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EVALUATION OF ANTIFERTILITY ACTIONS OF ETHANOLIC EXTRACT OF WHOLE PLANTS OF *SOLANUM TORVUM Linn*. IN FEMALE ALBINO RATS

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ABSTRACT

Solanum torvum Linn. commonly known as Wild egg plant (*Solanaceae*) called synonym *Solanum largiflorum* as, is an annual herb found in throughout India. Ethnomedicinally the plant is used to treat reproductive problems and several disorders. In the current study the antifertility activity of the ethanolic extract of whole plants of *Solanum torvum* (STE) was investigated. In the in female rats the antizygotic, blastocystotoxic and abortifacient activity were investigated after administration of STE - 200 and 400mg/kg/oral. The results suggest that the *Solanum torvum* ethanolic extract, exerted antifertility and antiestrogenic effects in female rats. The results revealed that ethanolic extract of *Solanum torvum* (STE) treatment caused reduction in reproductive organ weights, number of implants.

Key words: *Solanum torvum*, antifertility and antiestrogenic effects, anti implantation effect.

INTRODUCTION

The earth's population in 1800 was 1 billion, having taken all of human history to reach that mark. Only 2 centuries later, the global population is 6 billion, half of which lives in cities. Population size is clearly an important factor in measuring environmental impact. The combined effects of population growth, consumption, overuse, wastage and misuse of resources has strained the capacity of the earth to sustain life. That's why the study and control of human population is very important today. (1) Population explosion is the leading cause of poverty and pollution in developing countries. Several potential approaches for infertility have been investigated over a long period, including chemical, hormonal and immunological approaches. However, no suitable method has emerged that is effective and free from as other countries. (2).

Fertility regulation with plants or plant products has been reported in the ancient literature of Indigenous systems of medicines. In our county as well as in the world, there are several medicinal

plants associated with antifertility properties like *Polygonum hydropiper Linn, Citrus limonum, Piper nigrum Linn, Juniperis communis, Achyanthes aspera, Azadirachta indica, Tinospora cordifolia etc* (3). A large number of plant species with Antifertility effects have been screened in China and India, beginning about 50 years ago and were subsequently fortified by National and International agencies. Despite the great development of organic synthesis, currently 25 % of prescribed drugs worldwide are still derived new drugs for diseases that continue to lack a cure. Herbal medicines have a strong traditional or conceptual base and the potential to be useful as drugs in terms of safety and effectiveness leads for treating different diseases. According to WHO more than 80% of world's population relies on traditional herbal medicine for their primary health care. (4)

The leaves of the *S.torvum* are simple, alternate, broadly ovate, elliptic, variable in size, margins with broad lobes, deeply cut in juvenile phases, shallow in mature leaves, apex acute to obtuse, base somewhat sagittate to articulate, equal or oblique. The stems are with stout, reversed, reddish or pale yellow prickles, sometime basal stellate hairs. The flowers of *S.torvum* are small, white in colour with large clusters, simple, glandular hairs on axes; corolla bright white, to 2.5 cm across, lobed about 1/3 of its length; lobes not recurved; stamens with prominent anthers. Sepals lanceolate, sparingly hairy. Corolla glabrous, stellate pubescence abaxially. Filament 0.1cm long, anthers 0.4-0.7cm long. Ovary and style glabrous, 0.6- 0.8cm long. Berry yellow, smooth, 1-1.5cm long, calyx lobes present. The fruits are berries that are yellow, thin-fleshed and contain numerous flat, round, brown seeds. Seeds are numerous, drab brownish, flattened, discoid, 1.5-2 mm long slightly reticulate, self- compatible. (5,6)

