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# The Protective Effect of Ethanol Extract of the Stem Bark of *Tamarindus indica* L. on Mifepristone-induced Threatened Miscarriage in Female Wistar Rats

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**Abstract**

Threatened miscarriage, characterized by vaginal bleeding before 20 weeks of gestation, affects up to 25% of pregnancies and often results in pregnancy loss. Current treatment options remain limited, with insufficient empirical evidence supporting their efficacy and concerns about adverse effects. This study evaluated the miscarriage protective effect of ethanol extract of the stem bark of *Tamarindus indica* in female Wistar rats. Mifepristone (50 mg/kg) (dose) was used to induce threatened miscarriage in rats. Groups of rats were administered graded doses of the extract (100, 200 and 400 mg/kg), and parameters including duration and volume of uterine bleeding, pregnancy outcomes, litter size, and litter weight were assessed. The extract significantly reduced uterine bleeding in a dose-dependent manner, with 400 mg/kg showing optimal efficacy. In early gestation, bleeding duration and volume decreased from 3.8 ± 0.3 days and 3.5 ± 0.4 mL in the mifepristone group to 1.1 ± 0.2 days and 0.8 ± 0.1 mL in the extract (400 mg/kg) treatment group. In mid-gestation, values decreased from 4.8 ± 0.4 days and 5.0 ± 0.5 mL to 2.0 ± 0.3 days and 2.1 ± 0.3 mL in the extract (400 mg/kg) group. At 400 mg/kg, the extract achieved 75% pregnancy success, with mean litter size of 8.3 ± 1.1 and mean litter weight of 5.5 ± 0.3. Ethanol extract of *Tamarindus indica* stem bark demonstrated protective effects against mifepristone-induced miscarriage in Wistar rats, sustaining pregnancy to term with favorable outcomes.

**Key words**: Threatened miscarriage, *Tamarindus indica*, rats, mifepristone, pregnancy.

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## Introduction

Threatened miscarriage is defined as vaginal bleeding before 20 weeks of gestation in a pregnancy confirmed by positive urine and/or blood pregnancy tests, without passage of products of conception or evidence of fetal or embryonic demise (Mouri *et al.,* 2022). Approximately 25% of pregnant women experience some degree of vaginal bleeding in the first and second trimesters, and about half of these cases result in pregnancy loss. The bleeding is usually mild to moderate, while pain may present as intermittent cramps, suprapubic discomfort, pelvic pressure, or lower back pain (Mouri *et al.,* 2022).

Many factors, including chromosomal abnormalities, hormonal imbalances, uterine structural defects, infections, and maternal diseases such as diabetes mellitus and thyroid disorders, predispose pregnancies to miscarriage. Autoimmune conditions, poor diet, advanced maternal age (≥40 years), and lifestyle factors such as smoking, alcohol consumption, drug use, chemical exposure, and placental problems also contribute (Akaneme, 2007; Cleveland Clinic, 2024). Miscarriage may be classified as early, missed, incomplete, or recurrent (Miscarriage Australia, 2026).
The treatment options recommended for threatened miscarriage include watchful waiting, hormonal testing which may lead to the prescription of progesterone to support the growth of the baby (Clevelanclinic.org, 2024), bed rest, treatment with HCG and the use of uterine muscle relaxant drugs (Sotiriadis *et al*., 2004; Li *et al*., 2017), and temporary abstinence from sexual intercourse has also been suggested (Akaneme, 2007). However, these protocols are limited by insufficient empirical evidence of efficacy and potential adverse effects such as nausea, headache, drowsiness, and liver toxicity. Vaginal administration of progesterone pessaries may also be inconvenient for women experiencing bleeding (Li *et al.*, 2017).

