



Antifertility activity of *Oroxylum indicum* (L.) Kurz: *In vitro* and *in vivo* study on human sperm and male wistar rats

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Oroxylum indicum (L.) Kurz, commonly called as Broken bones tree or Indian trumpet flower, belonging to Fam. Bignoniaceae, is traditionally used as a contraceptive by ethnic people of Tripura, North-East India. Here, we investigated the scientific basis for use of *O. indicum* as male antifertility agent by folklore healers. *In vitro* spermicidal activity of aqueous (AEOI) and methanolic (MEOI) extracts of *O. indicum* stem bark were studied on human sperm. The *in vivo* activity was experimented on male albino rats. The treated animals were allowed to mate and the pups delivered by female rat partners were counted. Phytochemical estimation of test samples was done using HPLC. The AEOI and MEOI treatments significantly decreased human sperm motility and viability. Test extracts have increased the hypo-osmotic swelling of sperm. Both the extracts were significantly declined the weight of reproductive organ. The MEOI treated rats have shown significant decrease in sperm motility and sperm counts. AEOI and MEOI treatment significantly reduced level of testosterone, but sharply raised dihydrotestosterone and prostaglandin in rats. Results testified the traditional claim for use of *O. indicum* as a male contraceptive agent, where MEOI have shown reversible action on male reproductive system leading to contraception without harming the libido.

Keywords: Baicalin, Broken bones tree, Contraceptive, Indian trumpet flower, Kampong, Oroxylin, Sperm motility, Testosterone

With a population of over 1.38 billion¹, birth control in India has been a subject of prime focus in the public health sector. While there is a plethora of contraceptives for women to choose from, the same is scarce for men. Considering the number of side effects associated with the use of female contraceptives, birth control measures for men is gaining widespread attention. However, in natural product research gossypol was unique breakthrough for male contraception, but hypokalaemia, non-reversible contraception and loss of male libido were major side effect treatise its clinical uses².

Numerous studies have been performed on animal models that suggest the potency of natural products in

reducing male fertility. *Oroxylum indicum* (L.) Kurz (Fam. Bignoniaceae), commonly called Broken bones tree, Indian trumpet flower or Kampong, is known to possess several activities such as antimicrobial, anti-inflammatory³, anticancer, antidiabetic and analgesic properties^{4,5}. It has also been traditionally used for contraception in women, though its effectiveness in controlling male fertility remains unexplored. One study by Chetry *et.al.*⁶ reported the inhibitory action of *O. indicum* towards testosterone induced prostate hyperplasia in rats. Taking clue from this, we designed the following study to investigate the effects of methanol and water extracts of the *Oroxylum indicum* stem bark for regulating the fertility parameters in male rats.

Methodology

Plant material and extraction

The stem barks of *Oroxylum indicum* were collected from Tripura, India in the month of July,

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2011 and authenticated (Voucher. AUS/ 2504) by Dr. Debojyoti Bhattacharya, Assam University Herbarium, Department of Life Science and Bioinformatics, Assam University, Silchar. The collected stem barks were then shade dried, pulverized and the powder (100g) obtained was soaked in petroleum ether to remove fatty and waxy materials. The remainder was then subject to successive Soxhlet extraction with methanol and water as solvents (1:4 w/v). Prepared methanolic and aqueous extracts were reduced in a vacuum evaporator (Buchi Rotavapor® R-210) at $40 \pm 1^\circ\text{C}$ and finally dehydrated in desiccators to get thick brown methanol (MEOI) and aqueous (AEOI) extracts. The extracts were dried in vacuum and the percentage yield of each was determined as 8.24% (MEOI) and 10.61% (AEOI), respectively using the formula: % yield = weight of the extract (mg)/weight of the whole tissue powder (mg) $\times 100$

Extracts were then stored in a refrigerator at $8 \pm 2^\circ\text{C}$ for use in the *in vitro* and *in vivo* studies⁷. The AEOI and MEOI were dissolved in normal saline (pH 7.4) to obtain various concentrations (10, 20, 40, 80 and 100 $\mu\text{g/mL}$) of the extracts, which was used to perform the *in vitro* studies.

Evaluation of spermicidal potential (*in vitro*)

Semen sample collection and bioassay protocol

The human sperm used for this bioassay according to the procedures of the institutional ethical committee of National Institute of Health and Family Welfare (NIHFW), New Delhi, India. Human ejaculates have been obtained from male partners of patients those were referred to Infertility clinic of Department of Reproductive Biomedicine (RBM), NIHFW, New Delhi, India. Human sperm samples have been obtained from the male subject those had sexually abstinence for 72 to 96 h. At least 30 min before used all samples were liquefied at 37°C . Samples those shown a sperm count of more than $50 \times 10^6 \text{ mL}^{-1}$ and motility more than 60% were included for further study. Routine semen investigation such as concentration, motility, and morphology of sperms was done as per the Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction⁸. The motile sperms were counted using fresh unstained semen sample until assesses 200 spermatozoa. Experiment was carried out in triplicate for improving counting accuracy, procedures. The semen sample of same donor's was aliquot equally for further *in vitro* experimentations.

