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REPEATED DOSE ORAL TOXICITY STUDY OF ETHANOLIC EXTRACT OF OF *SOLANUM TORVUM* IN RATS USING - OECD 407 GUIDELINES.

K. BALAMURUGAN [*].

Assistant Professor, Department of Pharmacy, FEAT, Annamalai University, Annamalai Nagar, Chidambaram - 608002, Tamil Nadu, India. E-mail: placementbala@yahoo.co.in

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ABSTRACT

various parts of *Solanum torvum (Solanaceae)*extracts have has diverse ethno medicinal uses like 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. Despite its potential therapeutic uses, the toxicity profile of *Solanum torvum* has not been evaluated. This study assessed the sub-acute toxicity effects of whole plants of *Solanum torvum* ethanolic extract (SOE). The sub-acute toxic effect of the SOE (1000 mg/kg) was investigated by administration for 28 consecutive days as per the OECD 407 guideline. The weekly body weights were recorded. The animals were euthanized on the 29th day, and blood samples were obtained for the hematological and biochemical investigations. The heart and liver were subjected for histological examinations.

Key Words: OECD 407 guidelines, *Solanum torvum*, repeated dose oral toxicity study.

INTRODUCTION

The World Health Organization (WHO) estimates that 80 percent of the population of some Asian and African countries presently uses herbal medicine for some aspect of primary health care and approximately 25% of modern drugs used in the United States have been derived from plants.(1) *Solanum torvum L.* belongs to family *Solanaceae,* in Hindi known as Bhurat; it is a bushy, erect and spiny perennial plant usually 2 m in height and 2 cm in basal diameter, but may reach 5m in height and 8 cm in basal diameter. The shrub usually has a single stem at ground level, but it may branch on the lower stem. *Solanum torvum* contains a number of potentially

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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.(2,3,4)

The various parts of *Solanum torvum* extracts has diverse ethno medicinal uses, (5) a thoughtful attempt has been made here to explore herbal medicines that have a strong traditional or conceptual base and the potential to be useful as antifertility effects in terms of safety and effectiveness. A thoughtful attempt has been made here to explore herbal medicines that have a strong traditional or conceptual in terms of safety and effectiveness. The present study was an attempt to investigate the effects of the whole plants of *Solanum torvum* ethanolic extracts (SOE) for the preliminary test in repeated dose oral toxicity study in rats using OECD 407 guidelines before the antifertility actions in female rats.

MATERIALS AND METHODS

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 - 641 003. 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 sub-acute toxicological assessment by suspending in distilled water in Tween 80 (2%) as suspending agent was given at doses of 1000 mg/ kg.

Wistar rats having weight of 180- 220 gm were kept in quarantine for 10 days under standard husbandry conditions (27 ^{0}C, RH 65 ±2 %) for 12 h 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 IAEC,C.L.Baid Metha College of Pharmacy, Chennai - 97, Tamil Nadu, (IAEC / II / 02 / CLBMCP / 2013 dated 21.01.2013). (6)

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REPEATED DOSE 28-DAYS ORAL TOXICITY STUDY:

The study was carried out on adult female young virgin Wistar rats (180-220 g. body weight) between 8 and 12 weeks old, each group containing 6 animals were housed individually in labeled polypropylene cages. Animals were allowed free access to standard pellet diet and tap water *ad libitum*. They were maintained in controlled laboratory conditions of 12 hrs dark/light cycle, 22±2°C temperatures and 45-60% humidity. After 2 weeks of acclimatization, the animals were divided into four groups of 6 each. Group –I, (control group) rats received tween 80 (2%) 1 ml/ kg/p.o suspension; Group –II, SOE treated rats.

Table- 1: Grouping of rats in repeated dose 28-days oral toxicity study

Sl.No	Groups	Treatment
1.	Group-I (Control)	Rats treated with tween 80 (2%) 1 ml/ kg/p.o suspension for 28 days.
2.	Group-IV (SOE treated)	Rats treated with ACE (1000mg / kg/ p.o.) for 1- 28 days.