The use of complementary and alternative medicine (CAM) to promote health is increasing worldwide, with women reported to use CAM more frequently than men. Surveys indicate that over 80% of women in the UK, 50% in Australia, 90% in Canada, nearly 25% in Denmark, and almost 50% in the USA rely on CAM (Lingjing *et al*., 2021). This trend is likely similar in Nigeria, particularly in local communities where traditional health-care providers, including birth attendants, remain the most accessible option and often incorporate medicinal plants into their practice. Literature surveys confirm that medicinal plants have long been used to manage threatened miscarriage in these communities. Such plants represent a promising source of novel therapeutic agents that could prolong pregnancy and effectively reduce miscarriage risk. However, these plants have not been extensively investigated for their effectiveness in female reproductive health, and management of threatened miscarriage. One of such plants is *Tamarindus indica* L.

*Tamarindus indica*, commonly called Tamarind is a perennial evergreen leguminous tree that belongs to the Fabaceae family. It is indigenous to tropical and sub-tropical regions of the world, and grows wild in African countries like Sudan, Cameroun and Nigeria (Mohammed, 2019). *Tamarindus indica*, has versatile application in the cuisine industry, and in traditional medicine (Morton, 1987; Kuma and Bhattacharya, 2008). All parts of the plant, has been used in Indian and African traditional medicine to treat various ailments, due to their rich phytochemical constituents (De Caluwe *et al*., 2010). *Tamarindus indica* has been used in traditional medicine to treat inflammation, diarrhea, colds, snake bite, abdominal pain, fever, helminthic infection, and wound healing. It has also been used in the management of diabetes, prevention of obesity and other chronic conditions (Akram *et al.*, 2022). Ethno-pharmacological evaluation of *Tamarindus indica* revealed that almost every part of the plant has therapeutic value (Ahsan *et al*., 2025). The seed of *Tamarindus indica* seems to be the part that is most extensively studied and reported scientifically. For instance, Sudjaroen (2025), reported the antioxidant activity of the extracts of the seed, attributing it to the high content of phenolic compounds that have the capability to scavenge free radicals. Other activities reported include, antibacterial activity against a wide range of gram-positive and gram-negative isolates (Doughari, 2006), anti-fungal activity against *Candida albicans* and *Aspergillus niger* (El-Siddig *et al*., 2006), antidiabetic activity (Maitin *et al*., 2004), Amaechina and Ekwe, (2022) reported the graded dose dependent blood pressure lowering effect of the leaves of *Tamarindus indica* in normotensive Wistar rats. While it has been reported that some traditions employ *Tamarindus indica* preparation to manage menstrual disorders, and as a restorative tonic during the postpartum period (El-Siddig, 2006), there is paucity of information on the scientific evaluation of its activity in threatened miscarriage and pregnancy outcome.

This study aimed to evaluate the protective effect of ethanol extract of the stem bark of *Tamarindus indica* on threatened miscarriage and pregnancy outcomes in female Wistar rats.

## Materials and Methods

### *Collection and Preparation of Plant Extract*

The stem bark of *Tamarindus indica* was obtained from Chaza village, Suleja in Niger State of Nigeria. The plant was identified and authenticated at the Nigeria Institute of Pharmaceutical Research (NIPRID) Abuja, with voucher no: NIPRID.H.7080. The stem bark was air-dried under shade for 21 days, reduced to coarse powder in a mortar, and comminuted to fine powder with an electric blending machine

The powdered material (500 g) was exhaustively extracted with 95% ethanol (Fharmatrends Nigeria Limited), using the Soxhlet apparatus. The extract was concentrated to slurry paste using a rotary evaporator, and then to dryness in an electric oven maintained at 35°C for 7days, to give a total yield of 23.6%. The extract was stored in an airtight sample bottle, from which appropriate weight was weighed and freshly made into a solution of specific concentration, for use during experiment.

*Phytochemical screening*

Qualitative phytochemical analysis for the identification of low molecular weight secondary metabolites like alkaloids, saponins, flavonoids, glycosides, and phenols and triterpenoids was carried out, following standard procedures (Sofowora, 1978; Trease and Evans, 1989).

