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Acute toxicity assessment of ethanolic leaf extract of *Macrotyloma uniflorum* L. on Wistar albino rats

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Abstract

Macrotyloma uniflorum L. belonging to the family fabaceae is traditionally used in folklore medicine for the treatment of various ailments. To validate its use in traditional medicine it is important to evaluate its toxicity in the animal system. The aim of this study is to determine the toxicity of ethanolic extract of *M. uniflorum* leaves. Acute toxicity test was conducted by single oral administration of 50, 250, 500, 1000 and 2000 mg/kg body weight of the animals and they were observed for toxicity signs for a period of 14 days. At the end of experiment, the effects of *M. uniflorum* on serum parameters such as glucose, urea, uric acid, creatinine, cholesterol, triglycerides, HDL, SGOT (Serum Glutamate Oxaloacetate Transaminase), SGPT (Serum Glutamate Pyruvate Transaminase) and total protein were analyzed. Histological analyses were also carried out. In acute toxicity study no signs of toxicity, behavioral changes and mortality were observed in the test groups of animal during the experimental period. The serum level of glucose, urea, uric acid, creatinine, SGOT, SGPT, and protein were not altered by the administration of *M. uniflorum.* Histopathology study indicates the normal architecture of liver and kidney. Overall, the results suggest that, the oral administration of *M. uniflorum* ethanolic leaf extract did not produce any significant toxic effect in rats. On the whole this study proved the safety of *M. uniflorum* on Wistar albino rats.

Keywords: Acute toxicity, *M. uniflorum*, Biochemical parameters.
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Introduction

Traditional and alternative medicines are extensively practiced in the prevention, diagnosis, and treatment of various illnesses. It has attracted increasing public attention over the past 20 years as this type of medicine is easily accessible in some regions.[1,2,3,4] Medicinal plants have played a considerable role in a variety of ancient traditional systems of medicine. They are rich sources of bioactive compounds and thus serve as an important raw material for drug production and have turned out to be a target for the search of new drugs.[5,6,7]

Toxicology is the significant aspect of pharmacology that deals with the adverse effect of bioactive essence on living organisms prior to the use as drug or chemical in clinical use.[8] As per the OECD guidelines, in order to ascertain the safety and efficiency of a new drug, toxicological studies are very vital in animals like mice, rat, pig, dog, rabbit, monkey etc. under various conditions of drug. Toxicological studies help to make resolution whether a new drug should be adopted for clinical use or not.[9] Administration of a certain chemical substance to a biological system can cause adverse effects in living organisms. In most cases these responses are preferred and useful, but there are a number of other effects which are not advantageous. In such situation determination of toxicological effect of a drug in biological system hold an important attention. The types of toxicity tests which are regularly performed by pharmaceutical manufacturers in the investigation of a new drug involve acute, sub-acute and chronic toxicity. Acute toxicity is involved in estimation of LD_{50} the dose which has proved to be

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lethal (causing death) to 50% of tested group of animals. Purpose of acute oral toxicity is usually an original screening step in the assessment and evaluation of the toxic characteristics of all compounds.[10]

Horse gram (Macrotyloma uniflorum (Lam.) Verdc. is a minor legume used as a pulse crop in India and has been found to be good nutritional quality. Horse gram seeds have recently been shown to prevent atherosclerosis in rats and may be a potential functional food for the prevention of hyperlipidaemic atherosclerosis.[11] *M. uniflorum* leaves and stem showed abundant amount of phytochemicals as secondary metabolites which participate in various pathophysiological conditions of different diseases.[12,13] The ethanolic extract of *M. uniflorum* stem shows good free radical scavenging activity.[14] Moreover, botanicals are enjoying widespread use of plants for treatment of several ailments, but still known about their toxicity and protection issues which are always a concern. Investigations on toxicity in medicinal plants provide evidence for the presence of substances that are offer potential human health benefits. However, it ought to be a vital requirement to determine the toxic effects of some of the substances contained in the plants.[15] The present study aims to determine the toxicity of ethanolic extract of *M. uniflorum* leaves using an acute oral toxicity test in animal models.

Materials and Methods

Collection of plant material

The plant specimens for the proposed study were collected from Kothavadi village, Coimbatore district, Tamil Nadu, India. The plant was taxonomically authenticated by Dr. G.V.S Moorthy, Botanical Survey of India, TNAU campus Coimbatore, with the voucher number BSI/SRC/5/23/2013-14/Tech/1309. The leaves were washed with running tap water and shade dried at room temperature for 10 days in the absence of sunlight and coarsely powdered using a mixer grinder. Then they were weighed and kept in an airtight container.