Solanum torvum contains a number of potentially pharmacologically active chemicals like sterolin (sitosterol-d-glucoside) and 0.1% gluco-alkaloid solasonine Steriodal sapogenins-sisalagenone and torvogenin, steroidal sapogenins, neochlorogenin, neosolaspigenin steroidal gluco-alkaloid, solasonine; and solaspigenin triacontanol, tetratriacontainic acid, 3-tritriacontanone, sitosterol, stigmasterol and campesterol (7). The various parts of *Solanum torvum* extracts have the following activities viz. analgesic, anti-inflammatory, angiotensin and serotonin receptor blocking activities, antidiabetic, anti-dote and for the treatment of fever, antifungal activity, antihypertensive, antioxidant, antibacterial, antiulcer, antiviral, arterial hypertension, cardio protective, erythropoietic, immunomodulatory, jaundice, leucorrhoea, malaria, metabolic correction activity, nephroprotective, wounds, tooth decay and reproductive problems (8). A detailed literature survey reveals that no systematic approach has been made in the past to study the antifertility activity of whole plants of *Solanum torvum,hence* the antifertility activity of the ethanolic extract was planned in the current study.

MATERIALS AND METHODS

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Wistar rats having weight of 150- 200 g were kept in quarantine for 10 days under standard husbandry conditions (25 ^{0}C, Relative humidity 60 ±2 %) for 12 hours in dark and light cycle respectively and were given standard food and water *ad libitum* All the experiments were performed as per the CPCSEA norms after obtained the approval of the Institutional Animal Ethical Committee (IAEC).

The whole plants of *Solanum torvum* was collected from Thondamuthur, Coimbatore district, Tamilnadu and was authenticated by Scientist of Botanical Survey of India, Agricultural University, Coimbatore.. The plants collected were washed in running water, dried under shade, segregated and pulverized by mechanical grinder and the powder was passed through No 20 sieve. The powdered material was successfully extracted with ethanol by hot continuous percolation method in Soxhlet apparatus for 10 hrs. The residue obtained was then utilized for evaluating antifertility activity assessment by suspending in distilled water in Tween 80 (2%) as suspending agent.

Acute oral toxicity study

Acute oral toxicity study was performed as per OECD – 423 guidelines, albino mice of either sex selected by random sampling were used for acute toxicity study. The animals were kept fasting for overnight and provided only with water, after which the extracts were administered orally at 5 mg/kg body weight by gastric intubations and observed for 14 days. If mortality was observed in 2 out of 3 animals, the dose administered was assigned as toxic dose. If mortality was observed in 1 animal then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for higher doses such as 50, 100 and 2000 mg/kg body weight. (9)

Anti implantation activity

Antiimplantation activity was determined as as per standard procedure. (10). Colony bred female albino Wistar rats of proven fertility (150- 200g) were maintained under controlled standard animal house conditions, with standard food and water *ad libitum*. Vaginal smears from each rat were maintained daily. Only rats with normal estrous cycle were selected for the experiment. The female rats were caged with male rats of known fertility in the ratio of 2: 1 in the evening of Proestrous and examined the following morning for the evidence of copulation. Female rats exhibiting the copulation plug or thick clump of spermatozoa in their vaginal smears were separated and that day was designed as day1 of pregnancy. Pregnant rats were divided into 9 groups, each containing 6 animals.

Group I- received Tween 80, 1% and served as control (p.o.) daily for 7 days from day 1 to day7.Group II- received 200 mg/ Kg STE (p.o.) daily for 7 days from day 1 to day 7 (Antiovulatory effect). Group III- received 400mg/ Kg STE (p.o. daily) for 7 days from day 1 to day 7

(Antiovulatory effect). Group IV- received 200mg/ Kg STE (p.o. daily) for 3 days, from day 1 to day3 (Antizygotic activity). Group V- received 400 mg/ kg STE (p.o. daily) for 3 days, from day1 to day 3 (Antizygotic activity). Group VI- received 200 mg/ Kg STE (p.o. daily) for 2 days from day 1 to day 2, which detects blastocystotoxic effect. Group VII- received 400 mg/ Kg STE (p.0. daily) for 2 days from day 1 to day 2, which detects blastocystotoxic effect. Group VIII- received 200mg/Kg STE (p.o. daily) for 4 days from day 6 to day 9, which detects anti implantation or early abortifacient activity. Group IX – received 400mg/ Kg STE (p.o. daily) for 4 days from day 6 to day 9, which detects anti implantation or early abortifacient activity. All the treatments were given orally. The rats were laprotamized under light ether anesthesia on day 10 of pregnancy and the numbers of implantations were recorded.

