Toxicity assessment of ethanol extract of Solanum villosum (Mill) on wistar albino rats.

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ABSTRACT

Purpose: To evaluate the potential toxicity of ethanol extract of the medicinal plant *Solanum villosum* (Mill). **Methods:** Ethanol extract of *S. villosum* administered orally at ranges of doses 100, 200, 400, 600 and 800 mg/kg/bw to assess its impact on biochemical indices of Wistar albino rats. Hematological profile, biochemical assays, antioxidant and lipid peroxidation assays were compared between control and experimental animals. An acute toxicity test was performed in rats at different concentration of ethanol extract of *S. villosum* in order to establish the approximate oral lethal dose (LD) 50. **Results:** No mortality occurred during the two weeks experimental period, in both control and experimental groups. The changes in biochemical parameters were statistically insignificant at $p<0.05$ levels. The treated rats showed that very less toxic symptoms only after 800 mg/kg/bw. These observations were supported by hematological and liver function markers. **Conclusions:** The medicinal plant *Solanum villosum* can be administered orally at a dose range of 200 mg/kg/bw was very effective and without any side effects. Ethanol extract of *S. villosum* is not toxic and therefore it may be used safely in clinical trials. It is the first documented report about the plant *Solanum villosum* (Mill) in the toxicity assessment study.

Keywords: *Solanum villosum*, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), bw – body weight, lethal dose.

1. INTRODUCTION

Herbal medicines are popular and extensively used worldwide. In many places, they offer a more wide available and more affordable alternative to pharmaceutical drugs and natural food supplements. The acute, sub-acute and chronic toxicity studies on medicinal plants should be obtained in order to increase the confidence in its safety to human, particularly for use in the development of pharmaceuticals drugs [1]. Herbal plants may be administered in most disease conditions over a long period of time without proper dosage monitoring and consideration of toxic effects that might result from such prolonged usage [2].

Upon administration of a chemical substance to a biological system, different types of interactions can occur resulting into a series of dose-related responses. In most cases these responses are desired and useful, but there are a number of other effects which are not advantageous, and which could be harmful to the patients. Toxicology is the aspect of pharmacology that deals with the adverse effect of bioactive substances on living creatures along with their diagnosis and clinical use [3].

In order to develop and establish the safety and efficacy level of a new drug, necessary toxicological studies are conducted on animals, e.g. mice, rat, guinea pigs, dog, monkey, usually under varying conditions and drug levels [4]. Depending on the duration of drug exposure to animals toxicological studies may be three types (acute, sub-acute and chronic toxicological studies) [5].

The plant, *Solanum villosum* (Mill) belongs to *Solanaceae* family. *Solanaceae* is to belongs, a cosmopolitan family containing many essential vegetables and fruits. Within this family, *Solanum* constitutes the largest and most complex genus; it is composed of more than 1500 species, many of which are also economically important throughout their cosmopolitan distribution. The section *Solanum*, centering on the species commonly known as the black, garden or common nightshade, *Solanum villosum* (Mill), is sub glabrous to villous annuals, up to 50 cm high. The leaves are wavy or lobed margined, the bases are cuneate, 4 – 10 and 4 – 8 cm wide, and the apex is acute. Stem rounded to angled, almost glabrous to pubescent with appressed hairs. Leaves rhombic to ovatelanceolate. Inflorescence simple, umbellate to slightly lax solitary cymes, 3 to 5 lowered, rarely 10 flowered. Berries usually longer than wide, occasionally globose, red, orange or yellow [6].

S. villosum have been shown to contain active compounds such as alkaloids, flavonoids, steroids, tannins, phenols and reducing sugars [1]. *S. nigrum* is an important ingredient in traditional Indian medicines. Infusions are used in dysentery, stomach complaints and fever. The juice of the plant is used on ulcers and other skin diseases.

The fruits are used as a tonic, laxative, appetite stimulant; and also for treating asthma and excessive thirst [7]. Traditionally the plant was used to cure tuberculosis [8].

