

Preclinical Studies of Exher, a Polyherbal Preparation for Uterine Tonic Activity

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ABSTRACT : Exher was evaluated for its oestrogenic effect using In vivo and In vitro experimental models, It's a polyherbal formulation indicated as uterine tonic. Oestrogenic effect of Exher-1 (250mg/kg p.o.) & Exher-2 (500mg/kg p.o.) was studied in normal and ovariectomized rats. Exher-1 & Exher-2 were administered as an aqueous suspension for a period of 21 days. The parameters studied in in vivo models include uterine weight (wet and dry weight), oestrogen and progesterone levels. The effect of Exher-1 (250mg/kg p.o. for 21 days) & Exher-2 (500mg/kg p.o. for 21 days) was also studied on normal rats for regular oestrus cycle. In-vitro studies with Exher (50-400 µg/ml of aqueous extract) on isolated uterus in non-gravid, non-oestrinised rats were carried out to find out whether the formulation possesses any oxytocin like activity. Administration of Exher-1 in normal rats significantly increased the dry uterine weight but not the wet uterine weight. It also resulted in marked increase of oestrogen levels with no change in progesterone levels as compared to control. Administration of Exher-2 in normal rats has failed to significantly increase the dry and wet uterine weight. Exher-1 & Exher-2 treatment in ovariectomized rats did not show any increase in uterine weight and oestrogen concentration. The rats from both control and treated group showed normal oestrus cycle. Aqueous extract of Exher had no significant effect on the in-vitro acetylcholine-induced contraction and uterine motility. Exher possess oestrogenic activity only in the presence of functional ovary and is devoid of any progestational activity.

KEYWORDS : Exher, uterine tonic, ovariectomized, oestrogen, progesterone, uterus, oxytocin.

I. INTRODUCTION:

Ayurveda, an ancient system of Indian medicine has mentioned several herbs that are useful in the treatment of various uterine disorders. Many plant drugs have been proved for their beneficial effects in treating Uterine fibroids, Irregular menses¹, Abnormal uterine bleeding¹, Premenstrual syndrome and Polycystic ovarian diseases. Exher, is a poly herbal formulation consisting of various useful herbs and bhasma which have shown beneficial effects in the treatment of various uterine disorders. Abnormal uterine bleeding is a commonly encountered disorder in women's reproductive stage² and approximately one fifth of the female patients visiting a general gynecologist with this problem are elderly women who are on the verge of post menopause, constitute about 50% and adolescents constitutes one fifth of all female patients³. Studies on dysmenorrhea shows 7.2% in the first year and 26% five years later having painful menstruation problem. In the adolescent girls aged 10 to 20 the frequency of dysmenorrhea has increased from 36% to 56% between 13-14 years and 17-20 years and also approximately 88% had premenstrual tension and almost 45% had menstrual pain in adult women⁴. Thus the treatment of menstrual distress during adolescence is of great importance. Wide spectrum of drugs are available for the treatment of dysmenorrhea and premenstrual syndrome, but majority of them are hormones that are known to possess untoward side effects⁵. Exher is a non-hormonal polyherbal preparation formulated by Srushti Herbal Pharma, Bangalore with different aqueous plant extracts, which are useful in various menstrual disorders such as puberty menorrhagia, dysmenorrhoea, premenstrual syndrome, irregular periods, abnormal bleeding and threatened abortion. It includes Saraca ashoka, Aloe vera, Tribulus terrestris, Hemidesmus indicus, Trigonella foenum-graecum, Symplocos racemosa, Cyperus rotundus, and Asparagus racemosa as its main constituents.

Saraca indica is well known in ayurvedic medicine for its use as a stimulant to the endometrium and ovarian tissue. It is also largely used as a remedy in various ailments of the uterus especially as a uterine haemostatic in menorrhagia, uterine fibroids etc⁶. Aloes compound from Aloe Vera was found to be beneficial in cases of functional sterility and disturbed menstrual function. The saponin glycoside obtained from Asparagus racemosus exhibited anti-oxytocic activity. Symplocos racemosa has been reported to be useful in treating uterine disorders⁷. The present study is undertaken to evaluate the uterine tonic activity of these herbs and minerals in combination as a formulation for uterine disorders.

Development of drugs for uterine tonic activity is most helpful for the treatment of uterine disorders. These drugs not only cure the uterine disorders but they also improve the living condition of millions of women by decreasing the side effects. The goal of research is for curing uterine disorders through herbal formulation is to free the patients from severe side effects from HRT, synthetic drugs and to obtain economical treatment. As the herbal formulation can be safely used for a long periods, they may be better suited for uterine disorders. The present study demonstrates the role of Exher in prevention and curing of Abnormal uterine bleeding, Uterine fibroids, Infertility, Abortion, and Oligomenorrhea. This has a potential implication in further development of herbs and mineral formulations as a cure for uterine disorders.