Sample extraction

50g of dried plant powder was weighed and extracted in 250 ml of ethanol in an orbitory shaker for 72hrs. Repeated extraction was done with the same solvent till clear colorless solvent is obtained. Obtained extract was evaporated to dryness by using a rotary vacuum evaporator at 40-50°C and stored at 0-4°C in an air tight container for further use.

Preparation of animals

Adult female albino rats weighing about 150-180 g were obtained from the animal house of Karpagam University, Coimbatore and were used for the study. Rats were housed in polycarbonate cages in a room with a 12 hrs day-night cycle, at constant temperature of 22°C and humidity of 45-64%. During the experimental study rats were fed on pellets (Gulmohur Rat Feed, Lipton India, Bengaluru) with free access to tap water. The rats received humane care according to the criteria outlined in Principles of Laboratory Animal Care, 1985. The study was approved by Institutional Animal Ethics Committee (IAEC) and the experiments were conducted according to the ethical norms and IAEC guidelines.

Acute toxicity studies of the ethanolic plant extract

Wistar albino rats weighing 150-200g were used for the acute toxicity study. They were randomly distributed into one control group and five treated groups, containing six animals per group and were on standard normal diet provided with water *ad libitum*. They were allowed to acclimatize for seven days to the laboratory condition before the experiment. The acute oral toxicity testing was carried out in animals under the Organization for Economic Cooperation and Development (OECD) guidelines.[16]

Experimental design

Animal were randomly divided into 6 groups of 3 rats (n = 3):

Group 1: Control group receives a normal pelleted diet

Group 2: Received 50mg/kg body weight of the *Macrotyloma uniflorum* ethanolic extract orally

Group 3: Received 250mg/kg body weight of the *Macrotyloma uniflorum* ethanolic extract orally

Group 4: Received 500mg/kg body weight of the *Macrotyloma uniflorum* ethanolic extract orally

Group 5: Received 1000mg/kg body weight of the *Macrotyloma uniflorum* ethanolic extract orally

Group 6: Received 2000mg/kg body weight of the *Macrotyloma uniflorum* ethanolic extract orally

After the administration of the extract, the animals were observed individually for a period of 24 hrs for the signs of toxicity or mortality. They were continuously observed for 4hrs to detect any changes in autonomic or behavioral responses, spontaneous activity, irritability, corneal reflex, urination and salivation. Any mortality during the experimentation period of 14 days was also recorded. The percentage in mortality in each group was noted. In acute toxicity study even though the rats appeared healthy and normal, it is mandatory to monitor the rats daily for 1 to 2 weeks to find out the delayed toxicity. The experiments were carried out for 14 days.

Collection of serum sample

After the experimental regimen, the animals were sacrificed by cervical decapitation under chloroform anesthesia. Blood sample of each animal was collected separately and centrifuged for 10 min. at 2500 rpm. The serum supernatant was collected then used for the determination of serum constituents and serum enzyme activities.

Biochemical parameters assayed for acute toxicity studies

Biochemical parameters such as Glucose, Urea, Uric acid, Creatinine, Cholesterol, Triglycerides, HDL, SGOT (Serum Glutamate Oxaloacetate Transaminase), SGPT (Serum Glutamate Pyruvate Transaminase), and total protein levels were estimated using kit methods.

Histopathological studies

The toxicity was confirmed through histopathological studies on liver and kidney of rats. The tissue was fixed in 10% formalin immediately after removal from the animal to avoid decomposition for one hour. It was dehydrated by three changes of 500 ml of acetone and cleaned off from acetone by three changes of 500 ml of xylene for 3 hrs. Embedding in paraffin wax was carried out by removal of water using alcohol from 10-30% and then stained with hemotoxylin, which has an aqueous base. The sections were dehydrated using increasing concentrations of alcohol and then stained with eosin. They were then treated with diphenylxylene (DPX) and examined under the microscope to find the morphology.[17]

Statistical Analysis

All the data were expressed as Mean ± SD Statistical comparisons were performed by one way analysis of variance (ANOVA) using the Duncan's multiple range tests. A value of $p<0.05$ was considered statistically significant. The data were analysed using SPSS version 16.0.