*Acute toxicity study*

Acute toxicity study on the extract of the stem bark of *Tamarindus indica* was carried out in two phases as described by Lorke (1983).

*Animal Experiment*

Thirty-two (32) adult non-gravid female, and sixteen (16) male Sprague-Dawley rats (190-230 g) were purchased, and maintained in the Animal House of the Department of Pharmacology and Toxicology, University of Benin. The animals were fed with standard animal feed pellets, and allowed access to water *ad libitum*, under ambient temperature and natural day and night light cycle. Ethical approval was obtained from the Faculty of Pharmacy Ethical Committee for animal experiments, and all the animals were handled in compliance with the Public Health Service policy on humane Care and Use of Laboratory Animals (Uchendu *et al*., 2025).

*Mating and Grouping of Animals*

A modified protocol of Liu *et al.,* (2015), and Camilleri and Sammut, (2023) was used for this procedure. The female rats were randomly selected and grouped into nine (9) groups of four animals each, which were each paired with 2 male rats. Successful mating was confirmed by physical examination of vaginal smear for the presence of sperm, and if present was taken as the day-1 of gestation. The animals were grouped as follows:

Group A: Normal group of 4 female animals mated, and not Treated, Group B: Pregnant group of 4 female animals not treated; Groups C, D and E: Each with pregnant 4 animals, administered 100, 200 and 400 mg/kg of the extract respectively, from day-1 of gestation, through to term, while mifepristone (50 mg/kg) was administered on day-7 of gestation. Groups F, G and H: Each with 4 pregnant animals administered 100, 200, and 400 mg/kg of extract respectively, from day-1 through to-term, and mifepristone on day 12 of gestation. Hemi-shaped cotton buds of equal weights wrapped with paraffin were placed into the vagina of the female rats, to verify the successful induction of abortion (Liu *et al.,* 2015).

*Assessment of Duration and Volume of Uterine Bleeding: Preparation of cotton balls plug-in*

To assess the duration and volume of uterine bleeding, cotton was made into balls of equal size, and wrapped with hemi-shaped paraffin, for insertion into the vagina as plug-in. the cotton balls were used as improvised pads for the purpose of trapping bleeding.

*Evaluation of duration and volume of bleeding*

The cotton balls were inserted into the vagina of the female Wistar rats from the day eight of the gestation period, and replaced at specific time intervals. Insertion of cotton balls was stopped after the cessation of vaginal bleeding, and all the blood-soaked cotton balls from each of the groups were appropriately labeled, and preserved in a refrigerator, to be used for the evaluation of duration and volume of bleeding accordingly.

The evaluation of duration and volume of uterine bleeding was evaluated, using the alkaline-hematin technique, which is a common method used clinically, and in research to measure the quantity of uterine blood collected (Magnay *et al*., 2010; Liu *et al*., 2015). The principle is that the addition of an alkaline solution (sodium hydroxide) to blood leads to the conversion of the hemoglobin present in the blood to alkaline hematin, which can be detected spectrophotometrically, by comparing the absorbance of the blood collected from the cotton balls, to a standard blood sample of a known volume.

*Preparation of samples for spectrophotometric analysis*

On day 14 of the gestation period, 0.02 mL of blood sample was collected from the caudal vein and added to 4 mL of 5% sodium hydroxide (NaOH) solution, and mixed thoroughly. This was used as the standard (V1). (Camilleri and Sammut, 2023). To prepared the sample for analysis, the cotton balls from each of the groups were washed repeatedly using 5 mL of NaOH, until the solution was colourless. The solution was poured into a beaker, and the final volume of NaOH used for the wash was recorded, and served (V2).

*Spectrophotometric analysis*

A T80 + UV/VIS spectrophotometer was set at a fixed wavelength of 546 nm. The machine was first calibrated to zero (0.00) absorbance, using a blank cuvette with only 5% NaOH solution, after which the absorbance of the standard and all the samples from the respective groups were measured.