The extract was administered at a fixed time daily for 28 days and observed twice daily for morbidity/mortality and gross behaviour activity. Body weights of the animals were measured weekly. On the 29th day, after an overnight fast, the rats were anaesthetized with ether and blood samples were collected. The haematological, biochemical analysis and hormonal assays were performed. Diagnostic reagents or kits consisting of Cholesterol (Autozyme, Accurex Biomedical, Mumbai), Hormonal kits (Elecsys 2010 Modular Analytics, E 170, Cobase 411), SGOT & SGPT (E- Merck India Ltd. Worli, Mumbai), Total protein (Ecoline-,E- Merck (India) Ltd., Worli, Mumbai) and other kits acid phosphatase, albumin, alkaline phosphatase, ALP, ascorbic acid, cholesterol, creatinine, GGT, glucose, glycogen, LDH, sialic acid, total bilirubin, triglycerides, urea and uric acid were obtained from Agappe Diagnostics, Ernakulam, Kerala, India. The reagents required for investigating the haematological parameters were obtained from Masters Bio-Tech (P) Ltd., Bangalore, India. The organ weights were recorded and necroscopy and histopathological studies were also carried out. (7)

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RESULTS:

Table-2 Results of gross behavior studies of SOE at a dose of 1000mg/kg . p.o. in rats.

Sl.no	Effect on CNS:	Time (hrs)											
		$\frac{1}{2}$	1	$1\frac{1}{2}$	2	$2\frac{1}{2}$	3	$3\frac{1}{2}$	4	5	6	12	24
Spontaneous motor activity:													
1.	Ataxic gait.	-	-	-	-	-	-	-	-	-	-	-	-
2.	Bizarre behaviour	-	-	-	-	-	-	-	-	-	-	-	-
3.	Chronic convulsions	-	-	-	-	-	-	-	-	-	-	-	-
4.	Convulsions	-	-	-	-	-	-	-	-	-	-	-	-
5.	Grooming behaviour	-	-	-	-	-	-	-	-	-	-	-	-
6.	Lying flat on the back	-	-	-	-	-	-	-	-	-	-	-	-
7.	Lying flat on the belly	-	-	-	-	-	-	-	-	-	-	-	-
8.	Lying flat on the side	-	-	-	-	-	-	-	-	-	-	-	-
9.	Narcosis	-	-	-	-	-	-	-	-	-	-	-	-
10.	Opisthotonus	-	-	-	-	-	-	-	-	-	-	-	-
11.	Restlessness	-	-	-	-	-	-	-	-	-	-	-	-
12.	Rolling and jumping	-	-	-	-	-	-	-	-	-	-	-	-
13.	Sleeping	-	-	-	-	-	-	-	-	-	-	-	-
14.	Straub's phenomenon	-	-	-	-	-	-	-	-	-	-	-	-
15.	Timidity	+	+	+	+	+	+	+	+	+	+	+	+
16.	Tonic convulsions	-	-	-	-	-	-	-	-	-	-	-	-
17.	Tremors	-	-	-	-	-	-	-	-	-	-	-	-
18.	Twitches	-	-	-	-	-	-	-	-	-	-	-	-
19.	Writhing	-	-	-	-	-	-	-	-	-	-	-	-
Effect on reflexes:													
20.	Corneal reflexes	-	-	-	-	-	-	-	-	-	-	-	-
21.	Pain following stimulation	-	-	-	-	-	-	-	-	-	-	-	-
22.	Pinna reflex	+	+	+	+	+	+	+	+	+	+	+	+
Effect on autonomic nervous sySOEm:													
23.	Cyanosis	-	-	-	-	-	-	-	-	-	-	-	-
24.	Defecation	-	-	-	-	-	-	-	-	-	-	-	-
25.	Eyelids(closure/exophthalamus)	-	-	-	-	-	-	-	-	-	-	-	-
26.	Lacrimation	-	-	-	-	-	-	-	-	-	-	-	-
27.	Piloerection	-	-	-	-	-	-	-	-	-	-	-	-
28.	Pupil diameter (constriction/dilatation)	-	-	-	-	-	-	-	-	-	-	-	-
29.	Salivation	-	-	-	-	-	-	-	-	-	-	-	-

