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Antifertility activity of *Oroxylum indicum* Vent. stem bark on female Wistar rats

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Plant based traditional medicines are being used by the diversified populations of North-East India (NE India) for numerous human ailments and birth control since ancient times. Different ethnic communities of the Indian state Tripura have been traditionally using fresh stem bark of *Oroxylum indicum* (L.) Vent. for birth control. Thus, the aim of this research was to justify pharmacologically the traditional use of *Oroxylum indicum* stem bark for birth control. The *ex-vivo* uterotonic potential of four different extracts *viz*., ethyl acetate (EAOI), acetone (ACOI), methanolic (MEOI) and aqueous (AEOI) extracts (10 µg/50 µL) of *O. indicum* stem bark was carried out using uterine tissue and 4%, 16%, 53% and 89% uterine contraction, respectively was observed. Hence, 200 mg/kg/day dose of MEOI and AEOI were investigated on female rats for *in-vivo* abortifacient and anti-implantation activity and the level of different hormones released were estimated. In addition, acute-toxicity of the MEOI and AEOI were carried out on rats of either sex. The AEOI extract showed height potential for both aborticide (**p<0.01) and anti-implantation effect (**p<0.01) in compared to MEOI extract. It was noticed that there was a significant decline (**p<0.01) in gonadotropic releasing hormone (GnRH) level in anti-implantation model and major elevation (**p<0.01) in luteinizing hormone (LH) level of anti-implantation and abortifacient model in both standard and treatment group, where Ethinylestradiol (0.1 mg/kg/day, P.O.) used in standard group and the treatment group received AEOI. In acute toxicity studies, both the test samples of MEOI and AEOI have not exhibited any toxic effect up to 2000 mg/kg dose. Based on the pharmacological aspect, the present study justifies the traditional claim for *O. indicum* as an antifertility agent and identifies the potential of AEOI as an excellent and safe source of antifertility agent.

Keywords: Antifertility agent, Contraceptive, Medicinal plants, *Oroxylum indicum* (L.) Vent.

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The human population has been experiencing a massive surge especially the developing countries are at the receiving end and this population surge have created an acute pressure on natural, socio-economic as well as the cultural resources[1]. Fertility regulation of humans has emerged as a major concern for the developing countries, not only in the medical perspective but also on the social aspects as well. Nowadays most commercially available fertility controls agents are synthetic in nature, data suggest these agents can show a variety of unwanted health problem like hormonal imbalance, high blood pressure, augmented risk of cancer, diabetes and obesity[2].

Therefore, there is an overwhelming demand for a safer yet effective alternative and no other option can be better than herbal contraceptives. Herbal drugs have been associated with humans since the beginning of civilization and many plants have found their place as well-known fertility regulators in various records of folklore and traditional knowledge[3-6]. *Oroxylum indicum* Vent. has occupied its place as one such plant and therefore used in this study to evaluate its antifertility activity on albino rats. In our field survey[3], it has been observed that ethnic practitioners of *Darlong* community in Tripura used the plant *Oroxylum indicum* Vent. of Bignoniaceae family for birth control. This study has been planned to explore the mechanism of its fertility regulation. *Oroxylum indicum* has sword shaped

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large capsules, purple flowers and elliptic leaves while the bark is soft having light grayish brown coloration[7,8]. Apart from this the various other reported properties of *Oroxylum indicum* extracts are antimicrobial, antifungal, anti-inflammatory and anti-neoplastic properties[7,8]. A large variety of phytochemicals has been reported from this plant like oroxylin A, chyrysin, tannic acid, scutellarein-7-rutinoside, ursolic acid, aloe-emodin, baicalein (5, 6, 7-trihydroxy flavone) and its 6 and 7-glucuronides, scutellarein and its 7-glucuronides, anthraquinone, traces of alkaloid, sitosterol and galactose, biochanin-A, and ellagic acid[8-13].

Materials and Methods

Plant

The *Oroxylum indicum* Vent stem barks were collected from various parts of Tripura, NE India during the month of July 2011, which were authenticated by referring with Herbarium (AUS/2504) maintained in the Assam University. The stem bark after cleaning was dried under shade and crushed into bristly powder for further course of work.

Extraction

The powdered *Oroxylum indicum* (OI) stem bark was initially defatted with petroleum ether and subsequently successive extraction was done with ethyl acetate, acetone, methanol and water consecutively as solvents in the ratio of 1:4 (w/v) using Soxhlet extractor. All the respective extracts staring from pet-ether extracts (PEOI), ethyl acetate extracts (EAOI), acetone extract (ACOI), methanolic extracts (MEOI) to aqueous extract (AEOI), were concentrated using Buchi Rotavapor® (R-210) and dried in vacuum desiccators. All the extracts were stowed in fridge (8±2°C) after drying and further used for *in-vivo* and *in-vitro* studies.