Results and Discussion

Herbal drugs have established greater attention as an alternative to medical therapy and the demand for these herbal remedies has significantly increased recently. Their utilization is frequently based on long-term clinical experience.[18,19] Based on historical research; the oral route administration is the most suitable and commonly used method for acute toxicity. Even though the absorption might be slow, this method costs fewer and is painless to the animals. Since the crude extracts are administered orally, the animals were fasted prior to the administration of the dose, because food and other chemicals in the digestive tracts may affect the reaction(s) of the compound. All the procedures were performed based on the appropriate OECD guideline.[20]

In the acute toxicity study, the rats were treated with different concentrations of *M. uniflorum* leaf extract in the range of 50, 250, 500, 1000, 2000 mg/kg b.wt which did not produce signs of toxicity, behavioral changes, and mortality in the test groups of animal as compared to the control during 14 days of the acute toxicity experimental period. During acute toxicity evaluation, none of the animals died at the maximum dose administered i.e. 2000 mg/kg b.wt. *M. uniflorum*. The acute toxicity study showed that animals fed by oral gavages tolerated the limit dose of 2000 mg/kg body weight of ethanolic extract of *M. uniflrum* leaves. There were no visible signs of acute toxicity during the 14 days of observation. Absence of death at all doses up to 2000 mg mg/kg showed that the LD_{50} of the extract is greater than 2000 mg extract/kg body weight. These results showed that a single oral dose of the extract showed no mortality of these rats even under higher dosage levels indicating the high margin of safety of this extract.

The results of current study showed no adverse

changes in physical parameters throughout the dosing period. There was no significant change in the mean body weight of the animals in treated groups as compared to vehicle treated control group at the end of treatment (Figure 1). This indicates that the administration of the crude extract does not possess any toxicity on the growth of the animals. Skin, fur, eyes, mucous membrane, behavioral pattern, salivation, sleep of the treated as well as the control animals were found to be normal. Tremors, lethargy, diarrhea and coma did not occur in any of the animals.

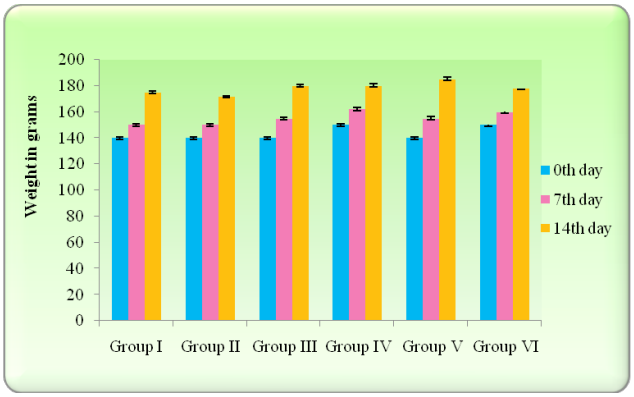


Figure 1. Effect of ethanolic extract of *M. uniflorum* on body weight of experimental rats.

In biochemical evaluation, there were no significant treatment related changes in blood glucose levels in treated and control groups (Table 1). The insignificant difference in urea, uric acid and creatinine levels between the treated groups and the control group probably indicates that the extract did not interfere with the renal capacity to excrete the metabolite (Table 1). Indeed, creatinine is known as a good indicator of renal function. Any rise in creatinine levels is only observed if there is marked damage to functional nephrons.[21]

In Table 2, 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 change the common lipid metabolism of the animals. Among the other parameters evaluated, SGOT and SGPT are well known enzymes which serve as biomarkers capable of predicting toxicity. SGOT is present in a wide variety of tissues which includes heart, kidney, skeletal muscle and liver whereas, SGPT is primarily localized in the liver.[22] The analysis of these parameters is important since there are several reports of liver toxicity related to the use of phytotherapeutic products.[23] The insignificant difference in SGOT and SGPT levels of treated and control rats were depicted in Table 3 and 4.

The synthetic capability of the liver was also conserved as shown by the serum and tissue protein concentrations. There were no noteworthy changes in total protein in rats treated with *M. uniflorum* in Table 5, which suggested that there was no indication of impaired renal function. Total protein measurements may be used to screen and help to diagnose kidney and liver diseases and many other conditions.[24] There were no significant changes in the serum parameters such as urea, uric acid, cholesterol, SGOT (serum) and SGOT (kidney), they were lying under the normal range. This study explores most of the biochemical parameters (i.e. Urea, uric acid, creatinine, protein, SGOT and SGPT) were not altered by the doses of *M. uniflorum* 50, 250, 500, 1000 and 2000 mg/kg and proved its safety on Wistar albino rats.