II. MATERIALS AND METHODS:

Animals: Rats are excellent subjects for uterine studies because Oestrogen dependent action on both rats and humans are similar but much more quickly in rats than in humans. Wistar rats of female sex were used for the study. The animals were procured from the Drug Testing Laboratory (DTL), Bangalore. Animal house was well maintained under standard hygienic conditions, at a temperature ($22\pm 1^\circ\text{C}$), room humidity ($60\%\pm 10\%$) with 12hr day and night cycle. The rats were provided with commercial food pellets and purified water ad libitum. Cleaning and sanitation work are done on alternate days. Paddy husk was provided as bedding material, which was changed every day. The cages were maintained clean.

Drugs: Srushti Herbal Pharma, Bangalore, provided aqueous extract of test drug Exher, a polyherbal formulation. Anesthetics Ketamine (Anket) and Diazepam (Valium) were purchased from pharmacy store. Dose of test drug was extrapolated from the human dose. Dose of Oestradiol for inducing uterotrophic effect was adopted from previous study⁷. Dose of anesthetics were chosen from "Experimental And Surgical Techniques In Rat" and adjusted suitably.

Composition of Exher:

Ingredients	Botanical name	Quantity
Kumari	<i>Aloe vera</i>	80mg
Shankapushpi	<i>Convolvulus microphyllus</i>	80mg
Ashoka	<i>Saraka ashoka</i>	120mg
Gokshura	<i>Tribulus terrestris</i>	100mg
Sariva	<i>Hemidesmus indicus</i>	80mg
Methi	<i>Trigonella foenum-graecum</i>	40mg
Lodhra	<i>Symplocos racemosa</i>	80mg
Musta	<i>Cyperus rotundus</i>	80mg
Shatavari	<i>Asparagus racemosa</i>	40mg
Kaseesa Bhasma	-----	40mg
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Acute oral toxicity study: The acute oral toxicity study was performed according to the OECD (Organization for economic co-operation and development) guidelines 425. Female albino rats of wistar strain (170-200g) were maintained under controlled standard animal house conditions with access to food and water ad libitum. The rats were acclimatized for 5 days and then fasted overnight, food but not water was withheld. Animals were weighed then limit and main test were performed. The limit test was carried out first at 5000mg/kg body weight for one animal and if animal died, main test was performed, if the animal survived two more animals were dosed, if both survive the test was terminated⁸.

Uterus weight in normal rats for uterotrophic effect: The Oestrogen and oestrogen analogues are known to act as uterotrophic by increasing the uterus weight. The Exher is known to possess oestrogenic effect. The isolated uterus is analyzed for dry and wet uterus weight⁹. The increase in uterus weight indicates the oestrogenic effect of Exher. Young female virgin Wistar rats weighing 170-200g were fed with standard rat feed and purified water ad libitum. Eighteen rats weighing between 170-200 gm were randomized into three groups of six animals in each group. The rats of group I received the vehicle (0.5% CMC in water) once a day orally daily at a dose of 10 ml/kg body weight for 21 days and served as control. The rats of group II and group III received 250mg/kg & 500mg/kg body weight respectively, Exher formulation as an aqueous suspension once a day orally daily at the same duration. After 21 days of treatment all the animals were weighed and sacrificed with overdose of ether anesthesia. The uteri were dissected, blotted, and weighed to obtain the wet and dry uterine (dried at 70°C for 24 hr) weights. Blood was collected and serum separated for estimation of oestrogen and progesterone through Radio Immuno assays. The uterine weights of all the groups were expressed in milligrams per 100gm of body weight and the response was calculated with relation to the vehicle⁹.

Uterus Weight In Ovariectomized Rats: In this model the Exher is evaluated for oestrogenic activity in the absence of functional ovary. The ovaries are responsible for producing endogenous oestrogen hormone. So the Exher is analyzed for its action as oestrogen derivative or analogue by estimating serum concentration of oestrogen and progesterone and for oestrogenic activity by dry and wet uterus weight. Young female virgin Wistar rats weighting 170-200g were fed with standard rat feed and purified water ad libitum. Each animal was anesthetized with Ketamine (60mg/ kg body weight) and diazepam (5mg/ kg body weight). Bilaterally Ovariectomy was performed for all the groups except for Sham group. Animals were randomized into 5 groups of 8 animals each and housed in pairs. Standard group was treated with beta Oestradiol 0.1 µg/animal, test groups were treated with Exher-1 (250mg/ Kg b. w). and Exher-2 (500mg/ Kg b.w.) while normal and control group-received vehicle only. After 21 days of treatment all the animals were weighed and sacrificed with overdose of ether anesthesia. The uteri were dissected, blotted, and weighed to obtain the wet and dry uterine (dried at 70°C for 24 hr) weights. Blood was collected and serum separated for estimation of oestrogen and progesterone through Radio Immuno assays. The uterine weights of all the groups were expressed in milligrams per 100gm of body weight and the response was calculated with relation to the vehicle⁹.