Animals

In this study, Wistar albino rats (150 to 200 g) of male and female sex was used. All the animals were obtained from Regional Institute of Medical Sciences (RIMS), Imphal. One week before starting the study the animals were acclimatized under laboratory environments. The polypropylene cages used for housing all rats under precise temperature of 27°C±2°C and 12 h dark/light cycle with free access of food and water *ad libitum*. Daily cleaning of the cages was done to maintained sanitary environment and extreme comfort. An approval for handling of experimental animal was purchased from Institutional Animals Ethical Committee (IAEC), IBSD, Imphal (Approval No.-IBSD/IAEC/ Trainee/Ph.cology/9 dated May 18, 2012) before the commencement of the experimentations.

Ex-vivo assays for uterine contractile activity

In this study, a female rat (250-400 g) confirmed with metaoestrus stage of estrous phase was used. For induction, 0.1 mg/kg S.C of Stilbestrol/ Diethylstilbestrol (DES) (Sri Krishna Pharmaceuticals Limited, Mumbai India) was used and waited for 24 h. After sacrificing the animals through cervical dislocation, the two uterine horns were dissected as well as cleaned from adjoining tissues. One horn of uterus was mounted on *De Jalon's* Solution (DJS) and continuously aerated with an aerator. 32±2°C bath temperature was kept. The uterine piece was positioned under optimal resting tautness of 0.75 g and equilibrated for 45 min prior to starting the investigation. Throughout the equilibration period, the mounted tissue was washed in 10 min of time intervals with *De Jalon's* Solution[14]. An isometrical force transducer (KYMOGRAPH, INCO E8REC DRUM) was used to measure the contraction. Oxytocin (0.01 mL) was used as standard drug to induce an isosmotic contraction of the uterine smooth muscle. The effect of the extracts EAOI, ACOI, MEOI and AEOI (10 μg/50 μL) on the uterine muscle with oxytocin (0.01 mL) was recorded, where 10:1(v/v) was the extracts and *De Jalon's* Solution ratio was maintained and concentration- response curves (CRC) were made. The test samples were mixed in the organ bath and kept for 5 min prior to the contact of the uterus to oxytocin, maintained 60s contact time. The responses of the extracts with oxytocin were compared with responses of oxytocin only (reference standard drug)[3].

Acute toxicity (ALD_{50}) study

Because of the higher intrinsic activity in combination with oxytocin MEOI and AEOI was selected for further *in-vivo* experimentations. The fixed dose method (Organization for Economic Co-operation and Development: OCED Guideline no. 423 and annexure 2b) of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) was used to carry out acute oral toxicity test for MEOI and AEOI. Prior to the experiment three rats in each group were kept and their food was withdrawn for the night. A suspension of the extracts was prepared using pure drinking water and administered orally at a dose of 2000 mg/kg in all animals[15,16]. The mortality and abnormal behavior were recorded after administration of the test samples.

Abortifacient activity

In this study, female Wistar rats (150 to 200 g) of confirmed fertility were kept with fertile male animals in 3:1 ratio for whole night. The next morning inspected vaginal smear of the female rats for confirmation of copulation. The female animals with presence of cluster of spermatozoa in the vaginal smears were selected for the experiment and considered as pregnancy day one[17]. Animals were segregated into 4 different groups consisting of 6 in each.

On the 10th day of pregnancy, laparotomy was done under anesthesia and the number of implantation sites was recorded by examining uteri. In ovaries, the number of corpora lutea was also counted. Animals left in cages after suturing abdomen and examined for vaginal bleeding. The group I (normal control) animals were given 0.5 mL drinking water/day. Group II (reference standard) received Ethinyl estradiol (0.1 mg/Kg/day). Test group III & IV (test-1 & Test-2) were given MEOI and AEOI as suspension (200 mg/kg prepared in pure drinking water/day) correspondingly from day 10 to 18 of pregnancy. On 21st day, again laparotomy was done under anesthesia and inspected for number of litters and percentage of resorption[18]. Subsequently, blood was collected by cardiac puncture. Serum was separated by refrigerated centrifuge at 380Xg for 10 min. Collected serum stored in -20°C refrigerator. The serum was analyzed after one week for estradiol (E2) and Progesterone, gonadotropic releasing hormone (GnRH), luteinizing hormone (LH), by using Enzyme-linked Immunosorbent Assay (ELISA) commercial kit, which read in 96 micro plate reader (MULTISKAN SPECTRUM, Thermo Scientific) and Prostaglandin-$F_2\alpha$ ($PGF_2\alpha$) and Prostaglandin-E_2 (PGE_2) were analyzed by Enzyme Immuno Assay (EIA) using EIA commercial kits[3].

