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Acute and Subacute Toxicity studies of the Ethanolic Extract of the Anti-Psoriatic Plant *Givotia Rottleriformis* Griff. Ex Wight. Bark

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ABSTRACT

The plant Givotia rottleriformis Griff. Ex Wight.. used in indigenous medicine in the treatment of rheumatism, dandruff and psoriasis. This study assesses acute and sub-chronic toxicity profile of ethanolic extract of Givotia rottleriformis bark using OECD guidelines. Toxicity was evaluated in Swiss albino mice after ingestions of the extract during one day (acute model) and in Wistar rats during 28 days (subacute model). The results showed that the LD50 of the extract is higher than 2000 mg/kg. There were no any behavioral alterations or mortality recorded in ethanolic extract of G. rottleriformis treated groups. In subacute treatment, there were no statistically significant changes in behavior, body weight, hematological and biochemical parameters. No detectable abnormalities were found in the histopathology of the selected organs. No mortality was recorded in experimental animals treated with the drug orally at a dose of 400 mg/kg. The results suggest that the plant seems to be destituted of toxic effects in mice and rat.

Keywords: *Givotia rottleriformis, Acute Toxicity, Sub-Acute Toxicity, Anti-Psoriasis.*

INTRODUCTION

The traditional systems of medicine such as the Ayurveda, Siddha and Unani have been a treasure trove for development of majority of modern medicines. Also the medicinal research relies on ethnobotany and ethnopharmacognocy for discovery of new molecules for that conventionally result in drugs developments [1]. World Health Organization (WHO) estimates that approximately 80 % of the developing world's population is using traditional medicine for primary healthcare [2]. However, there is a prevalent misunderstanding that herbal medicines are devoid of toxic effects [3]. Adverse effects of herbs have been reported including allergic reactions, hepatotoxicity [4], nephrotoxicity [5], cardiac toxicity [6], neurotoxicity [7] and even death [8] have been reported. Therefore, a pre-clinical toxicity study is indispensible to validate their safe medicinal use.

Givotia rottleriformis Griff. Ex Wight moderately sized tree of the family Euphorbiaceae distributed in limited areas of the forests of Tamil Nadu, Andhra, Karnataka, West Bengal and coastal Sri Lanka. The bark and seeds of the tree are used in indigenous medicine in the treatment of rheumatism, dandruff and psoriasis [9, 10]. The seeds yield oil that is valuable for lubricating fine machinery [11]. Phytochemical constituents of *Givotia rottleriformis* bark have been studied extensively and their analysis had revealed presence of flavonoids, alkaloids, steroids, tannins, terpenoids and saponins. The present study evaluates possible toxicity of the ethanol extract of *Givotia rottleriformis* bark using Economic Co-operation and Development (OECD) guidelines.

MATERIALS AND METHODS

Plant material:

The Plant specimen *Givotia rottleriformis* bark was collected from the forest of Athoor, Tamil Nadu for the proposed study was collected from Chennai, Tamil Nadu during the month of July 2010. It was identified and authenticated by the botanist Dr. P. Jayaraman, Director, Plant Anatomy Research Center, (PARC) Tambaram, Chennai. A voucher specimen No. PARC/2011/2140 has been deposited for further references.

Extraction:

About 500 gm of the bark of *Givotia rottleriformis* powder was extracted separately using a soxhlet apparatus with Ethanol (70% v/v) (18 h). The extracted solution was filtered and concentrated in a rotary evaporator under reduced pressure (rotary vacuum flash evaporator). The total extract (10 g) thus obtained was subjected to toxicity studies.

Experimental animals:

Healthy Wistar Albino rats of either sex, weighing about 250-200 g and Swiss Albino mice of either sex weighing 25-30 g were procured from Animal House. The entire process was approved by the Institutional Animal Ethical Committee which is certified by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) IAEC/52/2012. The animals were kept in clean and dry polycarbonate cages and maintained in a well ventilated animal house with 12 hrs light - 12 hrs dark cycle. The animals were fed with standard pellet diet and water was given ad libitum. For experimental purpose, the animals were kept fasting overnight but allowed for access to water.