2. MATERIALS AND METHODS

2.1. COLLECTION AND PROCESSING OF PLANT MATERIAL

The present study involves the use of the plant *Solanum villosum* (Mill), which is studied extensively for phytochemicals. No studies have been carried out on this plant to assess its phytochemical chemical activity. The plant, *Solanum villosum* (Mill) were collected from Thadagam hills at Coimbatore district, Tamilnadu, India. The specimen sample was identified and authenticated by Dr.G.V.S. Murthy, Joint Director, Botanical Survey of India, Southern Regional Centre, Coimbatore, Tamilnadu, India. The identification No. BSI/SRC/5/23/2014-15/Tech/255. The *Solanum villosum* leaves were collected washed thoroughly and shade dry on a filter paper. Dried leaves were taken and powdered for extraction. The shade dried powder material of the leaves of *S. villosum* was extracted with ethanol in a soxhlet apparatus. The ethanol extract was then distilled, evaporated, and dried under vacuum.

2.2.TOXICITY ASSESSMENT

Acute and toxicity studies was carried out according to Organization for Economic Cooperation and Development (OECD) guidelines 423 (OECD, 1987). Ten-week-old male Wister Albino strain rat weighing 210 ± 6.64 g were used for the study. The rats were procured from the Small Animal's breeding center of Kerala Agricultural University, Mannuthy, Thrissur. The rats were grouped and housed in polyacrylic cages with not more than six animals per cage, and maintained at temperature of 25 ± 2 °C; relative humidity of 55 ± 5%, 14 /10 h, dark/ light cycle, with free access to feed and water (ad libitium). The rats were acclimatized to laboratory conditions for 10 days before commencement of the experiment. All studies will be conducted in accordance with Committee for the Purpose of Control and Supervision on Experiments on Animals (659/02/a/CPCSEA), "CPCSEA Guidelines for Laboratory Animal Facility".

2.3.EXPERIMENTAL DESIGN

After the adaptation period, the animals were divided into six groups with six animals in each group. Rats in group I served as normal control while those in group II, III, IV, V and VI were fed orally with ethanol extract of *S. villosum* at doses of 100, 200, 400, 600 and 800 mg/kg/bw, respectively, for 14 days. After the experimental period, animals in different groups were sacrificed by cervical dislocation. Blood was collected in two different tubes from an incision made in the jugular veins. One tube had anticoagulant and the other tube without anticoagulant (to separate serum for various biochemical estimations).

2.4.HEMATOLOGICAL ASSAY

On the 14th day the blood samples were collected from external jugular vein under mild chloroform anesthesia for the estimation of hematological parameters like Hb [9], RBC, WBC [10], and platelets [11] counts were performed.

2.5.BIOCHEMICAL PARAMETERS ASSAYED

Biochemical parameters such as aspartate transaminase (AST) [12], alanine transaminase (ALT), alkaline phosphatase (ALP) [13], Bilirubin [14], Protein [15], Cholesterol [16], HDL [17], Triglycerides [18], Urea [19], Creatinine [20], Uric acid [21], were assayed.

2.6.ESTIMATION OF LIPID PEROXIDATION

Lipid peroxidation in liver was estimated colorimetrically by measuring thiobarbituric acid reactive substances (TBARS) by the method of Uchiyama and Mahara (1978) [22].

2.7.ASSAY OF ENZYMIC ANTIOXIDANTS

The centrifuged serum samples were used for the analysis of antioxidant enzymes such as super oxide dismutase (SOD) [23], and catalase (CAT) [24], were assayed.

2.8.STATISTICAL ANALYSIS

Results were expressed as mean ± SD of six animals in each group. Statistical significance was determined by one-way analysis of variance (ANOVA) and post hoc least-significant difference (LSD) test

3. RESULTS

An acute toxicity test was performed in Rats at different concentration of ethanol extract of *S. villosum* in order to establish the approximate oral lethal dose (LD50). No mortality occurred during the two weeks experimental period, in both control and experimental groups.

3.1.BODY WEIGHT

The results of the body weight of the rats in the toxicity studies are shown in the table 1. The data of the body weight showed that the there was no significant change in the entire period of the toxicity study. In addition

there was continual decrease in the body weights of the rats on increase the dose 600 and 800 mg/kg/bw, respectively, for 14 days. Furthermore, no rats died during the period of study.