Evaluation of sperm immobilization properties (*in vitro*)

Different concentrations of the extracts (AEOI and MEOI) were mixed thoroughly with human ejaculate (100-150 million spermatozoa/mL) in 1:1 (v/v) ratio as reported by Waller *et al.*⁹, 1980. A drop (10 μL) of each mixture was immediately taken on a glass slide and 5 fields were viewed under microscope with magnification of 400X for investigation of sperm motility. The semen sample mixed with test samples were incubated at 37°C for 30 min. The above said method was repeated for next set of experimentations.

Hypo-osmotic swelling test (HOS)

This test was done by using non-commercial patented (Indian Patent, 2991/DEL, Nov. 2009) HOS test kit⁹. The test was carried out in two sets: Control (sperm in normal saline) and Treatment groups. In treatment groups four different concentrations (10 $\mu\text{g/mL}$, 20 $\mu\text{g/mL}$, 50 $\mu\text{g/mL}$ and 100 $\mu\text{g/mL}$) of MEOI and AEOI were used. 5mL of sperm preparation was added to 50mL of HOS solution, mixed gently and incubated for 5 min at room temperature. 5mL of the stock solution was added to the two sets immediately after completion of the incubation time. Diversify sperm's tail coiling in random 100 sperm was indicator for swelling, that observed under a phase contrast microscope¹⁰.

In Vivo studies

Animals

In present study, wistar albino rats (150-200 g) and albino mice (20-25 g) of either used for *in vivo* studies. Animals were procured from the Regional Institute of Medical Sciences (RIMS), Imphal. The animals were housed in polypropylene cages; standard 12 h dark/light cycle and optimum temperature ($27^\circ\text{C} \pm 2^\circ\text{C}$) was maintained. The standard chack food was fed to all animals under study and water *ad libitum*. Ensured hygienic housing and optimum comfort for animals by cleaning litter in the cages on daily basis. Institutional Animals Ethical Committee (IAEC), IBSD, Imphal (approval No.-IBSD/IAEC/ Trainee/Ph.cology/9) approval was taken for handling the animals before beginning of the experimentations.

Determination of acute toxicity (ALD₅₀)

In the present study, AEOI and MEOI was assessed for acute toxicity on albino mice (as per approval of IAEC, IBSD, Imphal), kept under prescribed conditions. The animals were kept for fasting for 4 h before starting experiment. OCED Guideline

423- Acute Oral Toxicity- Acute Toxic Class Method was adopted for this study. The AEOI and MEOI extracts orally administered as a suspension (purified water used as a solvent at 20% w/v concentration). Behavioral abnormalities and mortality were recorded for 48 h after ingestion a single dose of 2000 mg/kg by the respective group of animals¹¹.

Evaluation of antifertility potential of AEOI and MEOI on male rats (*In vivo*)

Male antifertility potential of test products was evaluated as per WHO Protocol MB-50, 1983⁵ with innovative modifications. According to the protocol, male rats divided into the following three groups with 12 rats each after confirmed their fertility: Gr. I, Control group (1 mL normal saline); and Gr. II & III, Treatment group (200 mg/kg of AEOI and MEOI, respectively) p.o. for 14 days. About 50% animals from group I, II & III (n=6) were made as Recovery groups (animals selected randomly and kept to recover from the possible effect of the respective test samples after the withdrawal of treatment on 14th day of experiment/treatment for further 14 days under normal food and water).

On the 14th day of treatment, male rats from groups I, II & III (n=6) were introduced to female rats in a ratio of 1:2 (male: female) for a single night. Successful mating of male and female animal was assured in following morning by detecting spermatozoa in the vaginal smear using a microscope and vaginal plug of females. The impregnated female rats were separated and allowed to deliver at time; counted numbers of pups delivered by each female rat⁷. On the 15th day after confirmation of mating, the weight of the male rats was noted and 50% of animals (treatment groups, n-6) from the Gr. I, II and II were sacrificed. The accessory sex organs were weighed, caudal epididymal sperm was counted (growing spermatozoa with head & tail), motility and morphology were studied as per the reported method. The changes in morphology of animal sperms under treatment in *in vivo* system, also compared with the changes of human sperm morphology under treatment with same extracts in *in vitro* condition^{8,12}. The motile and immotile sperms in per unit area was calculated for determining percentage motility⁸. On 15th day of experiment, blood samples were collected from all sacrificed animals and allowed to coagulate. The serum was separated from coagulated blood by centrifugation at 2000 RPM for 10 min. The sera were aliquoted and

stored at -20°C until used for biochemical estimation (Day 1).