Anti-implantation activity

In this study, female Wistar rats (150 to 200 g) of confirmed fertility were kept with fertile male animals in 3:1 ratio for whole night. The next morning inspected vaginal smear of the female rats for confirmation of copulation. The female animals with presence of cluster of spermatozoa in the vaginal smears were selected for the experiment and considered as pregnancy day one[17]. Animals at day 1 of pregnancy were segregated into 4 different groups, consisting of 6 in each.

The group I (normal control) animals were given 0.5 mL drinking water/day. Group II (reference standard) administrated Ethinyl estradiol (0.1 mg/Kg/day). Test group III & IV (test-1 & Test-2) given MEOI and AEOI as suspension (200 mg/kg prepared in pure drinking water/day) correspondingly from day 1 to 7 of pregnancy[18]. On 10th day of pregnancy, the animals were sacrificed and uterine horns were examined for counting implants[19]. Subsequently, blood was collected by cardiac puncture. Serum was separated by refrigerated centrifuge at 380Xg for 10 min. Collected serum stored in -20°C refrigerator. The serum from each group were examined for the presence of Estradiol (E_2), Progesterone, GnRH and LH by the methods of Enzyme linked Immunosorbent Assay (ELISA) using ELISA commercial kits[3] and biochemical estimation done by kits (ERBA Mannheim, India).

Statistical analysis

Data were expressed as mean ± Standard Error Mean (SEM). Differences were considered significant at ***$p<0.001$, or **$p<0.01$ or *$p<0.05$ when compared test groups v/s control group. For statistical results, one-way analysis of variance (ANOVA) with Dunnett's test was performed using GraphPad InStat Version 3 (GraphPad Software).

Results

***Ex vivo* assays for uterine contractile activity**

All the test extracts (EAOI, ACOI, MEOI, and AEOI) have created tension (Fig. 1 & Fig. 2) in the uterine tissue and a synergetic effect was monitored on tautness of the tissue after adding oxytocin in organ bath. The extracts in combination with oxytocin attained higher intrinsic activity. Percentile agonistic activity of EAOI, ACOI, MEOI, and AEOI were calculated to be 4%, 16%, 53% and 89%, respectively (Table 1).

Acute toxicity study

Acute toxicity study of test extracts MEOI and AEOI did not show any abnormal symptom like respiratory discomfort, weight loss, dullness in eyes, anxiety, giddiness, diarrhea and any other alteration in the hair were observed in the animals under study primarily for 48 h and then 7 days continuous observation. Also, no mortality was observed at 2000 mg/kg body weight dose. Therefore > 2000 mg/kg body weight (BW) dose was considered as average lethal dose 50 (ALD_{50}), cut off the dose under Globally Harmonized Classification System 5 (safe dose) for Chemical Substances and Mixtures described in OECD guideline 423 (Annexure 2b and 3b). However, in the present study 1/10th of ALD_{50} cut off dose *i.e.,* 200 mg/kg body weight /days dose

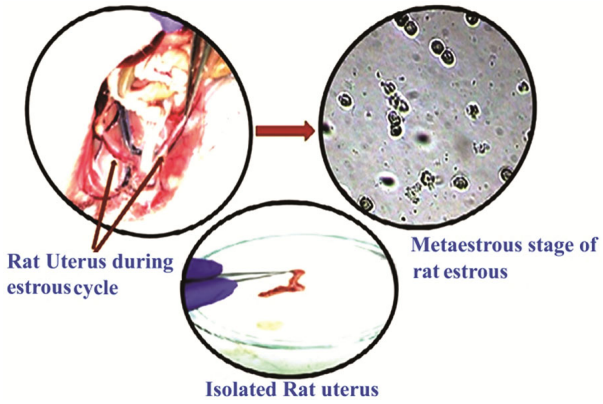

Fig. 1 — Metaestrous phase of rat uterus cycle and isolated rat uterus during estrous cycle observed in *ex -vivo* assays for uterine contractile activity

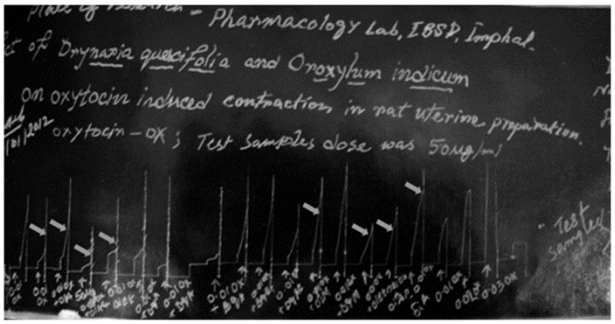

Fig. 2 — Kymograph showing synergistic activity of AEOI and MEOI on oxytocin induced rat uterine contraction observed in *Ex -vivo* assays for uterine contractile activity

Table 1 — *In vitro* uterine contractile activity

Sample	% Agonism
Oxytocin (10 µL)	-
Oxytocin (10 µL) + EAOI (10 µg/50 µL)	4
Oxytocin (10 µL) + ACOI (10 µg/50 µL)	16
Oxytocin (10 µL) + MEOI (10 µg/50 µL)	53
Oxytocin (10 µL) + AEOI (10 µg/50 µL)	89

was selected as therapeutic dose for *in vivo* experiments.