3.2.HEMATOLOGICAL ASSAY

Upon examination of the hematological profile (Table 2), Hb, PCV, RBC, WBC and platelet counts were found to be near normal in group II and III (treated with 100 and 200 mg/kg/bw) compared to the control group. The results indicate the non-toxic nature of the extract and between the two doses, 200 mg/kg bw was found to be more effective. Table 2 shows that the level of hemoglobin, packed cell volume, WBC, RBC and platelets counts. It was also noted that the significant decreased levels of hemoglobin, packed cell volume and RBC counts in group VI when compared to control groups.

3.3.BIOCHEMICAL ASSAY

Significant increases in ALP and Bilirubin were observed from Group VI (800 mg/kg/bw) as compared to normal control animals (Group I). The liver function test markers increased the levels of AST and ALT compared to control groups. (Table 3). Serum ALP and bilirubin are the most sensitive markers employed in the diagnosis of hepatic damage. Ethanol extract of *S. villosum* at doses of 100mg/Kg/bw and 200 mg/Kg/bw (Group II-III) did not show any effect on levels of ALP and bilirubin concentrations as compared to control animals (Group I). Leakage of enzymes into the surrounding confirms the toxicity of the dosage above 600 mg/kg/bw. The levels of protein was significantly decreased in $p<0.05$ levels in group VI compared to group I.

The lack of significant alterations in the levels of Total cholesterol, HDL and Triglycerides, which are good indicators of lipid metabolism, suggests that acute administration of extract does not altered the normal lipid metabolism of the animals. The urea, creatinine and uric acid levels were slightly increased in VI group when compared to control. As shown in Table 5, biochemical components such as urea, uric acid and creatinine in serum were determined after treatment and compared to that of control rats; there were slight changes which are statistically insignificant. This indicates that treatment of rats with the extract has no adverse effect on kidney functions.

3.4.LIPID PEROXIDATION AND ENZYMIC ANTIOXIDANTS

Increased in levels of LPO were seen from group VI (800 mg/kg/bw) when compared with control animals (group I) (Table 6). No significant differences were noted between groups II & III and the control (group I). The levels of SOD and CAT increases were seen from group II to IV (100 – 400 mg/kg/ bw) when compared to control group I. These results further show that dosage below 400 mg/kg/bw is nontoxic. So Ethanol extracts of *S. villosum* may be toxic at dosage above 800 mg/kg/bw.

4. DISCUSSION

Several compounds have been isolated from different fractions of *S.nigrum* which have shown pharmacological relevance to the observed effects of whole plant preparation of *S.nigrum*. Acetic acid, tartaric acid, malic acid and citric acid were identified as the major organic acids in *S.nigrum* [25]. Tartaric acid and citric acid however, were said to be most important in adaptive responses by *S. nigrum* to environmental stresses. High concentrations of solanine, a glycoalkaloid is found in most parts of Solanum species, but highest levels are found in unripe berries. However, when ripe, the berries are the least toxic part of the plant and are sometimes eaten without ill effects. Similarly, the solanine increases in the leaves as the plant matures. The absolute amount of alkaloid per leaf increased during leaf development, whereas, the concentration declined. Small unripe fruits of *S. nigrum* had a high concentration of solasodine, but both the concentration and the absolute amount per fruit decreases with fruit maturation [26].

Despite its beneficial effects *S. nigrum* may have some toxic effects. *S. nigrum* contains two main cytotoxic glycoalkaloids, solanine and solasodine, thus are poisonous when taken in large quantities with the highest concentrations of solanine occurring in the immature fruit but decreases on ripening [27]. Glycoalkaloids clearance takes more than 24 hours, which implicates that the toxicants may accumulate in case of daily consumption [28]. A condition that may be more marked during liver damage since glycoalkaloids concentrate mainly in the liver [29].

Daily clinical observations are of major importance as well as the final observations (end point) in repeated dose studies [30]. There were no mortality with any obvious signs of toxicity such as tremor, weakness and sluggishness, refusal of feeds, hair-loss and coma were observed in any of the animals throughout the duration of our observation.