Acute toxicity studies:

Acute toxicity study was performed according to OECD guidelines 423 (Organization of Economic Co-Operation and Development) [12]. It is a stepwise procedure with three animals of single sex per step. Depending on the mortality and morbidity status of the animal, on average of 2-4 steps may be necessary to allow judgment on the test substance. The procedure is to fix a minimal number of animals, which allows acceptable database scientific conclusion. The method uses different defined doses (5, 50, 500, 2000 mg/kg body weight) and the results allow a substance to be ranked and classified according to the "Globally Harmonized System" (GHS) for the classification of extracts which cause acute toxicity.

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Procedure:

Three healthy, Swiss Albino mice weighing 25-30 gms were selected for the study. The mice were fasted over-night and provided with water ad libitum. Following the period of fasting, the animals were treated with the ethanolic extract at the dose of 2000 mg/kg body weight orally. As most of the crude extracts possess LD_{50} value more than 2000 mg/kg body weight and this was used as starting dose. After oral administration, the mice were observed on hourly basis for 24hrs to access mortality and to detect any changes in the autonomic or behavioral responses viz. alertness, aggressiveness, spontaneous activity, irritability, tremor, corneal reflex, salvation, urination, respiration and convulsion etc.

The mice were observed regularly for 14 days to note the mortality or toxic symptoms. Since there was no death as per the guidelines, the study was repeated with the same dose to confirm the results. The flow chart depicts the procedure adopted for this method.

Sub-acute toxicity study:

In a 28-days sub acute toxicity study, sixteen either sex Wistar rats were divided into three groups of 6 rats each. Group I that served as normal control was administered with distilled water (p.o.) while groups II, III, were administered daily with the ethanolic extract of the *Givotia rotlleriformis* bark at a dose 200 and 400 mg/kg body weight based on the acute toxicity results respectively. The animals were then observed daily for gross behavioural changes and any other signs of subacute toxicity. The weight of each rat was recorded on day 0 and weekly throughout the course of the study, food and water consumption per mice was calculated. At the end of the 28 days they were fasted overnight, each animal was anaesthetized with diethyl ether, following which they were then dissected and blood samples were obtained by cardiac puncture into heparinised tubes. The blood sample collected from each rat was centrifuged with 3000 X g at 4°C for 10 min to separate the serum and used for the biochemical assays.

Haematological Parameters:

At the end of 28 days, blood samples were collected from overnight fasted animals through retro-orbital sinus puncture in ethylene diamine tetra acetic acid (EDTA) coated vials and plasma was separated by cold centrifugation (Plasto Crafts Superspin-R centrifuge) at 3000 rpm for 10 min. Blood was also collected for the analysis of haematological parameters such as white blood cell (WBC) count, red blood cell (RBC) count, haemoglobin (Hb) levels, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) using BC 2300 Haematology Analyzer (Shezhen Mindray Biomedical Electronics Co., Ltd., China).

Biochemical parameters:

Aspartate aminotransferase (AST), alanine aminotransferase (ALT) [13], total protein (liver damage) [14], alkaline phosphatase (ALP) [15] and urea and creatinine (kidney damage) [16] were analyzed using commercially available kits (Recon diagnostic Ltd., Vadodara, India). Also, plasma glucose and lipid profile [17] [total cholesterol (TC), triglyceride (TG) and high density lipoprotein (HDL-C)] were assessed and low density lipoprotein (LDL-C) and very low density lipoprotein (VLDL-C) were calculated.

Relative organ weights and histopathology:

Animals were later sacrificed by cervical dislocation under mild ether anesthesia for autopsy and liver, kidney, heart, lung and spleen were excised, rinsed in 0.9 % saline and weighed. After sacrifice, organ weights (lungs, heart, liver, kidney, brain, stomach, ovary, testes, and spleen) were recorded and relative organ weights (ROW) were calculated as follows.

$$ROW = \frac{\text{Absolute organ weight (g)}}{\text{Body weight on the day of sacrifice (g)}} X\ 100$$

Tissue pieces of vital organs (heart, liver and kidneys) were fixed in 10 % para formaldehyde for paraffin histology and processed in paraffin embedding as per the standard protocol. 7 µm thick sections of each tissue were stained with hematoxylin and eosin, and observed for possible histopathological damages.