Abortifacient activity

The potential of orally administrated aqueous and methanolic extract of *Oroxylum indicum* on pregnant female rats are presented in Table 2. Both the experimental extracts are found to have abortifacient activity. Administration of 200 mg/kg AEOI, MEOI resulted in 90.99% and 79.67% resorption index respectively with 61.41% and 25.82% post implantation loss (Table 2). There was reduction in sharp decrease in number of live foetus in AEOI, MEOI treated groups (Table 2). Survival ratio of AEOI treated animals was 42.40% when compared

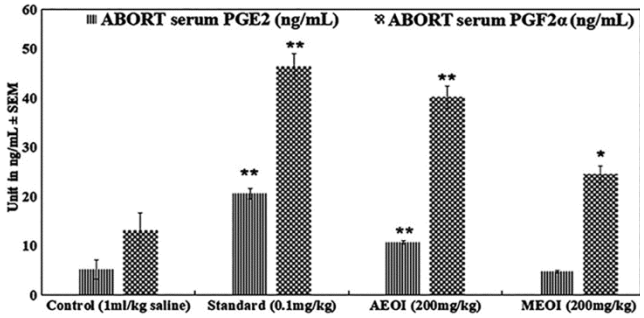

Fig. 3 — Effect of test samples (AEOI and MEOI) on Prostaglandin-$F_2\alpha$ ($PGF_2\alpha$) and Prostaglandin-E_2 (PGE_2) level estimated in abortifacient model. All data were expressed as Mean ± Standard Error Mean (SEM). Differences were considered significant at ***p<0.001, **p<0.01, *p<0.05, when compared test groups v/s normal (n=6)

with 100% in the normal animals (Table 2). Whereas, food and water consumption were unchanged in all group of animals. Live foetus count was reduced significantly (**p<0.01) in the AEOI treated animals whereas, the entire foetus was dead in the standard Ethinyl estradiol treated animals. There was substantial difference in implantation index amongst all the treatment (standard, AEOI and MEOI) groups compared to normal control group. Variances were recorded in the losses before and after implantation in all the treatment groups (Table 2).

In hormonal assay, there was significant increase (**p<0.01) in the $PGF_{2\alpha}$ level of all treated groups compare to normal animals. It was recorded that there was significant differences (**p<0.01) in the level of PGE_2 in AEOI treatment group when compared to normal animals (Fig. 3).

Anti-implantation activity

Animals under AEOI, MEOI and standard drug treatment have shown reduction in total number of implantations compared to normal animal group. Decline in survivability of fetus in AEOI treated animals was significant (versus control **p<0.01) than MEOI treated animals, whereas, 0% survival was recorded in animals under standard drug treatment (Table 3). On the contrary, there was significant reduction (**p<0.01) in uterus weight of AEOI treated group. Significant rise in liver weight (**p<0.01) was recorded from MEOI treated animals (Fig. 4 A & B).

Determination of hormones

Estradiol, Progesterone, LH and GnRH level in blood serum was estimated for both abortifacient and anti-implantation models. Significant decline (**p<0.01) in GnRH level was observed in anti-

Table 2 — Effect of test samples (AEOI and MEOI) on abortifacient activity

Parameters	Normal (1 mL distilled water/day)	Standard (Ethinyl estradiol 0.1 mg/Kg/day)	AEOI (200 mg/kg bw/day)	MEOI (200 mg/kg bw/day)
Maternal initial weight (g)	140.5±2.849	149.83±3.885*	153.16±1.014**	160.66±1.022**
Maternal final weight (g)	150.3±4.153	150.5±4.522	143.83±1.046**	176±1.461**
Number of live fetus	4.7±0.91	0±0**	1.2±0.40*	2.7±0.61
Number of dead fetus	0±0	2.17±0.70	2.83±0.65*	2.9±0.48
Survival ratio (%) {(Live/Dead)X100}	100	0	42.40	93.10
Number of rats that aborted	0	6/6	5/6	2/6
Percentage of rats that aborted (%)	0	100	83.33	33.33
Number of implantations	4.84±1. 39	2.21±3.43	3.11±1.39	3.64±1.39
Number of corpora lutea	5.76±1.31	5.27±1.54	5.97±2.23	5.68±3.97
Implantation index (%)	84.02	41.93	52.09	64.08
Resorption index (%)	0	98.19	90.99	79.67
Pre-implantation loss (%) {(No. of corpora lutea - No. of Implantation)/ No. of corpora lutea}X100	15.97	58.06	47.90	35.91
Post-implantation loss (%) {(No. of Implantation - Number of live fetus)/ No. of Implantation}X100	2.89	100	61.41	25.82