Body weight changes are an indicator of adverse side effects, as the animals that survive cannot lose more than 10% of the initial body weight [31,32,33].

Blood is a good indicator to determine the health of an organism. It also acts as pathological reflector of the whole body hence hematological parameters are important in diagnosing the functional status of exposed animal to toxicant [34]. The hematopoietic system is one of the most sensitive targets of toxic compounds and is an important index of physiological and pathological status in man and animals [35].

Damage and destruction of the blood cells are inimical to normal functioning of the body. It is an important index of physiological and pathological status of man and animal and the parameters measured are packed cell volume, hemoglobin, white blood cell count and platelets count [36]. The normal ranges of these parameters can be altered by the ingestion of some toxic plants [37].

The biochemical evaluation is important since there are several reports of liver and kidney toxicity related to the use of phytotherapeutic products [38,39]. In preclinical toxicity studies, renal changes are particularly liable to occur because of the high doses given and the fact that the kidneys eliminate many drugs and their metabolites [40, 41]. Among the biochemical parameters evaluated, AST, ALT and ALP are considered markers of liver function [42]. Hepatocellular damage is characterized by a mutual rise in serum levels of AST and ALT. But since ALT is localized primarily in the cytosol of hepatocytes, this enzyme is considered a more sensitive marker of hepatocellular damage than AST and within limits can provide a quantitative assessment of the degree of damage sustained by the liver [43]. ALP is most often measured to indicate bile duct obstruction [44].

Serum AST and ALT, are the most sensitive markers employed in the diagnosis of hepatic damage because these are cytoplasmic in location and are released into the circulation after cellular damage [45]. The site-specific oxidative damage of some of the susceptible amino acids of proteins is now regarded as the major cause of metabolic dysfunction during pathogenesis [46].

The cholesterol lowering effect of the plant extract is possibly associated with a decrease in intestinal absorption of cholesterol resulting in an increase in fecal excretion of neutral lipids [47]. It is well established that increased levels of blood cholesterol especially low density lipoprotein cholesterol (LDLc) is an important risk factor for cardiovascular complications since it favors lipid deposition in tissues including blood vessels. Evidences from lipid lowering trials have clearly established that reduction of total cholesterol or low density lipoprotein cholesterol (LDLc) is associated with decreased risk of atherosclerosis and coronary heart disease [48, 49]. Furthermore, Epidemiological studies have also shown an inverse correlation between high density lipoprotein cholesterol (HDLc) level and the risk of cardiovascular disease [50].

Membrane lipids succumb easily to deleterious actions of reactive oxygen species [51], and the measurement of lipid peroxidation is a convenient method to monitor oxidative damage [52]. Under normal physiological conditions, low concentrations of lipid peroxides are found in tissues. Free radicals react with lipids causing peroxidation, which further enhances lipid peroxidation. An increase in lipid peroxidation indicates serious damage to cell membranes affecting their fluidity as well as inhibiting several enzymes and cell function [53].

The enzymic antioxidants are the natural defense systems against lipid peroxidation. SOD and CAT enzymes are important scavengers of superoxide ion and hydrogen peroxide. These enzymes prevent generation of hydroxyl radical and therefore protect the cellular constituents from oxidative damage [54]. Superoxide dismutase (SOD) is one of the most important enzymes in the antioxidant defense system of the body. The major function of SOD is to catalyze the conversion of superoxide anion radicals (the first product of oxygen radical formation) to H_2O_2 and hence reduces the toxic effects due to this radical or other free radicals derived from secondary reactions. Catalase (CAT), which is present virtually in all mammalian cells, is responsible for the removal of H_2O_2 [55].

5. CONCLUSIONS

Based on this study, we conclude this is the first report of documentation about the plant on various biochemical investications using rats. The medicinal plant *Solanum villosum* can be administered at a dose range of 200 mg/kg/bw without any side effects. *Solanum villosum* contains two main cytotoxic glycoalkaloids, solanine and solasodine, thus are poisonous when taken in large quantities. Since, the toxicity studies in experimental animals cannot always be totally extrapolated to humans, and a reasonable estimate of the self-administered dose is difficult to make such as that applied during traditional use of this plant, additional clinical toxicological evaluations need to be performed to define a safe dose and protect the population from possible toxic effects of the plant. Since there are no toxic effects produced by the extract, further clinical studies would be conducted to prove the efficacy of *Solanum villosum* extract in the treatment of various diseased complications.