Statistical analysis:

Values were represented as mean ± SEM. Data were analysed using one-way analysis of variance (ANOVA) and group means were compared using the Tukey-Kramer Multiple Comparison test using Instat-V3 software. *P* values < 0.05 were considered significant.

RESULTS AND DISCUSSION

Acute toxicity study:

During the acute toxicity study, the ethanolic extract was administered orally and animals were observed for mortality and behavioral responses. There was no mortality observed even at 2000 mg/Kg for the extract and at 500 mg/Kg for the compounds. All the animals were found to be normal and there were no gross behavioral changes till the end of the observation period. This observation revealed that the ethanolic extract of the *Givotia rotlleriformis* bark was found to be very safe up to 2000 mg/kg of body weight known as maximum tolerated dose (MTD) by acute toxicity model study as per OECD guidelines 423. Hence from this 1/10th and 1/5th of MTD was selected and the effective doses were fixed as 200 and 400 mg/kg for the further pharmacological studies. Similarly, the isolated compound was found to be very safe up to 500 mg/kg of body weight known as maximum tolerated dose (MTD) by acute toxicity model study as per OECD guidelines 423. Hence from this 1/10th of MTD was selected and the effective doses were fixed as 200 and 400 mg/kg for the further pharmacological studies.

Sub acute toxicity study:

The results of haematological investigations conducted on day 28, revealed following significant changes in the values of different parameters investigated when compared with those of respective controls; however, the increase or decrease in the values obtained was within normal biological and laboratory limits.

Sub-acute oral toxicity studies have provided information on drugs that can possibly pose health risks. Twenty eight days of oral administration of ethanol extract of *G. rotlleriformis* did not result in death of the animals. No sign of observable toxicity was detected during the experimental period. Rats treated with various doses of ethanolic extract of *G. rotlleriformis* had a progressive weight gained. This increase in weight is significantly different ($P< 0.05$) from that of the control (**Table 1**). The progressive increase in body weight at doses of 200 and 400 mg/kg of rats during 28 days of drug administration may indicate the improvement of the nutritional state of the animal. The growth response effect could be as the result of increased food and water intake.

The levels of serum analytes such as glucose, cholesterol, AST and ALT, ALP, total protein, albumin, urea and creatinine were not significantly different between the control and the experimental groups of rats when fed with ethanol extract (**Tables 1**). High levels of AST and ALT are reported in liver diseases or hepatotoxicity [18] (Brautbar and Williams, 2002). Plasma AST, ALT and bilirubin of ethanol extract of *G. rotlleriformis* treated groups were comparable thus indicative of normal functional status of liver. Renal dysfunction can be assessed by concurrent measurements of urea and creatinine and their normal levels reflect at reduced likelihood of renal problems [19] (Davis and Bredt, 1994). In the present study, changes in plasma urea and creatinine levels in ethanol extract treated groups showed no significant differences on a dose dependent manner indicating a normal renal function. The results were tabulated in **Table 2**.

The haematopoietic system is one of the most sensitive targets for toxic compounds and hence it is mandatory to record any possible alterations resulting from a test substance [20]. Change in haematological parameters has a higher predictive value, when the data of drug toxicity on animal studies are translated for clinical usage [21] (Adeneye and Adokiye, 2008). A normal haematological profile of ethanol extract treated groups also further justified the non-toxic nature of plant extract. The results were tabulated in **Table 3**.

Food consumption of control and treated animals was found to be comparable throughout the dosing period of 28 days. Organ weight data of animals sacrificed at the end of the dosing period was found to be comparable with that of respective controls. No abnormal changes were observed in organ mass with respect to

body mass of ethanol extract fed rats when compared with control. (**Table 4**).