Male rats of the recovery groups (50% of animals from the group I, II and II, n=6) were also introduced to female rats in a ratio of 1:2 (male: female) for a single night on the 14th day of recovery (28th day of experiment) to study the reversable effect of male antifertility products under study. Successful mating of male and female animal was confirmed in next morning by observing spermatozoa in the vaginal smear using a microscope and vaginal plug of female rats. The impregnated female rats were separated and allowed to deliver at time; the number of pups delivered by each female rat was noted⁵. Again, on the 30th day of the experiment (Day 14 of recovery) the recovery group animals were sacrificed, serum was separated for biochemical estimation (Day 14).

Serum collected from Gr. I, II and III were analysed for Testosterone, Dihydrotestosterone (DHT) by the methods of Enzyme-linked Immunosorbent Assay and Prostaglandin F_{2α} (PGF_{2α}), Prostaglandin E₂ (PGE₂) by the method of Enzyme Immuno Assay (EIA) using commercial analytical rat kits (LSBio, USA) and estimated SGOT, SGPT, BIT, Triglyceride, Urea and Uric acid using commercial kit (Erba Mannheim, India).

Estimation of cholesterol in seminal plasma

Cauda epididymal fluid samples were obtained from a proximal and distal part of the cauda of animals according to the micro-puncture technique¹³. Each part was then flushed with 2 mL of normal saline. The samples were then centrifuged at 380 g for 10 min and the supernatants stored at -20°C. The total cholesterol content was estimated by using the technique of Eliasson, 1966 with slight modification¹⁴. In a test tube 2.5 mL of test reagent containing a mixture of 4.0 g of toluene-p-sulphonic acid, 60 mL of acetic anhydride and 40 mL of acetic acid, was gently mixed with 0.5 mL of concentrated sulphuric acid. Then the test tube was allowed to cool at room temperature (25±2°C). To that aliquot (0.1 mL) of seminal plasma was added, mixed well and the colour was allowed to develop in dark condition for 30 min. The green colour formed was then measured at 570 nm (Thermo Multiscan Spectrum) against water as a blank. A standard curve was made using standard cholesterol and total cholesterol content was calculated from that curve¹⁴.

Statistical Analysis

Data were expressed as mean \pm Standard Error Mean (SEM). Variances were considered significant at $***P < 0.001$, or $**P < 0.01$ or $*P < 0.05$ when compared test groups v/s control group. For numerical results, one-way analysis of variance (ANOVA) with Dunnett test (compare rest vs. control) and Student-Newman-Keuls multiple comparison test was performed using GraphPad InStat Version 3 (GraphPad Software Inc) and Origin Pro Ex software for statistical analysis.

Quantification of oroxylin-A and baicalin present in both MEOI and AEOI extracts. by HPLC method.

A validated HPLC method reported by Zheng & Dong¹⁵ was used with slight modification as per the conditions of NPC laboratory, IBSD, Imphal. The HPLC system - LC20AD and CBM-20A (Shimadzu Corporation, Kyoto, Japan) consisted of Diode array detector (SPD-M20A) and a reverse phase C18, (250 \times 4.6 mm, 5 mm particle sizes) Column (Mark, Germany) was used. Running conditions included: injection volume, 10 μ L; mobile phase, HPLC grade 70% methanol and 30% water. The flow rate was maintained 0.9 mL/min and elutes monitored at 272 nm in PDA detector. The methanolic extract (MEOI) and aqueous extract (AEOI) of *O. indicum* were dissolved in 100% HPLC grade methanol, vortex and filtered through an ultra-membrane filter (pore size 0.45 μ m; E-Merck, Darmstadt, Germany) prior to injecting in HPLC (stock solution 1mg/mL). The Standard compounds i.e. Oroxylin-A and Baicalin (Sigma) were dissolved in 100% HPLC grade methanol and filtered prior to injecting in HPLC (stock solution 1mg/ml) under similar conditions¹⁵.

Results

In vitro sperm immobilization activity

The *in vitro* sperm motility assay helped determine spermatozoal motility in the presence of test samples (AEOI and MEOI) at different time intervals. While both the test extracts caused significant ($**P < 0.01$) decrease in spermatozoal motility in a concentration-dependent manner (Fig. 1), MEOI showed higher activity amongst the two extracts.