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30.	Secretion of sweat	-	-	-	-	-	-	-	-	-	-	-	-
31.	Urination	-	-	-	-	-	-	-	-	-	-	-	-
Effect after manipulations:													
32.	Auditory stimulus response	+	+	+	+	+	+	+	+	+	+	+	+
33.	Catalepsy in induced position	-	-	-	-	-	-	-	-	-	-	-	-
34.	Escape after touch	+	+	+	+	+	+	+	+	+	+	+	+
35.	Paralysis of fore paws	-	-	-	-	-	-	-	-	-	-	-	-
36.	Paralysis of hind limbs	-	-	-	-	-	-	-	-	-	-	-	-
37.	Writhing reflex	-	-	-	-	-	-	-	-	-	-	-	-

(-): Normal or non response, (+): positive response

Table-3 : Results of the hematological parameters of SOE (1000 mg/kg/oral) inrats

Test	Control	SOE (1000 mg/kg/oral)
WBC (thous/mcl)	8.46±1.87	9.65±2.52
RBC (mill/mcl)	8.64±2.58	8.45±2.04
Hb (g/dl)	15.94±3.46	14.68±2.4
MCV (fl)	58.64± 3.24	57.45±3.24
MCH (pg)	21.22±2.25	23.12±1.14
MCHC (%)	42.54±1.24	43.78±2.47
HT (%)	40.12±2.09	42.78±3.22
Lymphocytes (%)	44±2.9	45±1.84
Monocytes (%)	3±0.1.22	3.01±1.2
Heterophils (%)	45±2.60	43±1.22

Values are mean ± SEM; n=6 in each group, SOEwas compared with control group showed non- significant results.

Table 4:Results of the biochemical parameters of SOE (1000 mg/kg/oral) in rats.

BIOCHEMICAL PARAMETERS	Control	SOE (1000 mg/kg/oral)
Albumin (g/dl)	3.7±0.5	4.12±1.2
Alkaline Phosphates (U/l)	86.24±2.4	65.14±1.2*
Creatinine (mg/dl)	0.6±0.05	0.7±0.21
Cholesterol (mg/dl)	72.04±0.14	142.22±1.54

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Glucose (mg/dl)	90.24±3.47	74.14±2.64*
Gamma Glutamyl Transferase (GGT) (U/l)	43.74±1.33	42.64±2.78
Lactate Dehydrogenate (LDH) (U/l)	136.45±5.8	147.54±2.31
Glutamic Oxaloacetic Transaminase (SGOT) (U/l)	68.24±4.2	69.24±2.54
Glutamic Pyruvic Transaminase (SGPT) (U/l)	26.14±1.74	29.48±1.94
Urea (mg/dl)	21.54±1.13	21.22±1.47
Uric acid (mg/dl)	5.24±0.44	6.04±1.11
Total bilirubin (mg/dl)	0.62±0.05	0.68±0.08
Total Protein (g/dl)	6.24±0.98	6.47±0.64
Triglycerides (mg/dl)	134.72±4.4	147.23±4.1

Values are mean ± SEM; n=6 in each group **SOE** were compared with control group albumin, creatinine, GGT, LDH, SGOT, SGPT, urea, uric acid, total bilirubin and total protein values were not altered significantly ALP, glucose values were significantly less and cholesterol level was significantly high.(*= P<0.05 significant).

Table-5: Results of serum hormonal parameters of SOE (1000 mg/kg/oral) treated rats.

Sl. No	**Groups**	**Oestradiol (Pg/ml)**	**ProgeSOEron e (Pg/ml)**	**Tri iodothyronine (µU/ml)**	**Thyroxin (µU/ml)**	**Thyroid stimulating hormone (µU/ml)**
1	Group-I (Control)	69.37±2.15	13.13±0.94	0.33 ± 0.11	1.28 ± 0.11	67.09 ± 1.12
2	Group-IV (SRE treated)	21.36±1.97**	06.58±0.94*	0.34 ± 0.21	1.57 ± 0.23	65.54 ± 1.61

Values are mean ± SEM; n=6 in each group SOE were compared with control group. Triiodothyronine, thyroxin, thyroid stimulating hormone values were not altered significantly while oestradiol, progeSOErone values were significantly less.(**= P<0.01) moderately significant,(*= P<0.05 significant).

