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ESTIMATION OF BLOOD, SERUM AND TISSUE PARAMETERS OF *Solanum torvum* EXTRACT IN FEMALE ALBINO RATS.

K. Balamurugan *
Assistant Professor, Department of Pharmacy, FEAT, Annamalai University, Annamalai Nagar, Chidambaram – 608002, Tamil Nadu, India.

ABSTRACT

Solanum torvum a important medicinal plant have various pharmacological properties and found throughout India. The antifertility activity of the ethanolic extract of *Solanum torvum* (STE) was seen earlier and the haematological parameters post treatment in rats was not altered. The biochemical parameters in STE treated rats showed albumin, creatinine, GGT, LDH, SGOT, SGPT, urea, uric acid, total bilirubin and total protein were not altered. The cholesterol level was high and the result was found statistically significant by compared to control group animals. The female hormone levels were altered significantly the thyroid hormone levels was not altered. The tissue biochemical parameters like cholesterol, estrogen and progesterone level are altered and there was reduction in the weights of reproductive organs. Results of tissue biochemical parameters study in the reproductive organ of STE treated rats altered the protein, glycogen, sialic acid, cholesterol, ascorbic acid, acid phosphatase and alkaline phosphatase. In conclusion the STE treated female rats indicate adverse effect on uterine milieu, making it unsuitable for implantation.

Key Words: *Solanum torvum,* biochemical parameters, hormone and tissue biochemical parameters.

INTRODUCTION

Fertility control is an issue of global and national public health concern. Many elements need to be considered by women, men, or couples at any given point in their lifetimes when choosing the most appropriate contraceptive method. These elements include safety, effectiveness, availability (including accessibility and affordability), and acceptability. Voluntary informed choice of contraceptive methods is an essential guiding principle, and contraceptive counseling, when applicable, might be an important contributor to the successful use of contraceptive methods. **(1)** There is an urgent need for research to develop new contraceptive modalities especially for men and also for women and to make existing methods more safe, affordable and acceptable. Current efforts in India to develop a male contraceptive are mainly directed towards development of antispermatogenic agents to suppress sperm production, prevention of sperm maturation, prevention of sperm transport through vas deferens or rendering these sperm infertile and prevention of sperm deposition. **(2)**

The current method of contraception results in unacceptable rate of unintended pregnancies. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. In India

medicinal plants have been screened for contraceptive potential and anti-fertility effects because of the health sector has always been concerned about the population explosion. So far herbal contraception has not reached the level of contraceptive protection as the pill, but it finds a path as alternatives for women who have difficulty with modern contraceptives. Numerous herbs have been used as abortifacient, contraceptives and modern scientific research has confirmed anti-fertility effects in at least some of the herbs tested in male and female animal models activity and the active agents. Many steroidal and non-steroidal compounds have been used as contraceptive and antiovulatory agent to control fertility. Though they act as potent antifertility agents, they are not free from marked side effects. **(3)**

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. 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 .**(4-5)**

Hence, search for a new potent antifertility substance with minimal side effects are in progress. And there were no documented evidence referring to the anti-fertility effects of whole plants extracts of *Solanum torvum*in animal studies. Thus, the present study was an attempt to investigate the effects of the areial plants of *Solanum torvum* ethanolic extracts on the antifertility actions in female rats and to study the various blood related parameters.

Collection of plant & Extraction procedure

The aerial plants of *Solanum torvum* was collected Thondamuthur, Coimbatore district, Tamilnadu. Authentication was done by the Scientists, Botanical Survey of India, Agricultural University, Coimbatore- 641 003. After authentification fresh plant materials of *Solanum torvumin , was* collected in bulk. The collected plants were washed in, dried under shade and pulverized by mechanical grinder and sieved. The powdered material was successfully extracted with ethanol (70% v/v) by hot continuous percolation in Soxhlet apparatus, filtrated through Whattman filter paper No. 40, the filtrates were evaporated to dryness and were subjected for following studies.

Experimental animals:

Healthy albino Wistar rats (female) after approval from the Institutional Animal Ethical Committee, C.L.Baid Metha College of Pharmacy, were used for the studies. Acclimatization, housing and feeding conditions were followed as per CPCSEA norms. The experiments were conducted on adult female young virgin Wistar rats the animals were randomly assigned for various experimental groups. Each group containing 6 animals were housed individually and were allowed free access to standard pellet diet and tap water *ad libitum*. They were maintained in

controlled laboratory conditions as per CPCSEA norms.