Hypo-osmotic swelling test (HOS)

Hypo-osmotic swelling enhanced ($*P < 0.05$) in MEOI and AEOI treated groups dose-dependently (Fig. 2). The highest concentration (100 μ g/mL) of test samples used showed the most prominent changes in human sperm with substantial morphological

distortions in the form of swelled sperm head, knot formation in the tail, bent tail and headless tail, tail coiling as shown in Fig 3. The EC₅₀ values were estimated as 59.25 and 58.12 μ g for AEOI and MEOI, respectively.

Acute toxicity study (ALD₅₀)

Animals administered with the highest dose (2000 mg/kg body wt.) of the test extracts (AEOI and MEOI) did not exhibit any signs of mortality and abnormality in body weight. Therefore 2000 mg/kg

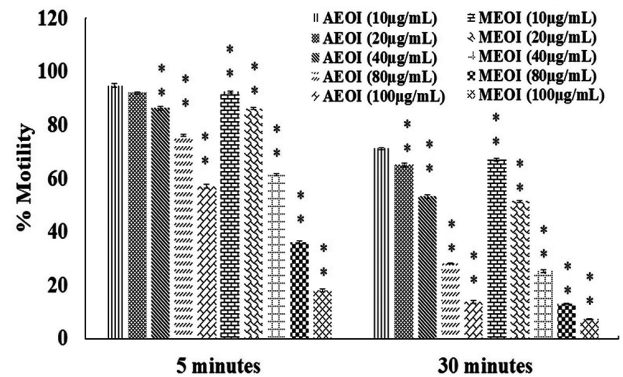


Fig. 1 — *In vitro* human sperm immobilization activity of methanol (MEOI) and aqueous (AEOI) extracts of *Oroxylum indicum* stem bark. [All data presented as Mean \pm Standard Error Mean (SEM). Variances were measured significant at $***P < 0.001$, $**P < 0.01$, $*P < 0.05$, where made comparison between test groups and Control (n=3)]

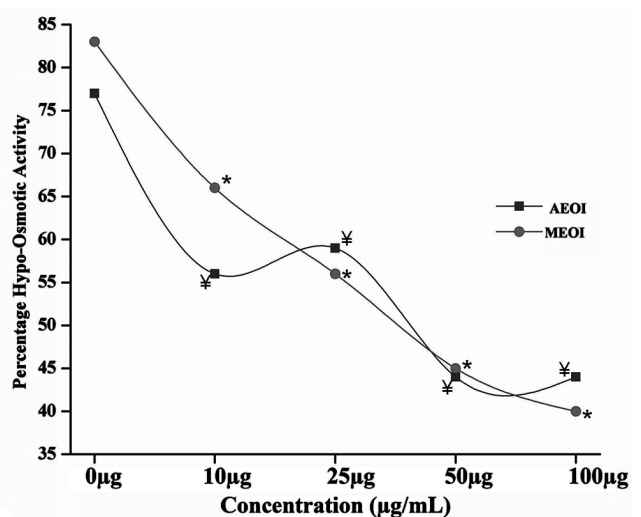


Fig. 2 — Hypo-osmotic swelling test (HOS) of methanol (MEOI) and aqueous (AEOI) extracts of *Oroxylum indicum* stem bark on human sperm. [All data presented as Mean \pm Standard Error Mean (SEM). Variances were measured significant at $***P < 0.001$, $**P < 0.01$, $*P < 0.05$, where made comparison between test groups and Control (n=3). \ddagger and * represent statistically significant variance (One-way ANOVA followed by Student-Newman-Keuls multiple comparison test) comparative to correspondence OM and OA control (0 μ L) group, respectively ($P < 0.05$)]

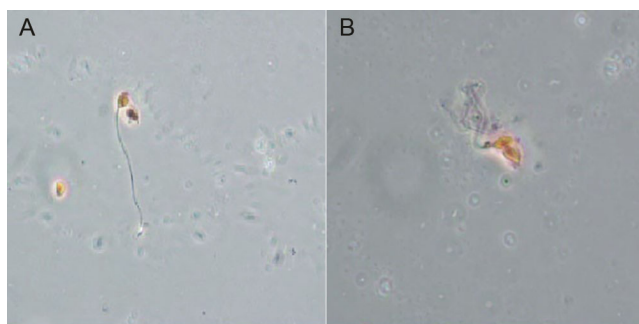


Fig. 3 — Treatment with methanol (MEOI) and aqueous (AEOI) extracts of *Oroxylum indicum* stem bark showing various human sperm morphological abnormalities in Hypo-osmotic swelling test (HOS). [Human sperm showed significant morphological distortions in the form of swelled sperm head, knot formation in the tail, bent tail and headless tail, tail coiling]