Table 1. Effect of *M. uniflorum* on biochemical parameters- Glucose, urea, uric acid and creatinine of control and treated rats

Contents	Glucose (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)
Group I	89.32 ± 0.29^{a}	17.72 ± 0.25^{a}	6.82 ± 0.03^{a}	`0.67 ± 0.02^{a}
Group II	89.50 ± 0.01^{a}	19.70 ± 0.26^{c}	7.64 ± 0.05^{b}	0.68 ± 0.01^{a}
Group III	89.55 ± 0.05^{a}	17.67 ± 0.20^{a}	6.64 ± 0.04^{a}	0.67 ± 0.01^{a}
Group IV	89.20 ± 0.59^{a}	18.61 ± 0.44^{b}	7.70 ± 0.01^{b}	0.67 ± 0.06^{a}
Group V	88.26 ± 0.86^{b}	17.00 ± 0.69^{a}	6.64 ± 0.04^{a}	0.65 ± 0.05^{a}
Group VI	88.20 ± 0.2^{b}	17.89 ± 0.34^{a}	6.39 ± 0.07^{a}	0.60 ± 0.06^{a}

Values are expressed as Mean ± SD (n=3), Mean in the same column with different superscript letters are significantly different, p<0.05

Table 2. Effect of *M. uniflorum* on biochemical parameters- Cholesterol, triglycerides and HDL of control and treated rats

Contents	Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)
Group I	166.92 ± 0.36^{a}	86.49 ± 0.54^{a}	66.26 ± 0.25^{a}
Group II	169.07 ± 0.16^{b}	86.77 ± 0.49^{a}	67.14 ± 0.77^{b}
Group III	166.83 ± 0.31^{a}	86.55 ± 0.62^{a}	67.67 ± 0.29^{b}
Group IV	166.78 ± 0.30^{a}	86.51 ± 0.50^{a}	66.23 ± 0.75^{a}
Group V	171.69 ± 0.76^{c}	84.97 ± 0.50^{b}	66.07 ± 0.06^{a}
Group VI	166.50 ± 0.50^{a}	84.48 ± 0.45^{b}	66.03 ± 0.15^{a}

Values are expressed as Mean ± SD (n=3), Mean in the same column with different superscript letters are significantly different, p<0.05

Table 3. Effect of *M. uniflorum* on SGOT of control and treated rats in vital organs

Contents	Serum (IU/L)	Liver (IU/L)	Kidney (IU/L)
Group I	10.70 ± 0.09[a]	7.28 ± 0.36[a]	6.31 ± 0.03[a]
Group II	10.46 ± 0.29[a]	7.06 ± 0.10[a]	6.35 ± 0.16[a]
Group III	10.47 ± 0.43[a]	7.63 ± 0.13[a]	7.36 ± 0.19[b]
Group IV	11.78 ± 0.08[b]	7.65 ± 0.17[a]	7.71 ± 0.06[b]
Group V	10.49 ± 0.35[a]	7.39 ± 0.34[a]	7.63 ± 0.10[b]
Group VI	11.65 ± 0.31[b]	7.51 ± 0.16[a]	6.26 ± 0.06[a]

Values are expressed as Mean ± SD (n=3), Mean in the same column with different superscript letters are significantly different, p<0.05

Table 4. Effect of *M. uniflorum* on SGPT of control and treated rats in vital organs

Contents	Serum (IU/L)	Liver (IU/L)	Kidney (IU/L)
Group I	10.68 ± 0.28[a]	12.40 ± 0.05[a]	7.80 ± 0.06[a]
Group II	10.22 ± 0.24[a]	12.29 ± 0.60[a]	7.45 ± 0.09[a]
Group III	10.15 ± 0.38[a]	12.38 ± 0.25[a]	7.31 ± 0.18[a]
Group IV	10.06 ± 0.15[a]	12.13 ± 0.15[a]	7.95 ± 0.19[a]
Group V	10.07 ± 0.13[a]	12.78 ± 0.22[a]	7.54 ± 0.10[a]
Group VI	10.12 ± 0.17[a]	12.44 ± 0.46[a]	7.43 ± 0.02[a]

Values are expressed as Mean ± SD (n=3), Mean in the same column with different superscript letters are significantly different, p<0.05