All data were expressed as Mean ± Standard Error Mean (SEM). Differences were considered significant at ***p<0.001, **p<0.01, *p<0.05, when compared test groups v/s normal (n=6)

Table 3 — Effect of test samples (AEOI and MEOI) on anti-implantation activity

Group	Body weight difference on 8th day	Weight of uterus (g)	Total no. of implants	Survivability of implants on Day 8th		% Survivability
				Live	Dead	
Normal (1 mL/kg saline)	17.67±4.09	1.10±0.13	4.33±0.5	4.33±0.49	0±0	100
Standard (0.1 mg/Kg)	5.0±1.69**	0.814±0.098	2.17±0.42**	0 ± 0**	2.17±0.48**	0
AEOI (200 mg/kg)	5.16±0.91**	0.63±0.08	3±0.25	0.7±0.33**	2.33±0.51**	22.33**
MEOI (200 mg/kg)	11.5±1.63	0.98±0.04	3.2±0.4	2.2±0.404	1±0.4	68.8

All data were expressed as Mean ± Standard Error Mean (SEM). Differences were considered significant at ***p<0.001, **p<0.01, *p<0.05, when compared test groups v/s normal (n=6)

implantation animal model under standard drug and AEOI treatment. Whereas, major elevation (**p<0.01) was recorded in LH level in the both anti-implantation and abortifacient models under standard and AEOI treatment. Sharp reduction in progesterone level was recorded in both the models under Standard, AEOI, MEOI treatment (Fig. 5 A- D).

Determination of serum biochemicals

Serum glutamic-oxaloacetic transaminase (SGOT), Serum glutamic pyruvic transaminase (SGPT), total bilirubin (BIT), cholesterol (CHO), triglyceride (TRI), alkaline phosphatase (ALP), urea and uric acid were estimated for anti-implantation activity. All the test groups have shown significant decrease in SGOT, SGPT, BIT, triglyceride levels, while ALP level was significantly higher (**p<0.01) in standard, AEOI and MEOI treated animals (Fig. 6 A- C).

Discussion

In this study the various parameters were evaluated which can serve as stepping stone to establish the potentiality and active mechanism of *Oroxylum indicum* (OI) as antifertility agent. The experiment was planned to evaluate the uterine contractile, abortifacient and anti-implantation activity of stem bark of OI. The acute toxicity studies confirmed safety profile of test extracts.

In *ex vivo* uterine contraction study all the extracts revealed their contractile property on isolated uterine

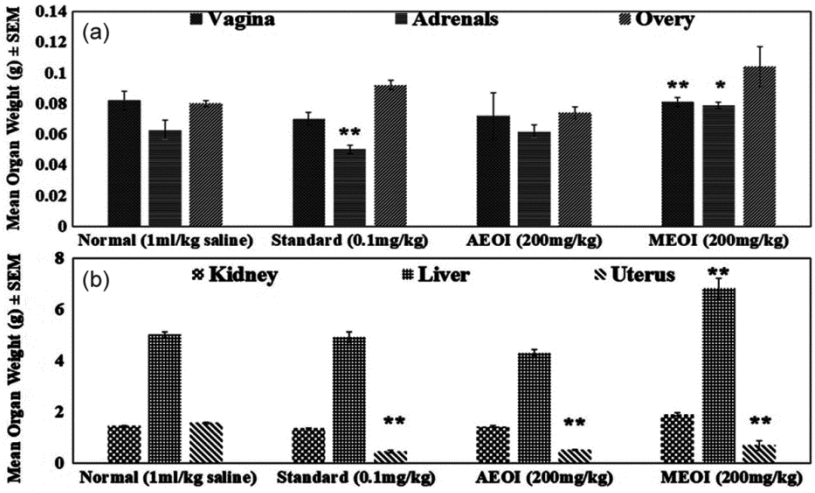

Fig. 4 — Effect of test samples (AEOI and MEOI) on different organ weight of the female rats (A. Vagina, Adrenals & Overy and B. Kidney, Liver & Uterus) estimated in anti-implantation model. All data were expressed as Mean ± Standard Error Mean (SEM). Differences were considered significant at ***p<0.001, **p<0.01, *p<0.05, when compared test groups v/s normal (n=6)