The final volume of uterine bleeding for each group was, calculated using the formula:

Final volume (ml) =

$$\frac{\text{Abs of the Standard (V1)} \times \text{Absorbance of Sample (V2)}}{\text{Abs. of caudal vein blood} \times \text{V1}}$$

*Effects of Pregnancy Outcome and Litter Viability*

Pregnancy outcome was evaluated by physical observation of the number of pregnancies that were protected, and ran through term in the treated groups of mifepristone-induced abortion, as compared to the to the untreated mifepristone-induced abortion group, while litter viability was assessed by observing the survival rate of the litter for a period of 9weeks.

*Statistical Analysis*

All data are analyzed by One-Way Analysis of Variance, using student T-test, and presented as mean ± standard error of the mean (SEM), with 95% confidence level, and $p < 0.05$ interval.

**Results**

*Phytochemical screening*

The results of the preliminary phytochemical analysis showed the presence of tannins, saponins, phenols, alkaloids, glycosides, flavonoids, and terpenoids, (Table 1).

*Acute toxicity study*

All the animals manifested no physical signs of toxicity, and no mortality was recorded at the phase I and II of the acute toxicity studies. This suggest that the $LD_{50}$ of the extract of the stem bark of *Tamarindus indica* is greater than 5000 mg/kg

*Effect of the extract on duration and volume of uterine bleeding in the early gestation period*

*Effect on duration of bleeding*

The extract caused a significant ($P < 0.001$) and graded dose reduction in the duration of uterine bleeding in the early gestation period (7 days). While the mifepristone-induced and not treated group bled for a period of 3.8 ± 0.3 days, the duration of bleeding reduced steadily and dose dependently in the treated groups, to 2.5 ± 0.4, 1.8 ± 0.3, and 1.1 ± 0.2 days, for the 100, 200, and 400 mg/kg respectively (Figure 1).

*Effect on volume of bleeding*

The effect of the extract on volume of bleeding followed the same pattern with the effect on duration of bleeding. The extract caused a significant ($P < 0.01$) dose- dependent reduction in the volume of uterine bleeding, by reducing the volume of bleeding from 3.5 ± 0.4 mL to 2.1 ± 0.3, 1.4 ± 0.2, and 0.8 ± 0.1 mL for the 100, 200, and 400 mg/kg groups respectively (Figure 2)

*Effect of the extract on duration and volume of uterine bleeding, on the (12 days) Mid- Gestation Period*
*Effect of extract on duration of bleeding*

The extract caused a significant ($P < 0.01$) dose-dependent reduction in the duration of bleeding in the mifepristone-induced groups in the mid-gestation period from 4.8 ± 0.4 days 3.5 ± 0.5, 2.6 ± 0.3, and 2.0 ± 0.3 days for the 100, 200, and 400 mg/kg of groups respectively (Figure 3).

*Effect of extract on volume of bleeding*

The mifepristone none treated group recorded a volume of uterine bleeding of 5 ± 0.5 mL, which was significantly and dose- dependently reduced to 3.9 ± 0.6, 2.8 ± 0.4, and 2.1 ±0.3 mL by 100, 200, and 400 mg/kg of the extract respectively (Figure 4)

*Effect of Tamarindus indica on Pregnancy Outcome in Early (7-day) Gestation Period*

The extract protected and sustained the pregnancy to terms in a dose dependent manner in the early (7-days) gestation period, with the dose of 400 mg/kg achieving the optimum viability of pregnancy in all the four female Wistar rats, as all the four pregnant female Wistar rats successfully carried their pregnancies to terms, and littered successfully (Figure 5).

*Effect of the extract on Mean Litter Number and Weights in Early (7-day) Gestation Period*

No litter was recorded in the 100 mg/kg group, which implies that the extract not have positive viability on pregnancy. The 200 and 400 mg/kg treated groups recorded normal weights of 5.7 ± 0.3, and 5.7 ± 0.2 grams respectively. These results compared positively with the mean litter weights of 5.8 ± 0.2 of the Normal group. Similarly, the mean litter numbers of 8 ± 1.0, and 9.5 ± 0.8 for the 200, and 400 mg/kg treated groups respectively compared positively with the mean litter number of 10.3 ± 0.9 of the control (Figure 6).