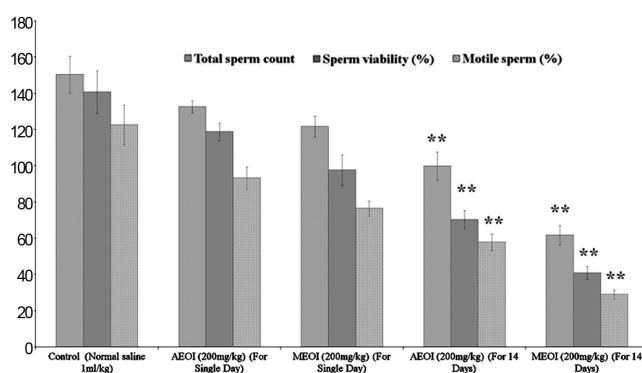


Fig. 4 — *In vivo* rat sperm immobilization activity of AEOI and MEOI extracts of *Oroxylum indicum* stem bark. [All data presented as Mean \pm Standard Error Mean (SEM). Variances were measured significant at *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, where made comparison between test groups and Control ($n=6$)]

dose was considered as average lethal dose 50 (ALD₅₀), whereby the cut off dose (safe dose), i.e., 1/10th of that dose (200 mg/kg) was selected for *in vivo* experiments.

Evaluation of antifertility potential of AEOI and MEOI on male rats (*In vivo*)

The results of *in vivo* anti-fertility test show no behavioural changes in the animals. Animals in the control group (Group I) showed normal sperm count, motility, and morphology, whereas animals treated with the test extracts for 14 days showed significant (** $P < 0.01$) decrease in sperm number, motility and viability in compares to the control (Fig. 4). The caudal epididymidal sperm parameters in the treated groups (AEOI and MEOI) showed evidence of spermatozoal toxicity. Further, these effects were more prominent in the MEOI treated animals when compared to the AEOI treated group. Several kinds of

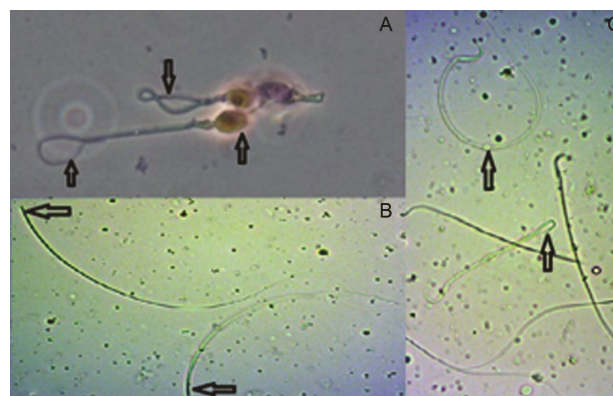


Fig. 5 — Treatment with methanol extract of *Oroxylum indicum* stem bark (MEOI) shows various rat sperm morphological abnormalities (*In vivo*). (A) represents human sperm where arrow pointing globose head, coiling of tail and bent tail; (B and C) represents rat sperm where arrow pointing headless sperm, coiling of tail and fusion of tail.

anomalous morphology of most of the sperms was observed including globular shape of head, tail coiling and tail fusion of two or more sperm (Fig. 5B & 5C), which was as similar as human sperm had abnormal morphology (Fig. 5A).

The no. of pups delivered through animals in the treatment group was 0.83 ± 0.307 (AEOI, 200 mg/kg) and 0.0 ± 0.0 (MEOI, 200 mg/kg), whereas no. of pups delivered through animals in the recovery phase was 2.33 ± 0.494 (AEOI, 200 mg/kg) and 2.00 ± 0.4 (MEOI, 200 mg/kg). This shows significant recovery of fertility after 2 weeks implying a reversible contraceptive effect (Fig. 6A). But, significant difference in body weight was not observed after treatment (Fig. 6B).

All the animals treated with the extracts had shown significant (** $P < 0.01$) reduction in the weights of accessory sex organs, including vas deferens, testis, prostate, and epididymis in comparison to the control animals (Fig 6C).