6. ACKNOWLEDGEMENTS

The authors are thankful to our Secretary and Joint Secretary of Kongunadu Arts and Science College, Coimbatore, Tamilnadu, India for providing facilities and encouragement.

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Table – 1 Mean body weights of the rats before and after 14 days of treatment with ethanol extract of *Solanum villosum*

	Body weights (grams)	
Groups	**Before treatment**	**After treatment**
Group l	210.00 ± 5.47	219.00 ± 3.74
Group ll	208.33 ± 6.83 ^{ns}	217.33 ± 6.02 ^{ns}
Group lll	209.17 ± 4.91 ^{ns}	218.00 ± 4.19 ^{ns}
Group IV	211.67 ± 2.58 ^{ns}	220.33 ± 2.33 ^{ns}
Group V	210.83 ± 5.84 ^{ns}	215.83 ± 5.84 ^{ns}
Group Vl	208.33 ± 7.63 ^{ns}	213.33 ± 7.63 ^{ns}

Values are expressed as mean ± SD of six animals in each group

Statistical Comparison: Group I vs Group II, III, IV, V & VI

* - Significant at 5% ($p<0.05$) ns – not significant

Table – 2 Effect of ethanol extract of Solanum villosum on the hematological parameters in blood of control and experimental rats

Groups	**Hemoglobin (g%)**	**PCV (%)**	**WBC(10^6 µl)**	**RBC(10^{12} µl)**	**Platelet(10^9 µl)**
Groups l	13.5 ± 0.36	40.16 ± 0.98	7.74 ± 0.44	6.94 ± 0.47	5.29 ± 0.45
Groups ll	13.41 ± 0.46 ^{ns}	40.16 ± 1.47 ^{ns}	7.71 ± 0.49 ^{ns}	6.90 ± 0.32 ^{ns}	5.38 ± 0.24 ^{ns}
Groups lll	13.36 ± 0.36 ^{ns}	40.50 ± 1.37 ^{ns}	7.70 ± 0.41 ^{ns}	6.72 ± 0.52 ^{ns}	5.49 ± 0.36 ^{ns}
Groups lV	13.41 ± 0.33 ^{ns}	40.00 ± 1.26 ^{ns}	7.90 ± 0.53 ^{ns}	6.58 ± 0.58 ^{ns}	5.66 ± 0.41 ^{ns}
Groups V	13.25 ± 0.27 ^{ns}	39.50 ± 0.54 ^{ns}	8.11 ± 0.24 ^{ns}	6.54 ± 0.52 ^{ns}	5.75 ± 0.52 ^{ns}
Groups Vl	11.83 ± 0.51 *	35.50 ± 1.76 *	8.11 ± 0.44 ^{ns}	5.58 ± 4.9 *	5.25 ± 0.52 ^{ns}

Values are expressed as mean ± SD of six animals in each group

Statistical Comparison: Group I vs Group II, III, IV, V & VI

* - Significant at 5% ($p<0.05$) ns – not significant

Table – 3 Effect of ethanol extract of *Solanum villosum* on the Liver function markers in serum of control and experimental rats

Groups	**AST (U/L)**	**ALT (U/L)**	**ALP (U/L)**	**Bilirubin(mg/dl)**	**Protein(g/dl)**
Groups l	62 ± 7.68	66.91 ± 6.77	88 ± 8.63	0.09 ± 0.024	6.55 ± 0.21
Groups ll	64 ± 6.14 ^{ns}	66.88 ± 3.97 ^{ns}	96 ± 6.14 ^{ns}	0.08 ± 0.013 ^{ns}	6.43 ± 0.31 ^{ns}
Groups lll	60 ± 5.62 ^{ns}	66.43 ± 1.82 ^{ns}	102 ± 6.69 ^{ns}	0.09 ± 0.026 ^{ns}	6.35 ± 0.38 ^{ns}
Groups IV	63 ± 6.21 ^{ns}	69.62 ± 2.05 ^{ns}	108 ± 6.99 ^{ns}	0.12 ± 0.036 ^{ns}	6.23 ± 0.23 ^{ns}
Groups V	61 ± 5.22 ^{ns}	66.63 ± 6.41 ^{ns}	103 ± 7.09 ^{ns}	0.12 ± 0.03 ^{ns}	6.12 ± 0.14 ^{ns}
Groups Vl	68 ± 6.19 ^{ns}	70.38 ± 7.30 ^{ns}	127 ± 8.42 *	0.31 ± 0.21 *	6.00 ± 0.23 *