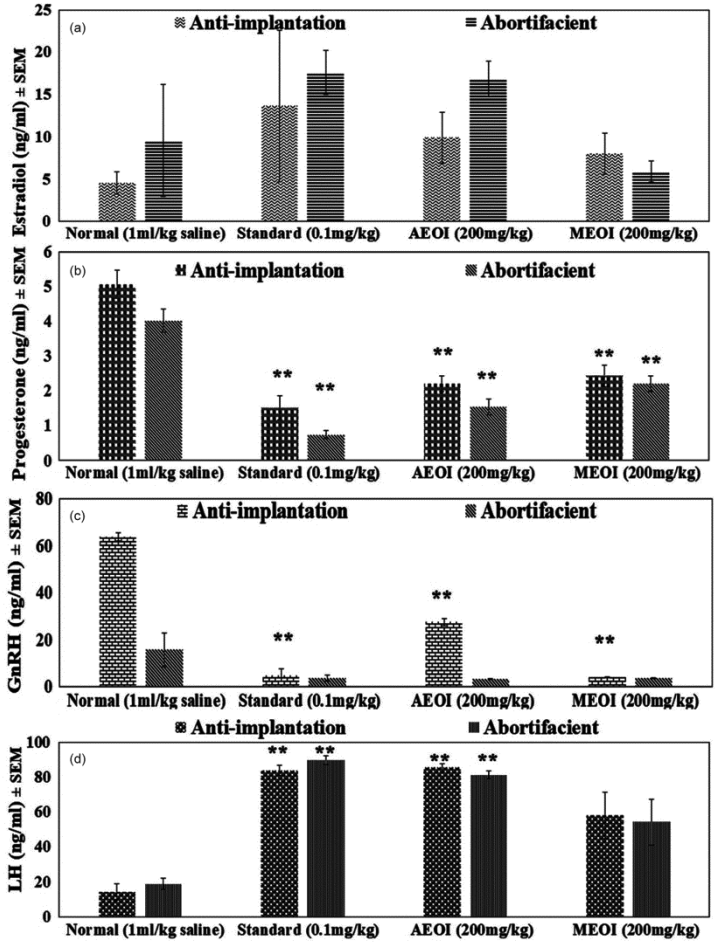

Fig. 5 — Effect of test samples (AEOI and MEOI) on different female hormones level [A. Estradiol (E2), B. Progesterone, C. Gonadotropic Releasing Hormone (GnRH), and D. Lutenizing Hormone (LH)] estimated in anti-implantation and abortifacient models. All data were expressed as Mean ± Standard Error Mean (SEM). Differences were considered significant at ***p<0.001, **p<0.01, *p<0.05, when compared test groups v/s normal (n=6)