A significant (** $P < 0.01$) reduction in serum testosterone level have recorded in both the treatment group of animals in comparison to the control animals. Also, a significant (** $P < 0.01$) upregulation in serum epididymal prostaglandin (PGE₂ and PGF_{2 α}) levels were observed in all treated groups in comparison to the control group of animals but no significant variation was observed in serum dihydrotestosterone level (Fig 7A). Treatment with test extracts significantly reduced the cholesterol content in seminal plasma of all the treated groups. MEOI showed the highest activity among

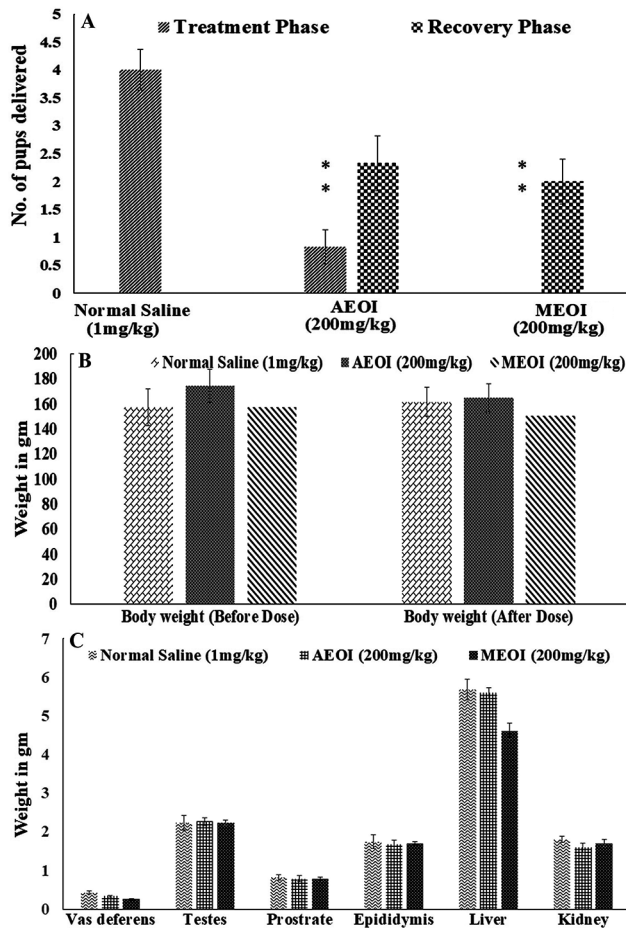


Fig. 6 — Effect of methanol (MEOI) and aqueous (AEOI) extracts of *Oroxyllum indicum* stem bark on (A) Anti-fertility test of male rats; (B) body weight; and (C) organ weight in *in vivo* model. [All data presented as Mean ± Standard Error Mean (SEM). Variances were measured significant at *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, where made comparison between test groups and Control (n=6)]

all groups in reducing the cholesterol content (80.72%) (Fig 7B).

Quantification of bioactive compounds in both MEOI and AEOI extracts by HPLC method.

We estimated the contents of bioactive compounds oroxylin-A and baicalin in the aqueous (AEOI) and methanol (MEOI) extracts of the *Oroxyllum indicum* stem bark using HPLC method. The contents of the compounds were estimated with respect to a standard curve of standard commercially available oroxylin-A and baicalin. The oroxylin-A content was estimated as 8.59% in MEOI and 7.144% in AEOI. The baicalin content was estimated as 4.46% in MEOI, and 12.1% in AEOI (Fig. 8 and Table 1 & 2).

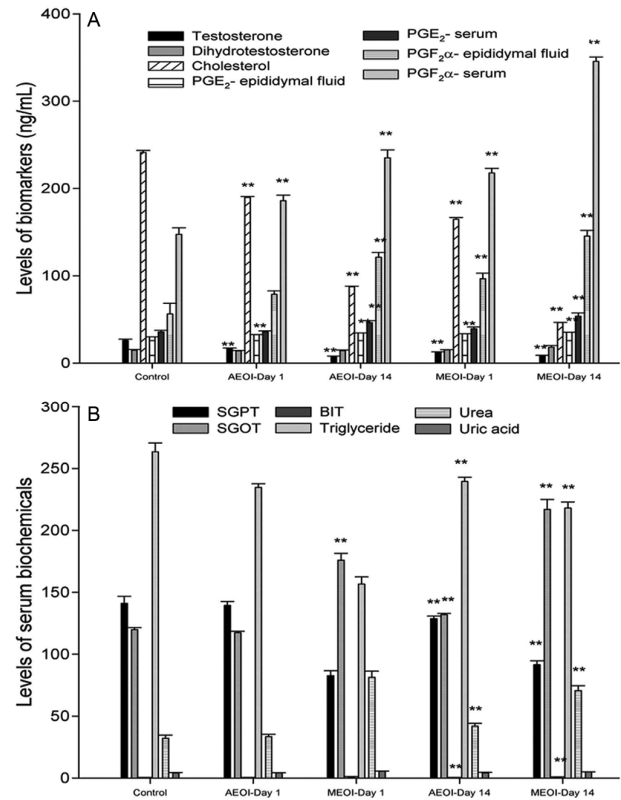


Fig. 7 — Effect of methanol (MEOI) and aqueous (AEOI) extracts of *Oroxyllum indicum* stem bark on (A) potential biomarkers; and (B) serum biochemicals of rats. [All data presented as Mean ± Standard Error Mean (SEM). Variances were measured significant at *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, where made comparison between test groups and Control (n=6)]

Discussion

In present study, all observation suggested that the methanol and aqueous extracts of *Oroxyllum indicum* stem bark has a sterile effect on the male rat reproduction system, which was passable for induction of rescindable sterility in male rats. Spermatozoa treated with the extracts (AEOI and MEOI) showed alterations in morphology and motility which was reflected that in *in vivo* studies, fertility rate of the animals those underwent 14 days treatment were significantly reduced.