Histopathological examination of brain, liver and lung did not reveal any significant abnormality when compared with control. (**Fig. 1**) Based on these findings, it can be concluded that the effect of ethanol extract of *G. rotlleriformis* (200 mg/Kg and 400 mg/Kg) treated via oral route over a period of 28 days have no toxic effect on rats. In light of these findings, we may conclude that ethanol extract of *G. rotlleriformis* is not toxic in all the doses studied herein. This study is the first report that evaluates toxicity of ethanol extract of *G. rotlleriformis* and defines it as non-toxic up to a dose of 2000 mg/kg body weight.

Table No. 1: The effect of the ethanol extract (70%v/v) of the *G. rotlleriformis* bark on weight changes in control and treated rats.

Group	Initial Weight	Body Weight (g)			
		1 Week	2 Weeks	3 Weeks	4 Weeks
Normal	158.20±10.20	162.11±7.78	162.66±12.22	163.32±14.02	165.68±9.88
EE200 mg/kg	160.25±8.22	163.55±14.12	172.15±11.45	185.14±12.30	190.20±10.46*
EE400 mg/kg	162.74±10.22	164.32±11.20	175.29±10.25	186.28±11.94	193.34±4.11*

EE: Ethanol Extract; n = 6; values are expressed as mean ± SEM

Data were analyzed by one-way ANOVA followed by Tukey multiple comparison test. The values are *P< 0.05 when compared against control.

Table No. 2: The effect of ethanol extract of the *G. rotlleriformis* bark on haematological studies

S. No	Parameters	Control	EE 200 mg/Kg	EE 400 mg/Kg
1	**RBC (10^2/µL)**	8.32±0.68	8.20±0.38	8.64±0.42
2	**Hb (g/dl)**	16.22±1.02	15.88±0.24	16.38± 0.16
3	**MCV (fL)**	64.61±6.66	60.54±5.88	61.22±5.98
4	**MCH (pg)**	18.98±4.62	17.86±1.68	19.12±2.28
5	**MCHC (gm/dl)**	36.61±2.86	36.84±2.54	36.08±2.22
6	**WBC (10^2/µL)**	8.2±2.08	7.7±1.88	7.9±2.46
7	**Neutrophils (%)**	22.24±2.54	23.68±1.82	23.72±1.26
8	**Eosinophils (%)**	1.22±0.4	1.38±0.86	1.28±0.66
9	**Basophils (%)**	0.0±0.00	0.1±0.02	0.00±0.00
10	**Lymphocyte (%)**	68.12± 0.24	68.86±0.26	69.02±1.12
11	**Monocyte (%)**	2.06±0.22	2.46±0.48	2.82±0.98

n = 6; values are expressed as mean ± SEM. **RBC**: Red blood corpuscle, **Hb:** Haemoglobin; **MCV:** Mean corpuscular volume, **MCH**: Mean corpuscular haemoglobin, **MCHC**: Mean corpuscular haemoglobin concentration, **WBC:** white blood corpuscle.

Table No. 3: The effect of ethanol extract of the *G. rotlleriformis* bark on Biochemical Profiles

Parameter	Control	EE 200 mg/Kg	EE400 mg/Kg
Glucose (mg/dl)	104.61±4.35	105.51±5.42	114.10±4.33
Cholesterol (mg/dl)	44.15±1.42	42.27±1.23	41.50±2.41
Triglyceride (mg/dl)	78.72±2.15	85.84±2.12	86.72±4.14
HDL (mg/dl)	105.12±8.04	104.8±8.71	113.12±7.27
LDL (mg/dl)	78.32±2.31	79.40±3.42	79.58±2.35
Protein (mg/dl)	7.11±0.30	7.82±0.28	7.71±0.32
Albumisn(mg/dl)	3.10±0.20	3.18±0.22	3.12±0.24
Globulin(mg/dl)	3.16±0.07	3.15±0.06	3.16±0.08
Creatinine (mg/dl)	0.20±0.04	0.27±0.04	0.24±0.05
Urea (mg/dl)	58.60±0.80	62.47±0.24	61.60±0.56
AST IU/L	54.10±1.12	55.21±2.31	55.10±2.20
ALT IU/L	25.42±1.76	26.10±2.28	22.18±2.36
ALP IU/L	62.26±3.30	65.12±3.11	62.10±1.54

n = 6; values are expressed as mean ± SEM. Data were analyzed by one-way ANOVA followed by Tukey multiple comparison test.