Table 5. Effect of *M. uniflorum* on protein of control and treated rats

Contents	Serum (g/dl)	Kidney (mg/g)	Liver (mg/g)
Group I	6.12 ± 0.29[a]	62.11 ± 0.25[a]	74.43 ± 0.03[a]
Group II	6.23 ± 0.01[a]	61.20 ± 0.26[a]	74.33 ± 0.05[a]
Group III	6.25 ± 0.05[a]	61.50 ± 0.20[a]	74.65 ± 0.04[a]
Group IV	6.29 ± 0.59[a]	62.21 ± 0.44[a]	74.12 ± 0.01[a]
Group V	6.15 ± 0.86[a]	62.46 ± 0.69[a]	74.32± 0.04[a]
Group VI	6.17 ± 0.2[a]	62.50 ± 0.34[a]	74.82± 0.07[a]

Values are expressed as Mean ± SD (n=3), Mean in the same column with different superscript letters are significantly different, p<0.05

There were no signs of toxicity seen in any of organ in histopathological analysis. Thus histopathological studies supports the protect nature of *M. uniflorum*. Histopathological analysis are shown in Figure 2, where the liver section of control and experimental animals showed showed normal architecture, clear lumen of central vein, no evidence of lesion, well preserved hepatocytes, sinusoidal veins and vacuoles . No damage was observed in the liver section of treated animals as well as control animals.

The kidney micrograph displayed in Figure 3 shows that no undesirable effects were observed in both control and the treated groups. The glomeruli and capsules appeared normal and the Bowman's space are also marked clearly. Histopathological examination of the kidneys of the rats treated with *M. uniflorum* exposed absence of inflammatory casts and glomerular congestion, consequently supporting the safety of the extract on renal system.

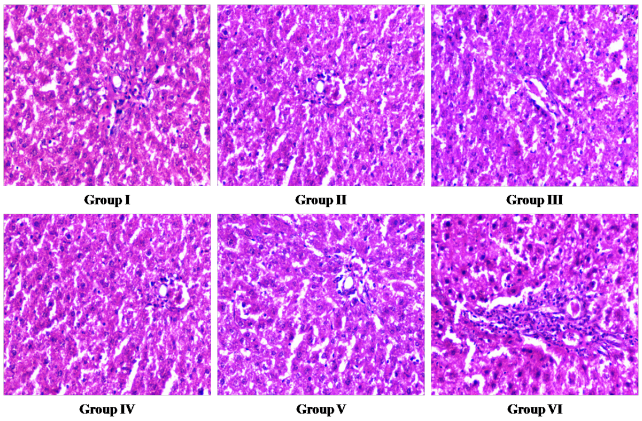


Figure 2. Effect of ethanolic extract of *M. uniflorum* on histopathology of liver in experimental rats.

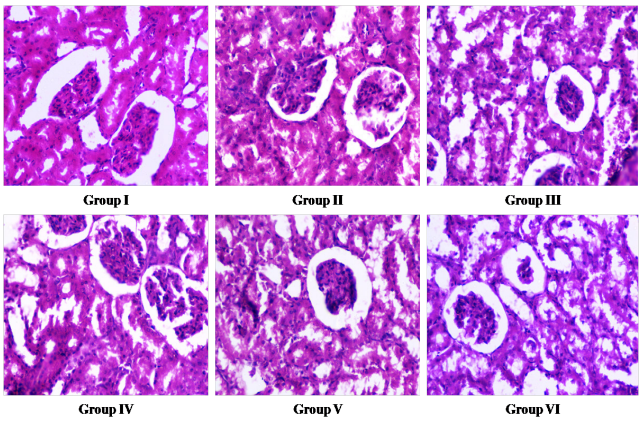


Figure 3. Effect of ethanolic extract of *M. uniflorum* on histopathology of kidney in experimental rats.

Conclusion

Observation from this study indicates that there were no significant undesirable effects seen in biochemical parameters as well as histology of vital organs. So, it may be concluded that the ethanolic extract of *M. uniflorum* did not induce any remarkable damage to the vital systems. In conclusion, the present investigation demonstrates that at doses consumed in the traditional medicine, the ethanolic extract of *M. uniflorum* may be considered as relatively safe, as it did not cause either mortality or produce severe toxicological effects on selected body

organs and biochemical indices of rats during the acute period of study.

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Conflict of Interest

We declare that we have no conflict of interest.

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