The maintenance of normal sperm morphology and motility are the key aspects towards optimal male fertility. In-vitro studies using human male ejaculate revealed a reduction in spermatozoal motility along with morphological distortions in the samples treated with the extracts. These changes occurred in a dose-dependently with the methanolic extract showing higher potency than the aqueous.

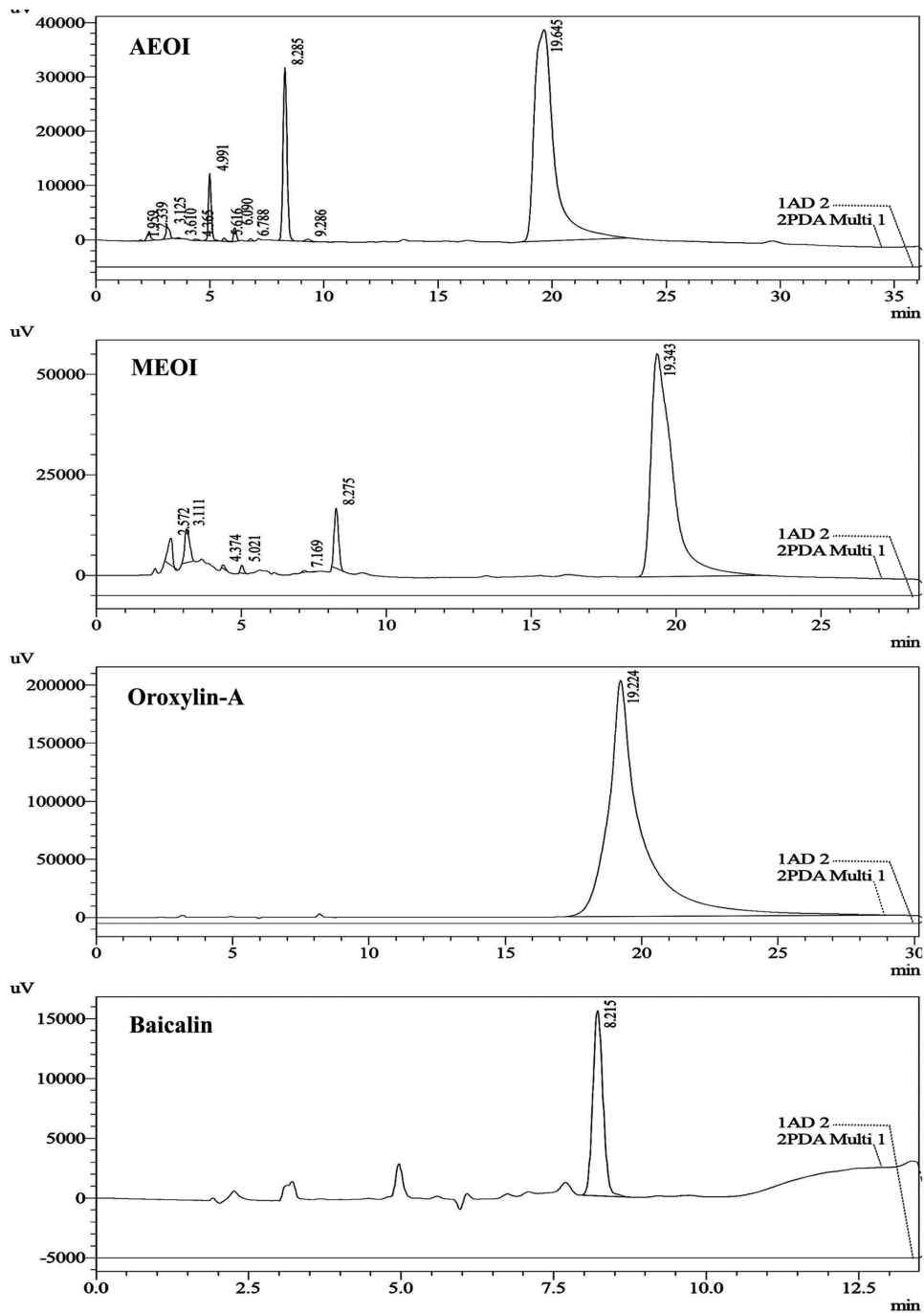


Fig. 8 — HPLC chromatogram of methanol (MEOI) and aqueous (AEOI) extracts of *Oroxyllum indicum* stem bark, Standard compounds Oroxylin-A (Sigma, USA) and Baicalin (Sigma, USA).