Vaginal Cornification in Rats: This is an early bioassay for oestrogenic activity based on epithelial proliferation. Vaginal cornification is the process of formation of cornified epithelial cells mediated by the hormone oestrogen, which can be the index of oestrogenic activity. The release of oestrogen hormone stimulates the production of cornified epithelial cells¹⁰. Young female virgin Wistar rats weighting 170-200g were fed with standard rat feed and purified water ad libitum. Eighteen rats weighing between 175-200 gm were randomized into three groups of six in each. Rats of group I, group II and group III received vehicle, 250mg/kg, and 500mg/kg body weight of Exher once a day orally daily for 21 days. Vaginal smear of both control and treated groups was examined microscopically daily between 10 - 11 a.m. to study the effect of Exher on the normal oestrus cycle. The vaginal smears are transferred to a glass slide they are evaluated microscopically according to the following scores: 0-dioestrus smear, mainly leukocytes, few epithelia cells. 1-mixture of leukocytes and epithelial cells, 2-proestrus smear, nucleated or nucleated plus cornified cells, 3-oestrus smear, cornified cells only.

Only animals showing score 2 or 3 are considered to be positive. The number of positive animals in each dosage group is recorded⁹.

Isolated Rat Uterus: In-vitro studies on non-gravid, non-oestrinized rat uterus is carried out to analyze oxytocin-like activity. The non-contractile response, decreased motility and anti-spasmodic activity of the formulation on in-vitro exposure of rats uterus indicates that this preparation can be useful in conditions associated with hypermotility of the uterus i.e. threatened abortion and dysmenorrhea. On day of the experiment, the degree of cornification was assessed by vaginal smear examination and rat found in oestrus cycle was employed for the experiment. Rats were sacrificed by cervical dislocation and two horns of the uterus were dissected out and separated. One horn of the uterus was mounted in a bath containing De Jalon's solution (NaCl - 9.0, KCl - 0.42, CaCl₂ - 0.06, NaHCO₃ - 0.5 and Glucose - 0.5 gm/L) at 37°C under 0.5 gm resting tension. The tissue was allowed to equilibrate for 20 minutes. Ten microgram concentration of oxytocin was added to the bath and the contractile response of uterus was recorded using a kymograph. The bath solution was drained completely and washed two to three times and filled with fresh solution. The response to the uterine tonic was also recorded using varied concentrations ranging from 50 - 400 µg of the aqueous extract. The effect of Exher on non-gravid and non-oestrinized rat uterus is evaluated for uterine motility. The number of uterine motilities per five minutes of control (distilled water) is compared with the number of uterine motilities per five minutes with the Exher. The effect of Exher on acetylcholine induced contractions is studied. The height of contractions of acetylcholine induced contractions is compared in combination with Exher and acetylcholine⁹.

Statistical Analysis: The data were analyzed statistically using Student's unpaired 't' test to find out the difference. The level of significance was set at $p < 0.05$ and considered as significant.

III. RESULTS:

The Exher formulation was found to be safe up to a dose of 5000mg/kg body weight, as on gross observation no untoward effects were seen. The normal rats were pretreated with Control, Exher-1, and Exher-2 for 21 days. The pretreatment with Exher-1 in normal rats significantly increased the dry uterine weight as compared to control. But the Exher-1 was unable to increase the wet uterine weight significantly when compared to control. The pretreatment with Exher-2 in normal rats failed to increase significantly both the dry and wet uterine weights, when compared to control rats. The pretreatment with Exher 1&2 in normal rats significantly increased the oestrogen concentration in the serum, but had no effect on the progesterone concentration in serum of Exher 1&2 as compared to control (Table 1).