Grouping of animals for estimation of haematological, serum and tissue biochemical activity of STE (5)

Group I- served as control received Tween 80, 2% for7 days from day 1 to day 7; **Group II** – received STE at 200 mg / kg for 7 days from day 1 to day 7. **Group III** – received STE at 400 mg/ kg for 7 days from day 1 to day 7. **Group IV**- received STE at 200 mg/ kg for 3 days from day 1 to day 3, which detects antizygotic activity. **Group V**- received STE at 400 mg/ kg for 3 days from day 1 to day 3, which detects antizygotic activity. **Group VI** – received STE at 200 mg/ kg for 2 days from day 1 to day 2, which detects blastocystotoxic activity. **Group VII**- received STE at 400 mg/ kg for 2 days from day 1 to day 2, which detects blastocystotoxic activity. **Group VIII** – received STE at 200 mg/ kg for 4 days from day 6 to day 9, which detects anti implantation or early abortifacient activity. **Group IX**- received STE at 400 mg/ kg (p.o. daily) for 4 days from day 6 to day 9, which detects anti implantation or early abortifacient activity. All the groups were treated through gastric lavage per oral.

Grouping of rats on different Phases of estrous cycles after treatment of ethanolic extract of *Solanum torvum* (STE)

Sl. No	Groups / Activity	Treatment
1	Control (1st day to 10th day)	Rats treated with tween 80 (2%) 1 ml/ kg/p.o suspension for 7 days.
2	Antiovulatory effect (1-7 days after mating)	Rats treated with STE (200mg / kg/ p.o.) for 1-7 days after mating.
3	Antiovulatory effect (1-7 days after mating)	Rats treated with STE (400mg / kg/ p.o.) for 1-7 days after mating.
4	Antizygotic activity (1-3 days after mating)	Rats treated with STE (200mg / kg/ p.o.) for 1-3 days after mating.
5	Antizygotic activity (1-3 days after mating)	Rats treated with STE (400mg / kg/ p.o.) for 1-3 days after mating.
6	Blastocidal activity (4th and 5th days after mating)	Rats treated with STE (200mg / kg/ p.o.) for 4th and 5thday after mating.
7	Blastocidal activity (4th and 5th days after mating)	Rats treated with STE (400mg / kg/ p.o.) for 4th and 5thday after mating.
8	Anti implantation activity (6th and 7 th days after mating)	Rats treated with STE (200mg / kg/ p.o.) for 6th and 7 thday after mating.
9	Anti implantation activity (6th and 7 th days after mating)	Rats treated with STE (400mg / kg/ p.o.) for 6th and 7 thday after mating.

Enumeration of Haematological / Serum bio chemical parameters

After the animals treatment with STE blood samples were collected and divided into part-I and part-II. To one part of the blood in test tube, two drops of sodium citrate (3.8%w/v) was

added and preceded for haematological parameters. Another part of the blood in the test tube was allowed to clot at room temperature, centrifuged at 4500 rpm for 15 min; the pure serum was collected from the supernatant and it was subjected to biochemical analysis.

Estimation of Haematological parameters

The following estimation of White blood corpuscle, Red blood corpuscle, Hemoglobin, Haematocrit, Mean corpuscular volume, Mean corpuscular haemoglobin, Mean corpuscular haemoglobin concentration were performed.**(6)**

Estimation of serum bio chemical parameters in rats

The following estimation of Albumin, Alkaline phosphatase and Creatinine by Tietz N. *et al.,* (7); Cholesterol by Allain C. *et al.,* **(8)**; Glucose by VH B, Ajay SS, GGT **(9)**, LDH by HU Bergmeyer, **(10)**; Glutamic Oxaloacetic Transaminase & Glutamic Pyruvic Transaminase by Mohanty S, *et al.,* **(11)**; Urea by Wheatherburn M.W, **(12)** ; Uric Acid & uric acid by Trivedi R.C. *et al.,* 1978 **(13)** ; Bilirubin by Defreese JD. *et al.,* and Total protein by Tietz NW, **(7)** and Triglycerides by Abdel-Rahman Z.., **(15)**