**Table 1:** The secondary metabolites present in the stem bark of *Tamarindus indica* Linn

| Phytochemicals | Results |
|---|---|
| Tannins | + |
| Saponins | + |
| Phenols | + |
| Alkaloids | + |
| Glycosides | + |
| Flavonoids | + |
| Terpenoids | + |

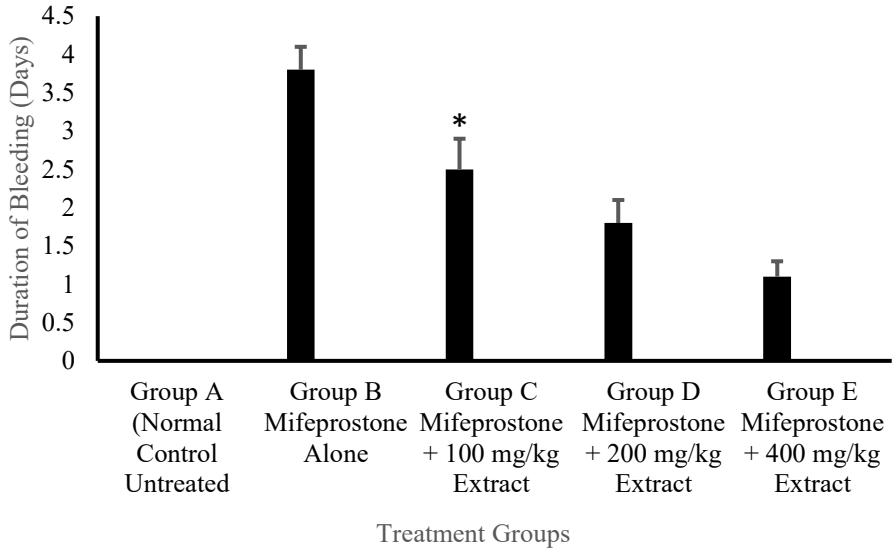


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**Figure 1:** The effect of the extract on duration of bleeding, in mifepristone-induced miscarriage on day 7of gestation, (n = 4), *P < 0.05, ** P < 0.01 **.

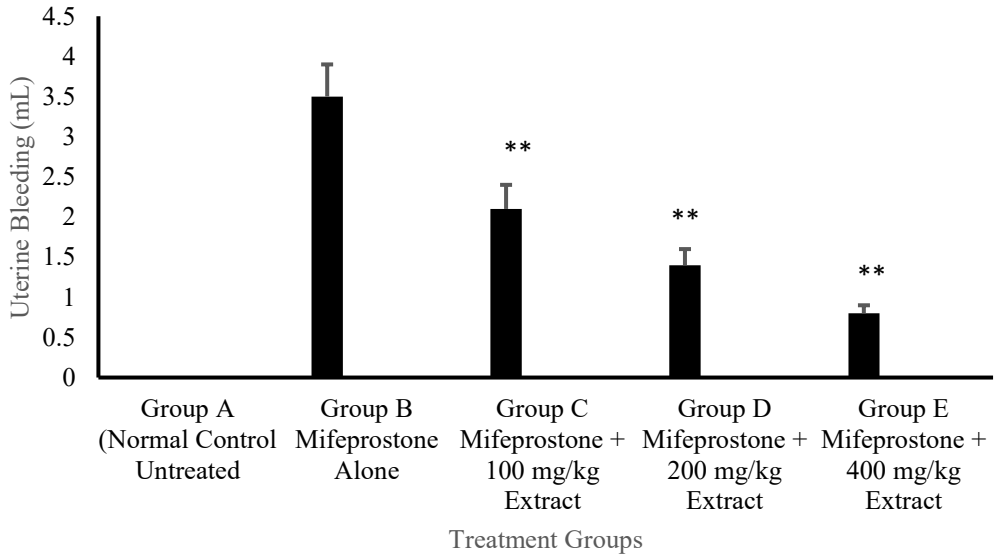


**Figure 2**: The effect of the extract on uterine bleeding volume in mifepristone-induced miscarriage on day7 of gestation, **P < 0.01, n = 4.