The pretreatment with Exher-1 in ovariectomized rats failed to increase significantly the dry and wet uterus weights of ovariectomized rats as compared to ovariectomized control rats. The pretreatment with Exher-2 in Ovariectomized rats failed to increase significantly both the dry and wet uterus weights, when compared to ovariectomized control rats. The pretreatment with Oestradiol in ovariectomized rats significantly increased both the wet and dry uterus weights of ovariectomized rats when compared to ovariectomized control rats. The pretreatment with Exher-1 in ovariectomized rats failed to increase significantly the oestrogen and progesterone concentration in the serum of ovariectomized rats as compared to ovariectomized control rats. The pretreatment with Exher-2 in ovariectomized rats failed to increase significantly the oestrogen and progesterone concentration in the serum of ovariectomized rats as compared to ovariectomized control rats. The pretreatment with Oestradiol in ovariectomized rats failed to increase significantly the oestrogen and progesterone concentration in the serum of ovariectomized rats as compared to ovariectomized control rats. The pretreatment with vehicle in normal control rats with functional ovaries failed to increase significantly the oestrogen and progesterone concentration when compared to ovariectomized control rats (Table 2).The pretreatment with Exher-1 for 21 days in normal rats did not affect the regular oestrus cycle. In the all the rats the duration of oestrus cycle was between 4-5 days. So there was no disturbance in regular oestrus cycle. The pretreatment with Exher-2 for 21 days in normal rats did not affect the regular oestrus cycle. In the all the rats the duration of oestrus cycle was between 4-5 days. So there was no disturbance in regular oestrus cycle. The pretreatment with vehicle for 21 days in normal rats did not affect the regular oestrus cycle. In the all the rats the duration of oestrus cycle was between 4-5 days (Table 3).In vitro studies on non-gravid, non-oestrinized rats are treated with Exher formulation of different concentrations ranging from 50µg/ml, 100µg/ml, 200µg/ml, 300µg/ml, 400µg/ml and 500µg/ml on isolated rat uterus failed to decrease the uterine motility significantly when compared with the normal uterine motility(Table 4). The Exher at a concentration of 100mg/ml failed to significantly inhibit the acetylcholine induced contractions significantly at a concentration ranging from 50 to 500 ng/ml of bath solution (Graph 1).

Table 1: Effect of Exher on uterine weight and hormones in normal rats

Groups	Uterine weight (mg/100gm b. wt)		Hormones	
	Wet weight	Dry weight	Oestrogen (pg/ml)	Progesterone (ng/ml)
Control (10ml/kg vehicle)	257.3 ± 12.45	47.45 ± 1.798	72.26 ± 23.15	19.11 ± 7.12
Exher-1 (250mg/kg)	266.9 ± 26.75	58.33 ± 5.127*	142.2 ± 14.22*	18.33 ± 4.59
Exher-2 (500mg/kg)	295.3 ± 16.28	54.57 ± 3.113	153.9 ± 17.75*	19.55 ± 6.54

Values are mean ± SEM (n=6), p<0.05 as compared to control group

Table 2: Effect of Exher on uterus weight and hormones of ovariectomized rats

Groups	Uterus weight (mg/100gm b. wt)		Hormones	
	Wet weight	Dry weight	Oestrogen (pg/ml)	Progesterone (ng/ml)
Ovariectomized control (10ml/kg vehicle)	27.94 ± 2.006	9.468 ± 0.7083	47.94 ± 5.425	4.133 ± 0.9102
Normal Control (10ml/kg vehicle)	182.0 ± 29.54***	39.82 ± 4.848***	72.26 ± 23.15	19.11 ± 7.129
Ovariectomized + Exher-1 (250mg/kg)	26.35 ± 3.301	12.26 ± 1.279	54.45 ± 13.50	1.883 ± 0.4115
Ovariectomized + Exher-2 (500mg/kg)	26.33 ± 3.978	10.97 ± 0.7653	52.42 ± 17.57	3.478 ± 1.621
Ovariectomized + Oestradiol (0.1µg/kg)	72.70 ± 18.75*	23.80 ± 2.299***	43.58 ± 8.702	2.522 ± 0.6870

Values are mean ± SEM (n=6), p<0.05 as compared to Ovariectomized control group

Table 3: Effect of Exher on rat oestrus cycle

Groups	Oestrus cycle			
	Dioestrus	Proestrus	Frankoestrus	Metaoestrus
Control	+	+	+	+
Exher-1 (250mg/kg)	+	+	+	+
Exher-2 (500mg/kg)	+	+	+	+