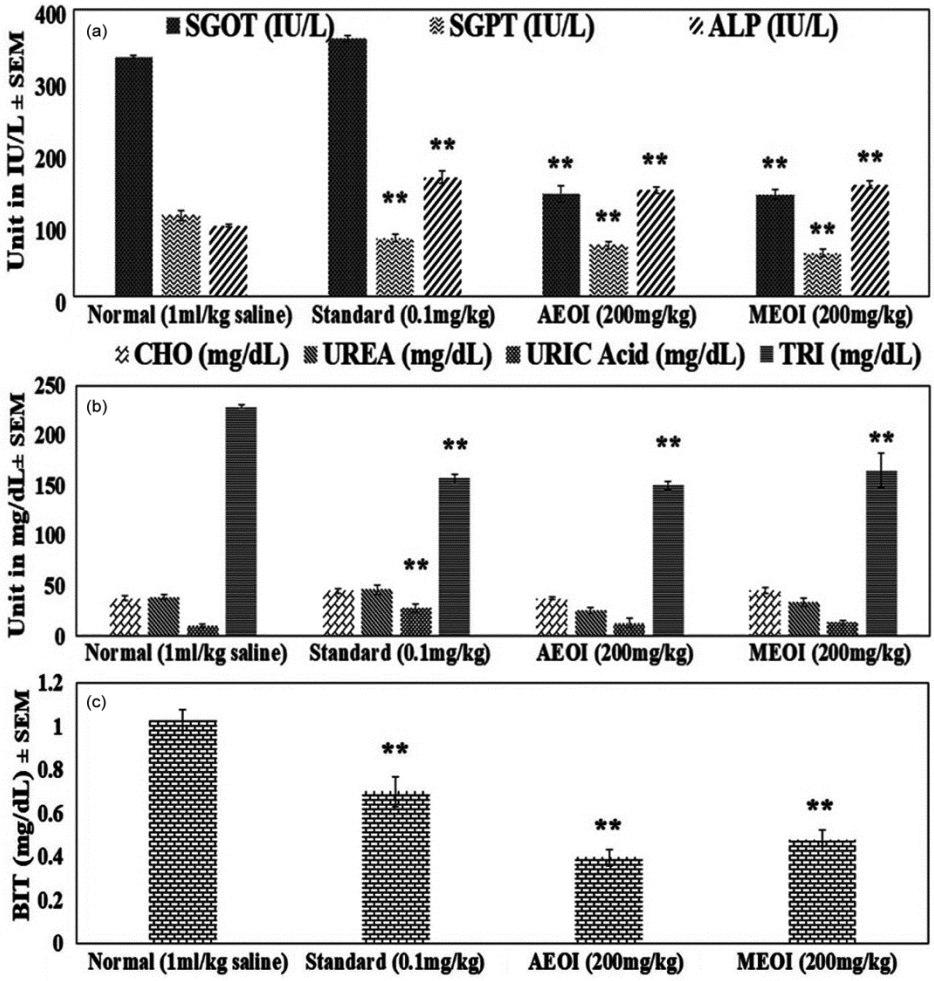

Fig. 6 — Effect of test samples (AEOI and MEOI) on different biochemical parameters of the female rats [A. Serum glutamic-oxaloacetic transaminase (SGOT), Serum glutamic pyruvic transaminase (SGPT), & Alkaline phosphatase (ALP); B. Cholesterol (CHO), Triglyceride (TRI), Urea & Uric acid and C. Total Bilirubin (BIT)] estimated in anti-implantation model. All data were expressed as Mean ± Standard Error Mean (SEM). Differences were considered significant at ***p<0.001, **p<0.01, *p<0.05, when compared test groups v/s normal (n=6)

muscle, having said so the findings of the study suggest that out of all the extracts AEOI and MEOI showed the higher contraction. Considering this fact, the further *in vivo* experiments were proceeded to study the antifertility efficacy of AEOI and MEOI.

A significant reduction in the number of live fetuses and percentage of implantation indexes has been observed on administration of the both the extracts, at the same it requires to noted that AEOI showed better results when compared to the MEOI. The results showed that in AEOI treated animals there was an increase in the percentage of embryonic losses after implantation and decline in fetal survival ratio, which indicated the abortifacient property of the extract. It is fact that post-implantation embryonic loss and decrease in fetal survival ratio are proportionate to abortifacient activity and these results are in concurrence with the earlier findings on abortifacient activity of *Alianthus excelsa*, *Spondius mombin*, *Alchornea cordifolia* etc[20-22].

In comparison to the normal animals, the extracts (AEOI, MEOI) treated animals have shown better anti-implantation activity. There were a decreased number of implantation sites as well as decline in the number of total implants in the extract treated groups, these results may be attributed to anti-zygotic and antiestrogenic property of the extracts or may be the result of interruption in endocrine-endometrial synchrony triggered by the extracts[23].

Embryo implantation depends on factors like ovum quality and endometrial receptivity. Several earlier studies are suggestive of the fact that plant extracts

triggered endometrial alterations leading to a non-receptive endometrium, making it unsuitable for implantion[17,24].

Estrogen plays a decisive role in blastocyst implantation; hence, failure of blastocyst implantation may be attributed to antiestrogens[25]. Administration of exogenous antiestrogens inhibits luteal function that can lead to pregnancy failure[26].

The role of steroid hormones is vital in the upkeep of pregnancy and growth of the fetus, in case of both humans as well as the non-human primates[27]. Progesterone maintains various homeostatic mechanisms related to preservation of gestation including suppression of maternal immunity preventing growth of fetus[27]. Estrogen plays a very important role in the upkeep of pregnancy increasing uteroplacental blood flow, thereby stimulating progesterone biosynthesis[28], whereas at the same time stress-induced implantation failure can be attributed to administration of minute doses of exogenous estradiol[29]. In our study, observed a sharp decrease in the level of progesterone in treated animals that might have caused cessation of fetus development[27], whereas increase in the level of estradiol suggests stress induced implantation failure. Golam *et al*.[23] also reported the blastotoxic effect and endocrine-endometrial dis-balance in *Pergularia daemia* treated animals, whereas, Gebrie *et al*.[30] demonstrated antizygotic and antiestrogenic property of *Rumex studeii*.

Pregnancy and fetal growth in primates not only require adequate steroid hormone concentrations but also an appropriate orchestration between maternal, placental, and fetal physiology.

According to many researchers' early pregnancy depends on a transitory period of steroid discharge by the corpus luteum, which is replaced entirely by placental steroid synthesis during mid-gestation period[31,32].

As the pregnancy advances fetal adrenal join with placenta to produce steroid hormones, hence any failure of pregnancy can be a direct consequence of interruption in steroid biosynthesis due to disruption in the functional communication flanked by placenta and fetal adrenal[27]. Our study supports the aforementioned works as on treatment, the synchrony of the steroid hormones including placental hormones were disrupted which suggests the failure of advanced pregnancy.