Estimation of serum hormonal assay:

The following estimation of Oestradiol by Tsang *et al.,* **(16)**; Progesterone by Scholler R *et al.,* and T3, T4 and TSH by Inada MK.**(18)**

Estimation of tissue biochemical parameters

Ovary and uterus were washed with chilled isotonic saline and the tissue homogenates were prepared in ice cold 0.1 M Tris-HCl buffer (pH 7.2) and used for the assay of clinical marker enzymes. The following parameters were studied in ovary and uterus of rats. Protein by Scopes RK *et al.,* **(19)**; Glycogen by Chun Y, Yin ZD. **(20)**; Sialic acid by Sugahara K, **(21)** ; Cholesterol by Nauck M *et al;* **(22)**; Ascorbic acid by Washko PW, *et al.,* (23) ; Acid phosphatise and Alkaline phosphatase by Oser, **(24)**.

Results of the hematological parameters of STE treated rats

Groups	WBC (thous/mcl)	RBC (mill/mcl)	Hb (g/dl)	HT (%)	MCV (fl)	MCH (pg)	MCHC (%)	Lymphocytes (%)	Monocytes (%)	Heterophils (%)
i-Control	8.07± 1.44	7.53 ±1.11	13.11 ±1.1	41.41 ±1.47	55.45 ±2.68	20.42 ±1.24	43.54 ±2.57	42±1.25	3±0.9	44±2.33
ii-Antiovulatory effect	8.72± 1.24	8.45 ±1.52	13.34 ±2.24	40.77 ±2.85	55.22 ±2.48	21.02 ±2.28	41.64 ±0.12	43±1.37	3±0.8	44±2.85

iii - Antiovulatory effect	8.14±1.42	8.04±1.47	14.24±2.11	41.67±2.25	57.64±2.62	21.64±1.48	41.24±0.46	45±1.64	3±0.5	44±2.61
iv - Antizygotic activity	8.22±1.54	8.15±1.36	13.11±2.54	41.54±1.84	55.74±2.64	21.86±1.82	42.24±2.72	43±2.84	3±0.6	42±2.34
v- Antizygotic activity	8.71±2.51	7.94±1.55	12.85±1.36	39.54±1.08	51.82±2.67	21.05±1.91	44.74±2.37	45±3.85	3±0.7	42±2.84
vi- Blastocidal activity	8.08±1.35	8.28±1.24	13.26±1.61	42.77±1.06	52.94±2.44	21.08±1.84	43.67±2.62	44±2.75	3±0.9	44±2.66
vii- Blastocidal activity	8.15±2.46	8.77±1.74	14.27±1.24	41.33±1.46	54.64±2.68	21.05±2.58	42.06±1.22	44±2.64	3±0.8	442±2.69
viii- Anti implantation activity	8.51±1.05	8.17±2.54	13.44±1.74	42.55±2.56	56.37±2.28	21.11±1.92	42.44±1.77	45±2.55	3±0.2	44±2.90
ix-Anti implantation activity	8.15±1.46	8.48±2.34	13.33±1.22	42.48±3.66	54.44±6.24	22.48±1.84	41.04±0.72	43±2.33	3±0.7	44±2.64

Results of the biochemical parameters in STE treated rats

Groups	Albumin (g/dl)	ALP (U/l)	Creatinine (mg/dl)	Cholesterol (mg/dl)	Glucose (mg/dl)	GGT (U/l)	LDH (U/l)
i- Control	3.71±0.5	86.24±2.4	0.6±0.05	72.04±0.14	90.24±3.47	90.24±2.47	136.45±5.8
ii- Antiovulatory effect	3.41±0.3	67.25±2.4*	0.6±0.12	152.11±2.45***	73.25±3.04*	96.44±6.41	135.14±4.5