Statistical analysis:

Statistical analysis were carried out using Analysis of variance (ANOVA) and the means separated using Duncan's new multiple range test. Data are presented as the mean +/- standard error of the mean (SEM).

RESULTS

Table I: Effect of aqueous ethanolic extract of *Solanum torvum* (200 and 400 mg/Kg) in different phases of estrous cycles in rats.

Sl.No	Treatment- (Dose mg/Kg Body Weight)	Mean days of Proestrous	Mean days of Estrous	Mean days of Metaestrous	Mean days of Diestrous
1	**Control**	1.83 ± 0.30	2.66 ± 0.21	4.33 ± 0.21	5.66± 0.21
2	**STE -200**	3.1 ± 0.01*	2.34 ±0.2*	2.22 ±0.12*	5.92 ± 0.12*
3	**STE -400**	3.01 ±0.11*	1.01± 0.04*	1.05 ± 0.11*	6.04 ±0.13*

Values are mean ± SEM; n=6 in each group ASE treated group was compared with control group a significant decrease in proestrous, estrous and metestrous; significant increase in diestrous phase. (*= P<0.05 significant)

Table -2: Anti-implantation activity of STE in female rats

Sl. No	Groups	Activity	No. of rats without implantation sites on Day 10	No. of implantation sites (Mean ± SEM)
1	i.	Control (1st day to 10th day)	0	10. 16 ±0.47
2	ii.	Antiovulatory effect (1-7 days after mating) 200 mg/kg/b.w p.o. treated rats.	3	1.52 ±0.21
3	iii.	Antiovulatory effect (1-7 days after	3	0.11 ±0.11

		mating) 400 mg/kg/b.w p.o. treated rats.		
4	iv.	Antizygotic activity (1-3 days after mating) 200 mg/kg/b.w p.o. treated rats.	3	1.51 ±0.12
5	v	Antizygotic activity (1-3 days after mating) 400 mg/kg/b.w p.o. treated rats.	3	0.62 ± 0.11
6	vi	Blastocidal activity (4^{th} and 5^{th} days after mating) 200 mg/kg/b.w p.o. treated rats.	1	1.51 ± 0.13
7	vii	Blastocidal activity (4^{th} and 5^{th} days after mating) 400 mg/kg/b.w p.o. treated rats.	3	0.31 ± 0.11
8	viii	Anti implantation activity (6^{th} and 7^{th} days after mating) 200 mg/kg/b.w p.o. treated rats.	2	1.13 ± 0.1
9	ix	Anti implantation activity (6^{th} and 7^{th} days after mating) 400 mg/kg/b.w p.o. treated rats.	2	0.10 ±0.11

Table -3: Results of tissue biochemical parameters study in the reproductive organ (ovary) of STE treated rats

Sl. No	Groups	Protein (mg/g)	Glycogen (mg/g)	Sialic acid (mg/g)	Cholesterol (mg/g)	Ascorbic acid (mg/g)	Acid Phosphatase (mgpi/g/h)	Alkaline phosphatase (mgpi/g/h)
1	i- Control	166.22±3.8	8.69± 0.23	0.964 ± 0.01	4.18± 0.15	14.45 ± 0.64	5.87 ± 0.21	6.21 ± 0.23
2	ii- Antiovulatory effect	169.14±1.7	4.11± 0.15***	0.601± 0.01*	11.39± 0.12***	6.99± 0.22*	2.39± 0.11**	3.22± 0.12**
3	iii - Antiovulatory effect	165.21±3.2	4.43±0.31***	0.611± 0.01*	10.13± 0.12***	7.14± 0.25*	2.38± 0.11**	3.34± 0.18**
4	iv - Antizygotic activity	171.11±2.5	4.24± 0.24***	0.612± 0.03*	14.26± 0.13***	7.47± 0.14*	2.87± 0.33**	3.14± 0.16**
5	v- Antizygotic activity	170.25±2.8	4.21± 0.11***	0.627± 0.01*	11.39± 0.17***	7.33± 0.14*	2.74± 0.24**	3.66± 0.17**
6	vi- Blastocidal activity	168.69±3.5	4.25± 0.11***	0.598± 0.01*	11.42± 0.11***	7.37± 0.19*	2.66± 0.04**	2.61± 0.11**
7	vii- Blastocidal activity	169.89±2.2	4.43± 0.17***	0.597± 0.03*	10.55± 0.13***	7.79± 0.14*	2.68± 0.14**	3.62± 0.13**
8	viii- Anti implantation	169.95±1.2	4.34± 0.16***	0.592± 0.01*	10.68± 0.14***	7.17± 0.19*	2.47± 0.21**	3.66± 0.12**