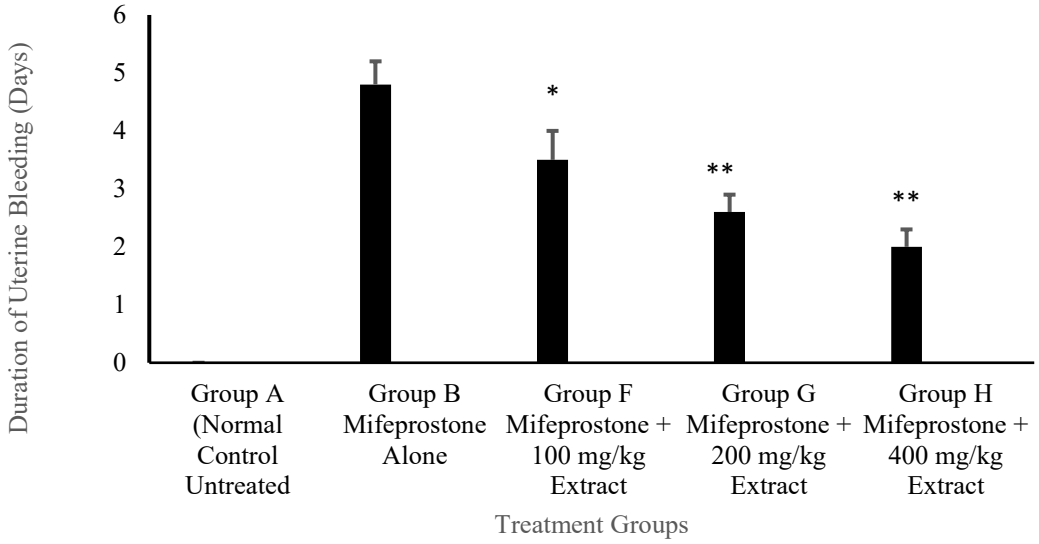


**Figure 3**: The effect of the extract on duration of bleeding, in mifepristone-induced miscarriage on day 12 of gestation. *P < 0.05, **P < 0.01 n = 4.