HDL: High density lipoprotein; **LDL**: Low density lipoprotein; **AST**: Aspartate transaminase; **ALT:** Alanine transaminase; **ALP:** Alkaline phosphatase

Table No. 4: Effect of ethanol extract of the *G. rotlleriformis* bark on organ weight

Organ	Control	EE 200 mg/Kg	EE 400 mg/Kg
Liver (g)	3.00±0.10	3.10±0.12	2.92±0.09
Heart (g)	0.30±0.04	0.30±0.04	0.29±0.02
Lung (g)	0.44±0.12	0.44±0.10	0.45±0.10
Spleen (g)	0.45±0.04	0.46±0.04	0.46±0.05
Ovary (g)	1.62±0.28	1.64±0.20	1.66±0.18
Testes (g)	2.32±0.12	2.34±0.11	2.30±0.10
Brain (g)	1.22±0.14	1.24±0.10	1.32±0.12
Kidney (g)	0.81±0.05	0.78±0.05	0.75±0.05
Stomach (g)	1.12±0.11	1.14±0.12	1.10±0.10

n = 6; values are expressed as mean ± SEM. Data were analyzed by one-way ANOVA followed by Tukey multiple comparison test. The values are NS-Non significant when compared against control.

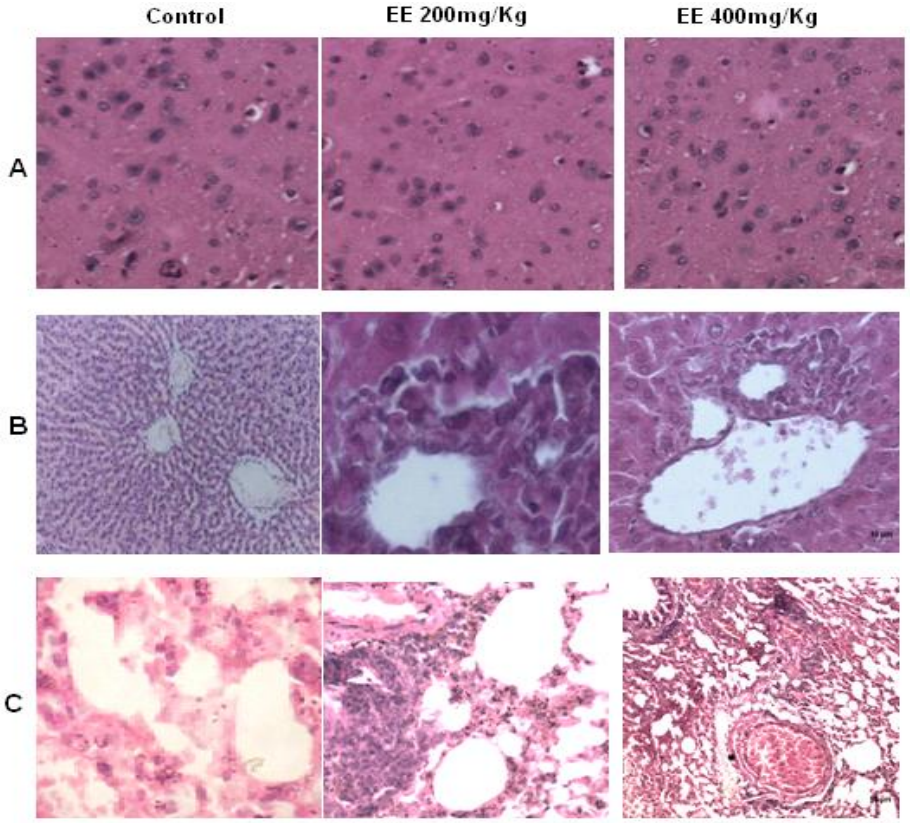

Fig. 1: Photomicrographs of the sections of the (A) Brain, (B) liver; (C) Lung of control, ethanol extract (EE 200 mg/Kg); ethanol extract (EE 400 mg/Kg) administered mice for 28 days

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