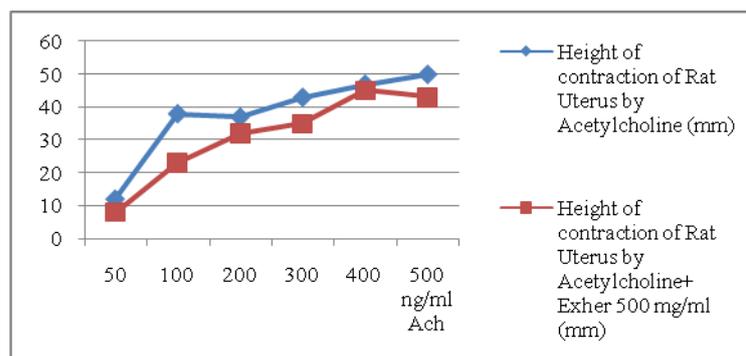
Values are mean ± SEM (n=6), p<0.05 as compared to Ovariectomized control group

Table 4: Effect of Exher on motility of rat uterus

Treatment (n=6)	No. Of contractions/5min
Control	4.667 ± 0.4216
Exher(50µg/ml)	3.833 ± 0.3073
Exher(100µg/ml)	4.333 ± 0.4944
Exher(200µg/ml)	4.667 ± 0.7601
Exher(300µg/ml)	3.500 ± 0.3416
Exher(400µg/ml)	3.500 ± 0.5627

Values are mean ± SEM (n=6), p<0.05 as compared to Control group

Graph-1: Effect of Exher on Acetylcholine induced contraction on Rat uterus



IV. DISCUSSION:

Exher is evaluated for its uterine tonic activity using various parameters in the clinically important four different animal models. The primary changes in the uterine tissue are governed by the ovarian hormones oestrogen and progesterone^{10,11}. Administration of oestrogen is known to increase the wet weight and dry weight of the uterus significantly¹². A typical progesterone is also an active anti-oestrogen which is reported to decrease the uterine wet and dry weight¹³. The present findings in the study indicate that the Exher possess significant uterine tonic activity by increasing the rat uterus weight and oestrogenic activity in the normal rats which was further substantiated by the increase in the serum oestrogen levels.

The polyherbal formulation was devoid of any progestational activity which was further substantiated by the estimation of Progesterone in the serum¹⁴. The ineffectiveness of Exher in ovariectomized rats reveals that this polyherbal formulation do not have the oestrogenic effect in non-functional ovary, but it is effective only in the presence of a functional ovary probably due to increased oestrogen secretion¹⁵ from the ovaries by the exher. The regular oestrus cycle¹⁶ remained unaffected in all the rats treated with Exher. Increasing the ectopic uterine motility is the major reason for primary dysmenorrhea¹⁷. This motility is the basis for several symptoms including pain and primary dysmenorrhea^{18,19}. In-vitro studies of Exher on non-gravid, non-oestrinised rat uterus had no statistical significant effect on the contraction induced by acetylcholine there by indicating that the Exher do not alter the tone of uterus²⁰. This indicates that the Exher has neither oxytocin like activity nor anti-oxytocic activity^{21,22}. The drugs that produces non-contractile response, decreased motility and anti-spasmodic activity following in-vitro exposure of rats uterus can be useful in conditions associated with hypermotility of the uterus i.e. threatened abortion and dysmenorrhea. Several pathophysiological phenomena can cause dysmenorrhea. All types of dysmenorrheas are not fully understood but it seems irregularity in the peristaltic movement in the uterine muscle should be the major cause of dysmenorrhea¹⁸. Prostaglandins may be one of the groups that cause these pathophysiological disorders and so for several years prostaglandin inhibitors were employed to control symptoms of dysmenorrhea²³. The drugs which are known to decrease the uterine motility can also be used for the treatment of dysmenorrhea²⁴. But the poly herbal formulation Exher has not significantly reduced the acetylcholine induced contraction and uterine motility. So this formulation is doubtful in treating the conditions like threatened abortion. Further studies are required to evaluate its usefulness.

V. CONCLUSION:

The Exher was capable of increasing the oestrogen hormone secretion by acting on the functional ovary. In the absence of functional ovary the Exher has failed to produce the statistically significant oestrogenic effects. The oestrogenic effect was determined by increased uterus weight and oestrogen concentration in the serum. Exher is capable of preventing and curing uterine fibroids and abnormal uterine bleeding only in the presence of functional ovary. It is found to be ineffective in case of non-functional ovary. The Exher can be used in conditions like menstrual irregularities as it was found safe on rat oestrus cycle by maintaining the regular oestrus cycle. The Exher has failed to decrease the uterine motility and acetylcholine induced contractions significantly, so its usefulness in treating dysmenorrhea is doubtful. But the models used for evaluating the oestrogenic activity is of much shorter duration then the Exher prescribed for its therapeutic effectiveness. Hence its therapeutic effectiveness has to be revealed on long treatment with the formulation.

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