iii - Antiovulatory effect	3.25±0.4	69.22±2.4*	0.7±0.02	143.21±3.51***	75.34±2.22*	92.22±3.45	129.24±2.1
iv - Antizygotic activity	3.22±0.2	68.98±2.4*	0.7±0.05	148.22±2.45***	71.35±1.81*	93.64±6.14	138.07±4.6
v- Antizygotic activity	3.32±0.4	68.48±2.4*	0.6±0.11	146.34±4.45***	74.41±2.08*	94.44±2.13	142.09±6.6
vi- Blastocidal activity	3.14±0.6	69.14±2.4*	0.7±0.21	145.33±5.78***	70.42±4.96*	92.24±1.84	136.28±6.7
vii- Blastocidal activity	3.25±0.5	70.45±1.1*	0.7±0.34	144.66±2.49***	73.23±2.31*	91.14±1.66	135.12±4.6
viii- Anti implantation activity	3.11±0.3	69.22±3.5*	0.6±0.11	141.16±4.44***	71.08±2.07*	94.11±4.62	134.11±3.4
ix-Anti implantation activity	3.21±0.4	70.45±1.9*	0.7±0.22	141.81±4.45***	72.26±1.07*	95.21±2.54	139.22±2.6

Results of the biochemical parameters in STE treated rats

Groups	SGOT (U/l)	SGPT (U/l)	Urea (mg/dl)	Uric acid (mg/dl)	Total Bilirubin (mg/dl)	Total Protein (g/dl)	Triglycerides (mg/dl)
i- Control	68.24±3.2	26.14±1.74	21.54±1.13	5.24±0.44	0.62±0.05	6.24±0.98	134.72±4.4
ii- Antiovulatory effect	69.45±8.7	27.56±2.54	24.11±0.66	4.98±0.44	0.59±0.07	7.04±0.25	133.05±2.2
iii - Antiovulatory effect	69.58±0.9	28.54±1.32	24.69±2.24	4.58±0.44	0.62±0.05	6.78±0.42	138.11±2.4
iv - Antizygotic	69.74±2	26.45±1.	21.36±2.	5.64±0.	0.61±0.	6.64±0.	139.25±1.5

activity	.1	74	24	44	03	98	
v- Antizygotic activity	67.25±3 .2	28.64±1. 42	24.36±1. 11	6.01±0. 44	0.62±0. 04	7.01±0. 14	141.45±2.2
vi- Blastocidal activity	68.69±2 .7	25.78±1. 04	22.31±1. 09	5.01±0. 44	0.63±0. 02	6.85±0. 18	142.98±8.7
vii- Blastocidal activity	65.98±6 .8	28.64±1. 44	22.45±1. 77	4.65±0. 44	0.59±0. 01	6.34±0. 38	141.45±3.9
viii- Anti implantatio n activity	67.26±4 .3	27.41±2. 74	22.98±1. 38	5.14±0. 44	0.58±0. 01	7.14±0. 08	139.24±1.4 4
ix-Anti implantatio n activity	67.54±0 .3	26.44±0. 74	23.65±0. 81	5.24±0. 44	0.59±0. 02	6.84±0. 87	138.54±3.7

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

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)**
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
ii- Antiovulat ory 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**

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**
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**
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**
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**
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**
viii- Anti implantation activity	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**
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**

Results of tissue biochemical parameters study in the reproductive organ (uterus) of STE treated rats

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)**
i- Control	155.11± 2.4	7.69± 0.23	0.954 ± 0.01	5.18± 0.15	13.35 ± 0.37	6.07 ± 0.17	6.37 ± 0.42
ii- Antiovulatory effect	147.24± 2.7	3.15± 0.17***	0.722 ± 0.01*	10.09± 0.11***	6.17± 0.25*	2.29± 0.15**	3.14± 0.32**

iii - Antiovulatory effect	145.34± 3.2	3.41±0.32***	0.699 ± 0.02*	9.13± 0.11***	7.05± 0.25*	2.25± 0.13**	3.09± 0.08**
iv - Antizygotic activity	154.15± 2.5	3.21± 0.24***	0.648 ± 0.03*	9.26± 0.12***	7.08± 0.17*	2.31± 0.33**	3.47± 0.06**
v- Antizygotic activity	156.01± 3.8	3.24± 0.16***	0.624 ± 0.02*	9.39± 0.07***	7.34± 0.13*	2.61± 0.14**	3.78± 0.17**
vi- Blastocidal activity	152.09± 3.5	4.01± 0.11***	0.601 ± 0.01*	9.42± 0.15***	7.37± 0.11*	2.39± 0.14**	2.99± 0.01**
vii- Blastocidal activity	150.35± 4.2	3.42± 0.16***	0.597 ± 0.04*	9.55± 0.13***	7.05± 0.14*	2.55± 0.22**	3.49± 0.23**
viii- Anti implantation activity	151.44± 1.9	4.04± 0.11***	0.577 ± 0.02*	10.01± 0.24***	7.28± 0.15*	2.47± 0.27**	3.17± 0.22**
ix-Anti implantation activity	151.27± 3.1	3.51± 0.13***	0.608 ± 0.04*	10.06± 0.13***	7.22± 0.04*	2.41± 0.07**	2.98± 0.14**