Table 1 — HPLC profile of Oroxylin-A (Retention Time-19.358) and Baicalin (Retention Time-8.285)

Concentration (µg)		Area	
Oroxylin-A	Baicalin	Oroxylin-A	Baicalin
0.0125	0.0125	3649646	59753
0.0250	0.0250	8082041	93225
0.0500	0.0500	17169355	179917
0.1000	0.1000	32090898	319935

The body weight was not changed significantly, weight of other vital organs and behaviour of the animals upon oral treatment with the extracts, which demonstrates the general well-being of the animals, but the weight of the testis along with other accessory organs such as the vas deferens, prostate gland and epididymis were significantly decreased in

Table 2 — Quantification of Oroxylin-A and Baicalin in methanol (MEOI) and Aqueous (AEOI) extracts of *Oroxylum indicum* stem barks

Bioactive compound	Retention time		Area		Concentration of bioactive compound in 10 µg sample (10 µL)		Content (%)	
	MEOI	AEOI	MEOI	AEOI	MEOI	AEOI	MEOI	AEOI
Oroxylin-A	19.343	19.645	2837803	2368235	0.859 µg	0.7144 µg	8.59	7.144
Baicalin	8.275	8.285	156422	382621	0.446 µg	1.21 µg	4.46	12.10

comparison to the control animals after 14 days of treatment. The epididymal sperm count, motility was decreased and morphological abnormalities such as globular shape of head, tail coiling and tail fusion of two or more sperm was also seen. Decrease in the weights of the sex organs may be attributed to the reduced levels of testosterone as observed in the treatment group animals as it is already known that the various sex organs are androgen dependent for the maintenance of structure and function. Any change in the level of this hormone could also influence sperm motility and viability within the epididymis thereby affecting the overall reproductive function. However, the serum levels of dihydrotestosterone remained unaltered which is associated with the maintenance of male libido¹⁶.

Along with androgens, prostaglandins (PGs) are also involved in male reproduction. Higher concentration of PGs has been observed in the seminal fluid¹⁷ that stimulate influx of calcium through a receptor associated mechanism which facilitates the acrosome reaction. Prostaglandin E₂ (PGE₂) has been reported to stimulate sperm motility while Prostaglandin F_{2α} (PGF_{2α}) inhibits it^{18,19}. In this work, both the test products shown an increase level of PGE₂ and PGF_{2α}, however the effect was more prominent with the methanol extract. It is interesting to note the elevation in the serum PGE₂ and PGF_{2α} levels. It is plausible that the increase in PGF_{2α} could be responsible for the sterile effect of the rats during the treatment period since it is inversely correlated with sperm motility^{20,21} while increased PGE₂ could be responsible for facilitating the reversible action of *Oroxylum indicum* extract which corroborates with the findings in this study in which the male fertility was restored following two weeks of extract withdrawal (Fig 7). The reversible effect was witnessed through an increase in the pup numbers delivered by female rats inseminated by male rats of the recovery group in comparison to the pup number delivered by female rats impregnated by males of the treatment groups.

Cholesterol is an important component that acts as a precursor in the synthesis of androgens, therefore

reduced levels would indicate an inadequate level of androgen synthesis in the testis. Our study showed that animals treated with the test extracts had significantly reduced cholesterol content in seminal plasma in which the MEOI showed higher activity.

Conclusion

Both the aqueous (AEOI) and methanol (MEOI) extracts of the stem bark of *Oroxylum indicum* significantly (***P* < 0.01) decreased the weight reproductive organ in male rats. The MEOI treated rats showed significant (***P* < 0.01) decrease in sperm motility and sperm counts. AEOI, MEOI treatment significantly (***P* < 0.01) reduced level of testosterone, but sharply raised dihydrotestosterone and prostaglandin in rats. The results support the traditional claim for use of *O. indicum* as a male contraceptive agent, where MEOI have shown reversible action on male reproductive system leading to contraception without destructively effecting the libido. Reversal of fertility was also seen upon withdrawal of the treatment indicating reversible contraception. However, further investigations are essential to established the mechanism of action and to identify the specific roles of individual components found in the extracts those are responsible for imparting the anti-fertility activity.

Conflict of interest

Authors declare no competing interests.

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