	activity							
9	ix-Anti implantation activity	170.85±3.7	4.55± 0.11***	0.599± 0.03*	11.77± 0.03***	7.44± 0.14*	2.25± 0.41**	2.18± 0.12**

Values are mean ± SEM; n=6 in each group; STE treated rats were compared with control group rats the values of glycogen, sialic acid, ascorbic acid, acid phosphatase and alkaline phosphatase were decreased significantly; Cholesterol was increased significantly and the values Protein was not altered. (*= P<0.05 significant;**= P<0.01 moderately significant, *= P<0.001 highly significant.

Discussion:

On administration of ethanolic extracts of *Solanum torvum* (STE) in rats in the different phases of estrous cycles showed a significant decrease in proestrous, estrous and metestrous; increase in diestrous phase in a significant (P<0.05) manner. The studies indicated further increase of estrogen and there by the mean days of diestrous days are increased from the control animals, indicating the accumulation of estrogen levels. Estrogenic substances are known to cause infertility by shortening the time of transport of egg, disrupting estrous cycle, lowering the plasmic progesterone and decreasing pregnanediol which finally stops development of endometrium. The study revealed that STE produced estrogenic activity.

Endometrial glycogen is one of the most important factors for developing antiimplantation of blastocyst in early stages of gestation. Mobilization of glycogen during formation of deciduma is regulated through hormonal pathways wherein the action of estrogen is dominant. An increase in glycogen mobilization provides nutritive support to the developing blastocyst for their survival. In the present study, a significant decline (p<0.001) in the uterine glycogen content in STE at 200 and 400 mg/kg/po treated rats indicated poor nutritive support to the developing blastocyst for their survival. (11)

Sialomucoprotein, a derivative of sialic acid, forms mucous in the ovary and uterus which sticks around the blastocyst fluid and helps in the attachment of the blastocyst. In addition, sialic acid also acts as an immuno-barrier between the mother and embryo, thereby, has a vital role in embryo implantation in uterus. In the present study, a significant decrease (p<0.05) in the sialic acid content of ovary and uterus in extract treated female rats was observed (11).

Cholesterol is the precursor of sex hormones and is utilized during steroidogenesis. In the present investigation the cholesterol concentration of ovary and uterus were increased (p<0.001) after STE treatment, indicating non utilization of cholesterol by the system. Hence, reduced level of circulating estrogen contributes to altered physiology of female reproductive system. Thus, the present investigation suggests that STE exert antifertility and antiestrogenic activity in female rats (12)

Ascorbic acid plays an important role in many biological processes. It is a potent anti-oxidant, anti-inflammatory, anti-viral agent and an immune stimulant and is present in a wide variety of biological specimens. Due to the presence of a variety of other antioxidants in biological samples such as serum, most ascorbic acid assays show strong interference. In the present study, ovary and uterus ascorbic acid levels were decreased ($p<0.05$) after STE treatment. (13)

It is well established that alkaline and acid phosphatase are associated with the decidual cell reaction and play important role in implantation. A high acid phosphatase activity at the time of implantation is associated with its involvement in the preparation of the implantation chamber. A significant decline in ovary and uterus alkaline and acid phosphatase activity in STE treated mated female rats indicate adverse effect on uterine milieu, making it unsuitable for implantation. These results are in agreement with the findings of many workers who also observed impairment of implantation by virtue of decline in uterine acid phosphatase activity after treatment with plant extracts in mated female rats (14)