Premature ova expulsion from oviduct that resulted in hindrance of pregnancy has been observed on administration of compounds at low concentrations with estrogenic activity during early pregnancy[33,34]. In addition to this, reduction in number of implants may be due to the accelerated transportation of degenerated ova into uterus, which further results decreased fertility[33,35]. In the test group, a decrease in the number of implants has been observed which may due to the previously reported properties of the extract.

Antifertility activity shown by AOEI might have been possible due to its capacity to change the estrus cycle of the animal by affecting estrogen level[36].

Female reproductive hormones like progesterone, FSH, GnRH and LH in the body fluid ensure that the cycle is appropriate and creates a conducive environment for pregnancy, which includes follicular growth, ovulation, and corpus luteum formation and endometrial response including proliferative and secretory phase for implantation[37]. Therefore, measurement of these hormones helps in determination of ovulation and characterization of luteal phase defects. Deranged pregnancy in humans can be detected through quantitative determination of the hormone concentration in serum[37]. In our study, we have analyzed estradiol, progesterone, LH, GnRH and PGs etc levels in body fluids, since these can be easily co-related with the failure of pregnancy. Decline in the GnRH level in body fluid is a suggestion of disintegration in the reproductive cycle of the animals. Normal growth and functioning of the corpus luteum in most mammals require Luteinizing hormone (LH) from the anterior pituitary. Moreover, leuteolysis is associated with high serum LH concentration. In the present study, embryonic loss observed in the test group might be due to impaired luteal phase. Our study results have shown sharp increase in the levels of LH in test group animals in comparison to control group that could be the reason of pregnancy failure and this finding is supported by the report of *Jukic et al.*[38]. This result in particular suggests that OI might initiate abortion and anti-implantation by raising LH level[25,27,28].

The role of progesterone in maintaining implantation process throughout the pregnancy period is well documented[39]. Hence, drop in the progesterone level may have contributed to the abortifacient and anti-implantation activity of *Oroxylum indicum.* In the extract treated animals, endometrial disruption is observed which may be co-related to the decline of progesterone levels.

Reports of the various earlier studies are suggestive of the fact that decrease in progesterone synthesis is induced due to increase in the levels of PGF2α which further initiate luteolysis[40,41]. The role PGE2, PGF2α

in initiation of uterine contraction leading separation of fetal membrane from uterine wall is proven to be vital[42-45].

In our study, the level of PGE_2 and$PGF2\alpha$ were analyzed for both AEOI and MEOI extract treated animal groups and the results are indicating their abortifacient property.

The onset of parturition might have been accelerated due to the hormonal differences observed in the AEOI treated animals in comparison to control group. Our observations in present study was supported by the earlier reports[37,38,40].

Ex vivo uterotonic, contraction results by AEOI and MEOI showed synergistic effect with oxytocin thus indicating the role of extracts in abortifacient activity via acceleration of oxytocin function.

Thus, in this study we have revalidated the abortifacient and anti-implantation property of *O. indicum*.

Conclusion

This research investigation has established that the AEOI exhibited significant abortifacient and anti-implantation potentials that might be due to presence of phytochemicals in the *O. indicum* plant. The mechanism of abortifacient and anti-implantation activity of stem bark extract of *O. indicum* could be possibly due to altered implantation site and reproductive hormone levels and early uterine contractility leading to abortion. Thus, present study pharmacologically justified the traditional claim for the *O. indicum* stem barks as a fertility antidote. The future prospect of the work lies in discovery of the therapeutic efficacy of the plant as a valuable safe source of fertility regulating agent.

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Conflict of Interest

The authors have declared that there is no conflict of interest.

Author Contributions

BD has carried out Collection & extraction of test samples She also done *ex vivo* assay for uterine contractile activity, *in-vivo* acute toxicity, abortifacient activity, anti-implantation activity, hormonal assay, compiled research data, data analysis, prepared figures. MM has helped in hormonal assay, data compilation, analyzed data and also critically reviewed the manuscript. BG has helped in Hormonal assay, data compilation, data analysis and also critically reviewed the manuscript. AD helped for carrying out *in-vivo* antifertility studies. KN helped for carrying out spermicidal activities critically reviewed the manuscript. SD helped in ethnopharmacological survey, collection and drying of the samples at the collection site (Tripura). ADT helped in collection and extraction of test samples, mentored phytochemical experimentation. AC helped in extraction of test samples, mentored phytochemical experimentation and critically reviewed the manuscript. MDC conceptualized overall project, mentored overall project, and critically reviewed the manuscript. LD has designed overall antifertility works, supervised all *ex-vivo* and *in-vivo* experiments and wrote the manuscript.

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