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Table-6: Results of tissue weight analysis of SOE (1000 mg/kg/oral) treated rats.

Sl. No	Groups	Brain (g)	Heart (g)	Kidney(g)	Liver(g)	Uterus(g)
1.	Group-I (Control)	1.612±0.09	0.457±0.04	0.987±0.02	3.07±0.09	0.535±0.01
2.	Group-IV (SOE treated)	1.612±0.87	0.478±0.02	0.954±0.04	3.11±0.11	0.398±0.21*

Values are mean ± SEM; n=6 in each group SOEwere compared with control group brain, heart, kidney, liver values were not altered significantly uterus values was significantly less (*= P<0.05 significant).

Table-7: Results of macroscopic analysis of SOE (1000 mg/kg/oral) treated rats.

Sl.No	Groups	Brain (g)	Heart (g)	Kidney(g)	Liver(g)	Uterus(g)
1.	**Group-I (Control)**	N	N	N	N	N
4.	**Group-IV (SOE treated)**	N	N	N	N	AbN

N= Normal; Ab N = abnormal

Results of Sub-Acute Toxicological Study:

Repeated dose 28-days oral toxicity study was performed in adult female young virgin Wistar rats and the gross behavioural studies were observed after administering SOE at a dose of 1000mg/kg/oral and the spontaneous motor activity, effect on reflexes, effect on autonomic nervous system and effect after manipulations parameters were observed visually. The effects of drugs on the central and peripheral nervous systems can be easily recognized in normal animals. Several neurological and neuropsychological tests are described which can be used as first screen for behaviour abnormalities in mice or rats. Some of the plant extract contains potent drugs may act on ANS and CNS results in an increase or decrease activity in motor nerves which may alter the behavioural changes.(8)

Repeated dose 28-days oral toxicity study of the test compound SOE at 1000 mg/kg/oral does not altered the normal behaviour such as ataxic gait, bizarre behaviour, grooming

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behaviour, lying flat on the back, lying flat on the belly, lying flat on the side, narcosis, restlessness, sleeping, straub's phenomenon, timidity and also absence of chronic convulsions, opisthotonus, rolling and jumping, tonic convulsions, tremors, twitches and writhing. The results were compared with control animals and found to be normal (Table: 2). Further corneal reflexes, pain following stimulation and pinna reflex were also normal. Effect on autonomic nervous system such as cyanosis, defecation, eyelids (closure/exophthalamus), lacrimation, piloerection, pupil diameter (constriction /dilatation), salivation, secretion of sweat and urination were normal when compared to control group animals. Effect after manipulations such as auditory stimulus response, escape after touch, writhing reflex, paralysis of hind & fore paws and catalepsy in induced position were not observed. All the above parameters proved that the repeated dose 28-days oral toxicity study in rats after administering SOE at a dose of 1000mg/kg/oral respectively were safe and did not alter the normal behaviour of the treated rats.(9)

The hematological parameters such as WBC count, RBC count, haemoglobin, MCV, MCH, MCHC, HT, lymphocytes, monocytes and heterophils the results were not altered significantly when compared to control group animals (Table:3).(10) Serum biochemical parameters such as albumin, creatinine, GGT, LDH, SGOT, SGPT, urea, uric acid, total bilirubin and total protein were not altered significantly when compared to control group animals. (11) The level of ALP, glucose were less and cholesterol level was very high and the results were found statistically significant when compared to control group animals (Table:4).The hormonal levels of oestrogen, progesterone were altered significantly and the T3, T4 and TSH levels were not altered. The histopathological results supported the above findings (Table: 5).