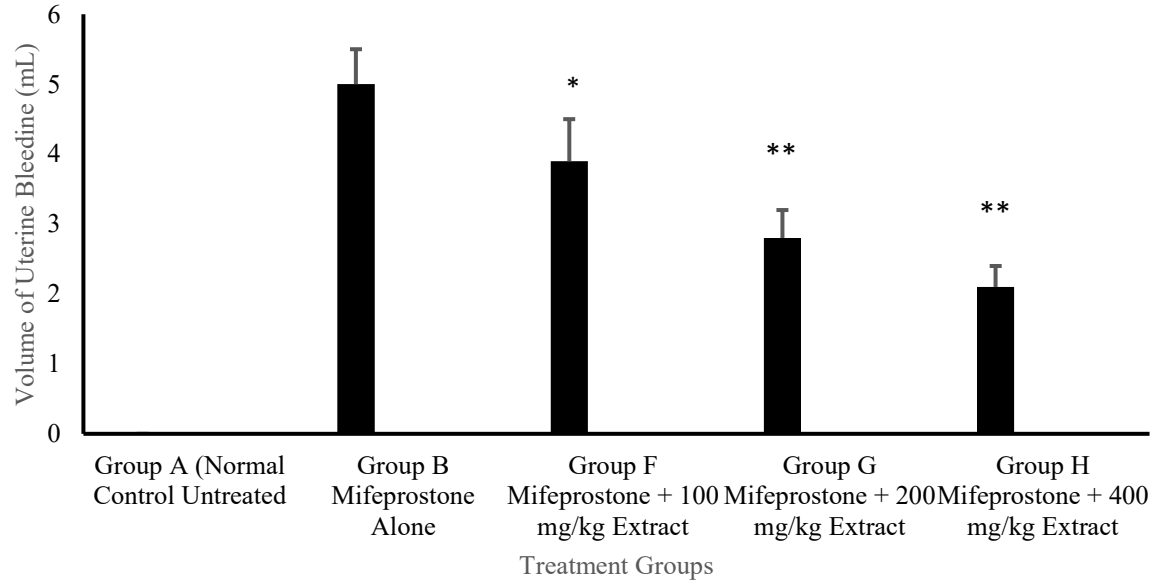


**Figure 4**: The effect of the extract on uterine bleeding volume in mifepristone-induced miscarriage on day12 of gestation, *P < 0.05, **P < 0.01 n = 4