Values are expressed as mean ± SD of six animals in each group

Statistical Comparison: Group I vs Group II, III, IV, V & VI

* - Significant at 5% ($p<0.05$) ns – not significant

Table – 4 Effect of ethanol extract of Solanum villosum on the Lipid profile in serum of control and experimental rats

Groups	Cholesterol (mg/dl)	HDL (mg/dl)	Triglycerides (mg/dl)
Groups l	67.79 ± 5.49	56.47 ± 2.48	32.72 ± 5.57
Groups ll	68.35 ± 4.51 ns	58.03 ± 2.17 ns	32.30 ± 5.09 ns
Groups lll	68.00 ± 4.16 ns	56.34 ± 2.59 ns	32.02 ± 4.13 ns
Groups lV	67.68 ± 5.11 ns	54.38 ± 3.51 ns	30.68 ± 3.72 ns
Groups V	66.06 ± 4.04 ns	55.75 ± 3.29 ns	31.41 ± 4.02 ns
Groups Vl	65.86 ± 5.04 ns	53.75 ± 3.39 ns	31.59 ± 4.62 ns

Values are expressed as mean ± SD of six animals in each group

Statistical Comparison: Group I vs Group II, III, IV, V & VI

* - Significant at 5% ($p<0.05$) ns – not significant

Table – 5 Effect of ethanol extract of Solanum villosum on the Lipid profile in serum of control and experimental rats

Groups	Urea (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)
Groups l	40.96 ± 4.82	0.48 ± 0.14	1.35 ± 0.24
Groups ll	41.27 ± 3.35 ns	0.47 ± 0.14 ns	1.32 ± 0.22 ns
Groups lll	41.87 ± 3.05 ns	0.50 ± 0.13 ns	1.41 ± 0.18 ns
Groups lV	40.75 ± 3.82 ns	0.51 ± 0.13 ns	1.40 ± 0.11 ns
Groups V	45.23 ± 4.56 ns	0.54 ± 0.18 ns	1.51 ± 0.13 ns
Groups Vl	43.71 ± 5.86 ns	0.59 ± 0.09 ns	1.50 ± 0.29 ns

Values are expressed as mean ± SD of six animals in each group

Statistical Comparison: Group I vs Group II, III, IV, V & VI

* - Significant at 5% ($p<0.05$) ns – not significant

Table - 6 Effect of *Solanum villosum* extract on lipid peroxidation and antioxidants such as SOD and catalase in serum of control and experimental rats

Groups	SOD	CAT	LPO
Groups l	17.34 ± 0.27	4.47 ± 0.40	3.81 ± 0.18
Groups ll	17.37 ± 0.46 ns	4.55 ± 0.39 ns	3.66 ± 0.71 ns
Groups lll	17.92 ± 0.32 ns	4.66 ± 0.36 ns	3.19 ± 0.16 ns
Groups lV	17.81 ± 0.40 ns	4.74 ± 0.33 ns	3.21 ± 0.29 ns
Groups V	17.65 ± 0.34 ns	4.86 ± 0.27 ns	3.25 ± 0.16 ns
Groups Vl	17.43 ± 0.37 ns	4.14 ± 0.62 ns	3.90 ± 0.59 ns

Values are expressed as mean ± SD of six animals in each group

Statistical Comparison: Group I vs Group II, III, IV, V & VI

* - Significant at 5% ($p<0.05$) ns – not significant

Units:

SOD - 50% inhibition of nitrite / min / mg protein

CAT – n M of H_2O_2 decomposed / min / mg protein

LPO – n moles of MDA formed / mg protein