The histopathological slides of myocardium 1000 mg/kg/oral treated rat showed the normal architecture, absence of oedema, nuclear fatty infiltration, inflammatory cells with normal architecture of myocardial fibres were seen which confirms the safety of AAEwhen compared to normal group cells (Fig:1-2).(12) The histopathological slides of liver 1000 mg/kg/oral treated rat hepatic cells has sinusoidal space, central vein showing normal architecture, absence of disarrangement, degeneration of hepatic cells, necrosis, sinusoidal haemorrhages and dilatations; conforms the safety of SOE when compared to normal group cells of liver (Fig:3-4). (13)

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HISTOPATHOLOGICAL RESULTS OF SOE (1000 mg/kg/oral) TREATED RATS

Photomicrograph of sections (H&E staining, magnificationX10)

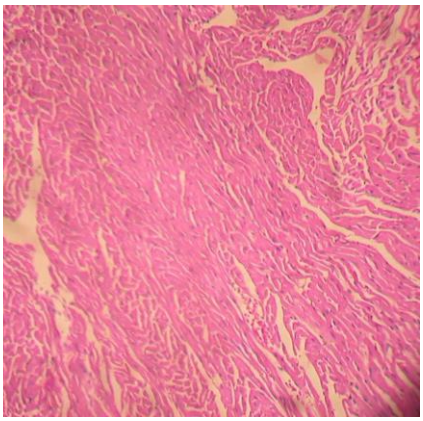	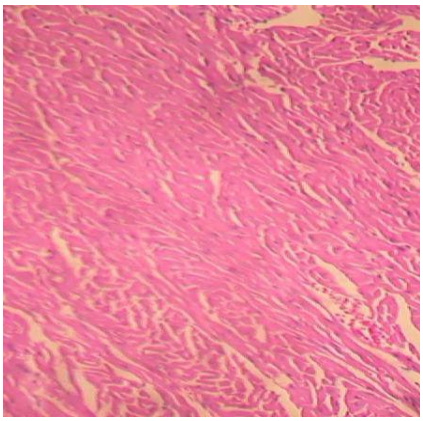
Fig: 1 Tween 80 (2%) 1 ml/ kg/p.o treated cardiac muscle of rats.	**Fig:2 SOE treated cardiac muscle of rats**
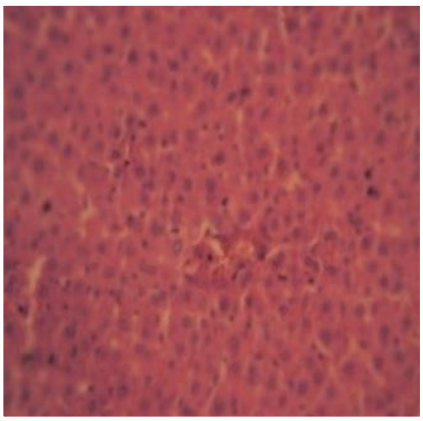	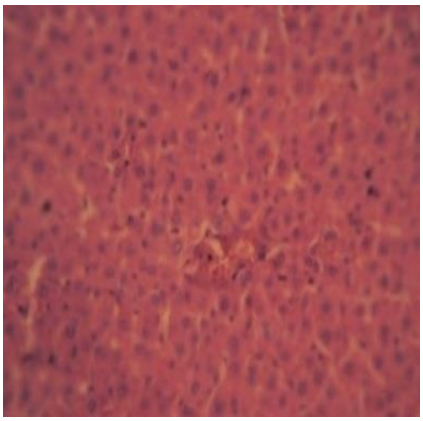
Fig: 3 Tween 80 (2%) 1 ml/ kg/p.o treated liver cells of rats.	**Fig: 4 SOE treated liver cells of rats.**

Conclusion:

In summary, repeated oral doses of ethanolic extract of *Solanum torvum* L., in wistar rats to rats for 28 days resulted in devoid of abnormalities in the hematological parameters, gross behavior studies, macroscopic analysis, serum hormonal parameters, biochemical parameters. Further, the assessment of histopathology of hepato, and cardiac are devoid of toxicity in nature at the level of ethanolic extract of *Solanum torvum* L at 1000 mg/kg per day. The study provides scientific evidence for the future studies in *Solanum torvum* L.

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Conflicts of interest: None declared.

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