribute to their potential therapeutic effects. Analyzing the shift in hormonal content and *Tamarindus indica*'s estrogenic makeup allowed researchers to report on the plant's antifertility and abortifacient effects. Changes in reproductive hormone levels will result in pregnancy failure, and estrogen hormones are essential for pregnancy. HAETI demonstrated abortifacient properties, which were further inferred from histopathological analysis of the uterus and ovaries. The *Tamarindus indica* plant can raise levels of estrogen, alkaline phosphate, and glycogen, which prevents implantation and other estrogenic effects. Our study's findings showed that *Tamarindus indica* fruit pulp hydroalcoholic extract has a potent contraceptive effect, which may be explained by the phytoconstituents of the plant's capacity to inhibit implantation and have an estrogenic effect. Further investigation on *Tamarindus indica* as a potential natural anti-implantation agent allow for new abortive drug and their molecular mechanism should be warranted.

CRedit authorship contribution statement

Mandeep Kaur: Investigation. **Avtar Chand Rana:** Visualization, Supervision. **Sunil Kumar:** Resources, Supervision. **Beena Kumari:** Writing – original draft. **Dinesh Kumar:** Data curation. **Ashok Jangra:** Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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