Discussion

The hematological parameters of STE 200 and 400 mg/kg/po treated rats complete blood counts were not altered significantly when compared to control group animals proving the safety of STE. The serum albumin level responsible maintaining and regulating the colloidal osmotic pressure of blood was not significantly altered indicating that STE does not have any adverse effect in the treated rats. Alkaline phosphatase is associated with the decidual cell reaction and play important role in implantation and a significant decline in uterine acid and alkaline phosphatase activity in extracts treated mated female rats indicated an adverse effect on uterine milieu and thus making it unsuitable for implantation. **(25)**

Administration of 200 & 400mg/kg/p.o. of STE in rats the serum creatinine level was not significantly altered. Cholesterol is needed as a structural element in all cell membranes, and is a building block for some hormones and other essential body functions ASE treatment the serum Cholesterol level was found to be significantly high. Glucose is the primary energy source for all animals and the human body a decrease in the circulating glucose content in ASE treated female rat indicates poor nutritive support to the developing blastocyst for their survival and hence the antifertility effects were seen. Further on the

administration of ASE, in rats the serum GGT, LDH, SGOT, SGPT, urea, uric acid, Bilirubin, total protein, serum triglycerides level was not altered. **(26-27)**

Estrogen is a steroid synthesized from both tissue and circulating cholesterol. During administration of STE at 200 & 400mg/kg b.wt./day in rats the oestradiol level was decreased in the circulating blood in extract treated female rats which indicates poor support to the developing embryo for their survival resulting in antifertility effect. Progesterone is a steroid synthesized from both tissue and circulating cholesterol. STE treatment in rats level of progesterone was decreased indicates poor support to the developing embryo for their survival and hence the antifertility effect is observed.**(28)** STE treatment the T3, T4 and TSH level was not altered which may be due to short period of administration of test drugs and the differentiation was found to be insignificant.

Administration of STE treated rats the tissue weight of liver, heart, kidney were not altered significantly; uterus and vaginal weights were altered significantly when compared to control group of animals. The decrease in estrogen and progesterone parameters of female reproductive organs and insufficient level of circulating estrogen and progesterone which was essential for maintenance of their physiological integrity and due to this uterus and vagina weights were decreased. **(29)**

In the present study, a significant decline ($p<0.001$) in the uterine glycogen content in STE treated rats indicated poor nutritive support to the developing blastocyst for their survival which can account for their antifertility action. Sialomuco protein, 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, a significant decrease ($p<0.05$) in the sialic acid STE treated female rats was observed. **(30)** Cholesterol is the precursor of sex hormones and is utilized during steroidogenesis cholesterol concentration of ovary and uterus was increased after STE treatment, indicating non utilization of cholesterol by the system. Ascorbic acid anti-oxidant, anti-inflammatory, anti-viral agent and an immune stimulant ascorbic acid can restore the ciliary mucus cells. In the present study, ovary and uterus ascorbic acid levels were decreased after ASE treatment. Alkaline and acid phosphatases are associated with the decidual cell reaction and implantation. 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.**(31)**

Conclusion:

In a nut shell, on administration of ethanolic extracts of *Solanum torvumin* , at the dose 200 & 400mg/kg b.wt./day altered the serum, tissue biochemical parameters and not altered haematological parameters. In conclusion the STE treated female rats indicate adverse effect on uterine milieu, making it unsuitable for implantation.

Ethical approval: IAEC, C.L.Baid Metha College of Pharmacy, Chennai, Tamil Nadu, (IAEC / II / 02 / CLBMCP / 2013 dated 21.01.2013). **Funding source:** None. **Conflicts of**

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