Conclusion

In a nut shell, on administration of STE at the dose 200 & 400mg/kg b.wt./day prevented pregnancy in the treated female rats by virtue of antifertility property with antiestrogenic activity. The uterine biochemical milieu serves various functions viz. it enables the spermatozoa to ascend to the site of fertilization within the oviduct. It provides adequate nutrition for the embryo during its various developmental stages. It maintains an appropriate environment for the physical and biochemical integrity of the blastocyte structure and it meets specific immunological requirements which are essential in the pre implantation phase. There may be a possibility that *Solanum torvum* ethanolic extracts may block the cholesterol bioconversion activities, by inhibiting dehydrogenase or desmolase Δ3-3β - hydroxysteroid in the mitochondrial of steroidogenic tissue, which in turn may influence the uterine microenvironment, making it hostile for implantation or for blastocyte to survive.

Conflicts of interest: None declared.

Ethical approval: IAEC, C.L.Baid Metha College of Pharmacy, Chennai - 97, Tamil Nadu, (IAEC / II / 02 / CLBMCP / 2013 dated 21.01.2013).

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REFERENCES:

1. Van Bavel J. The world population explosion: causes, backgrounds and projections for the future. Facts, views & vision in ObGyn. 2013;5(4):281.

2. Chian RC, Buckett WM, Jalil AK, Son WY, Sylvestre C, Rao D, Tan SL. Natural-cycle in vitro fertilization combined with in vitro maturation of immature oocytes is a potential approach in infertility treatment. Fertility and Sterility. 2004 Dec 1;82(6):1675-8.

3.Joshi SC, Sharma A, Chaturvedi M. Antifertility potential of some medicinal plants in males: An overview. Int J Pharm Pharm Sci. 2011;3(5):204-17.

4. Sheldon JW, Balick MJ, Laird SA, Milne GM. Medicinal plants: can utilization and conservation coexist?. Advances in economic botany. 1997 Jan 1;12:i-104.

5. Mohan M, Kamble S, Gadhi P, Kasture S. Protective effect of Solanum torvum on doxorubicin-induced nephrotoxicity in rats. Food and chemical toxicology. 2010 Jan 1;48(1):436-40.

6. Agrawal AD, Bajpei PS, Patil AA, Bavaskar SR. Solanum torvum Sw.—a phytopharmacological review. Der pharmacia lettre. 2010;2(4):403-7.

7. Yousafa Z, Wanga Y, Baydounc E. Phytochemistry and pharmacological studies on Solanum torvum Swartz. Journal of Applied Pharmaceutical Science. 2013 Apr 27;3(4):152-60.

8. Yuan-Yuan LU, Jian-Guang LU, Ling-Yi KO. Chemical constituents from Solanum torvum. Chinese Journal of Natural Medicines. 2011 Jan 1;9(1):30-2.

9. Schlede E. Oral acute toxic class method: OECD Test Guideline 423. Rapporti istisan. 2002;41:32-6.

10. Khanna U, Chaudhury RR. Antifertility screening of plants, Part- I: Investigations on Butea monosperma Lam (Kuntze). Indian J Med Res 1968;56:1575-80.

11. Yadav R, Jain GC. Effect of aqueous extract of seeds of Cassia fistula on the uterine biochemical milieu of female albino rats. Pharmacology. 2009;1:859-67.

12. Grummer RR, Carroll DJ. A review of lipoprotein cholesterol metabolism: importance to ovarian function. Journal of animal science. 1988 Dec 1;66(12):3160-73.

13 Griffiths HR, Lunec J. Ascorbic acid in the 21st century–more than a simple antioxidant. Environmental Toxicology and Pharmacology. 2001 Sep 1;10(4):173-82.

14. Naik SR, Thakare VN, Patil SR. Protective effect of curcumin on experimentally induced inflammation, hepatotoxicity and cardiotoxicity in rats: evidence of its antioxidant property. Experimental and Toxicologic Pathology. 2011 Jul 1;63(5):419-31.