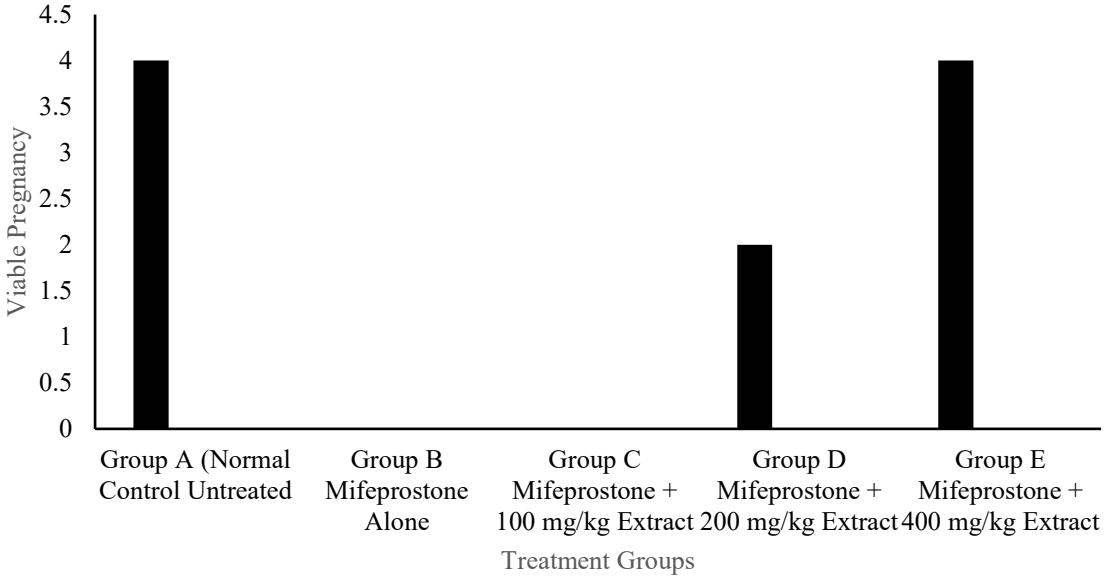


**Figure 5**: The effect of the extract on viability of pregnancy.

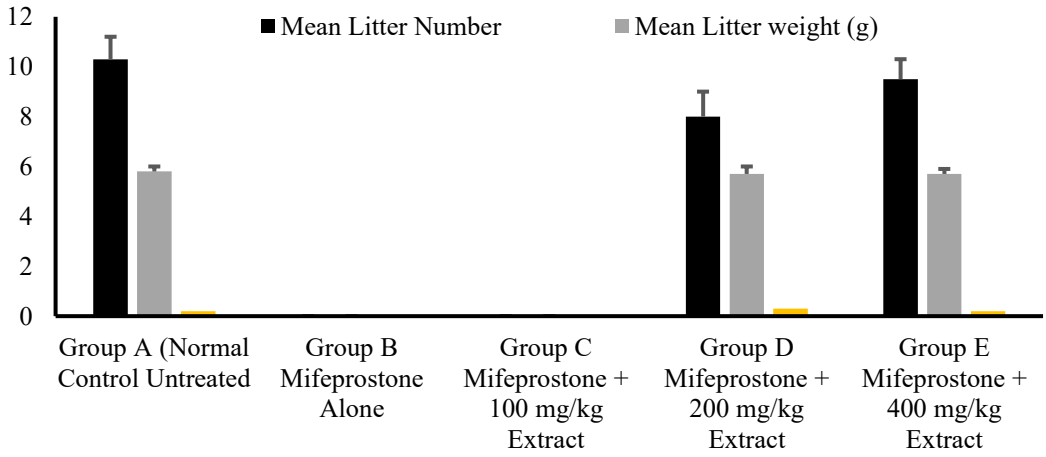


**Figure 6**: The effect of extract on mean litter number, and mean litter weights. n = 4.

**Discussion**

Miscarriage, defined as the loss of pregnancy before 20 weeks of gestation, occurs in approximately 10–25% pregnancies (Hristeva and Angelova, 2024), with vaginal bleeding often presenting as an early symptom of threatened miscarriage. This condition is a major source of anxiety for expectant mothers and contributes significantly to the financial burden of pregnancy care (Akpan *et al.,* 2022). Current treatment options for threatened miscarriage remain limited, with insufficient empirical evidence to support their effectiveness

(Akaneme, 2007). These limitations underscore the need for alternative therapeutic strategies, including the use of medicinal plants and herbs, which may offer viable options for pregnancy protection.

Our study demonstrated that ethanol extract of the stem bark of *Tamarindus indica* possesses promising potential in the management of threatened miscarriage. Specifically, the extract significantly reduced both the volume and duration of uterine bleeding in a dose-dependent manner. Furthermore, the 75% pregnancy success rate, the mean litter number, and the mean litter weight of the 400 mg/kg extract treated group compares positively with the results of control. These results strongly suggest the effectiveness of the extract of the stem bark of *Tamarindus indica*, in protecting threatened miscarriage, and ensure positive pregnancy outcome.

Mifepristone, also called RU-486, is a synthetic steroid that serves dual clinical purpose, in the management of Cushing syndrome associated hyperglycaemia, and in the termination of up to 10 weeks pregnancy (Autry and Wadhwa, 2024). In the induction of abortion as a model of threatened miscarriage, mifepristone binds with high affinity as an antagonist of progesterone receptors (PR-A and PR-B), and disrupts pregnancy by blocking progesterone activity, increasing uterine sensitivity to prostaglandins, promoting decidual breakdown, and facilitating cervical ripening and dilation (DrugBank, 2005; Autry and Wadhwa, 2024; Dr. Oracle, 2025). This action effectively prevents implantation, as progesterone is the primary hormone responsible for preparing the endometrium for implantation and sensitizing the body to the effects of prostaglandins by increasing their synthesis and decreasing their metabolism (Autry and Wadhwa, 2024). Although the molecular mechanisms underlying the protective effects of *Tamarindus indica* extract were not directly evaluated in this study, the ability of the extract to counteract mifepristone-induced bleeding and sustain pregnancy suggests possible antagonism of mifepristone's adverse effects. This may involve reducing uterine sensitivity to prostaglandins or potentiating progesterone activity, thereby supporting pregnancy maintenance.

## Conclusion

The findings of this study indicate that the, ethanol extract of the stem bark of *Tamarindus indica* has protective effects against mifepristone-induced miscarriage in female Wistar rats. The extract not only reduced uterine bleeding but also sustained pregnancy to term with favorable litter outcomes. These results suggest that *Tamarindus indica* possesses promising potential for use in the management of threatened miscarriage within ethnomedicine.

## Declaration on conflict of interest

The authors declare no conflict of interest.

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