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Toxicological Evaluation of *Murraya Paniculata* (L.) Leaves Extract on Rodents

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Abstract: Problem statement: Present study was aimed to explore the acute and sub-acute toxicities studies with orally administered 50% ethanolic leaves extract of *Murraya paniculata*. **Approach:** Acute toxicity (oral single dose, 50-2000 mg kg^{-1}) includes any mortality and CNS and ANS toxicities in mice while, sub-acute toxicity (100, 200 and 400 mg kg^{-1}, orally and once daily for 28 days) includes any change in body weight, food and water intake and other biochemical, hematological and histopathological changes in tissues (liver, kidney, spleen, stomach, heart and lung) in rats. **Results:** Acute oral administration of *M. paniculata* extract (2000 mg kg^{-1}, single dose) did not show any mortality and CNS and ANS toxicities. Similarly sub-acute oral administration (100, 200 and 400 mg kg^{-1} for 28 days) in rats did not show any change in body weight, food consumption, water intake and biochemical, hematological and histopathological parameters compared to control untreated group. **Conclusion:** The conclusions of the present study indicate *Murraya paniculata* to be safe in its oral effective dose.

Key words: Red Blood Cells (RBC), White Blood Cells (WBC), Carboxymethyl Cellulose (CMC), Sub-acute toxicity, *Murraya paniculata*, Acute toxicity

INTRODUCTION

The herbal and natural products of folk medicine have been used by men since the beginning of the human race. However, the general acceptability of herbal medicines has been limited by a lack of defined chemical characterization, dose regimen and adequate toxicity data to evaluate their safety. The indiscriminate increase in the use of plant extract is further aggravated by the belief that herbs are safe simply because they are natural in origin (Gesler, 1992). Plants produce bioactive compounds which act as defense mechanisms against predators and at the same time, may be toxic in nature (Roch *et al.*, 2001). Therefore, it has become imperative to assess the safety of plants used for medicinal purposes for possible toxicity.

Murraya paniculata (Linn.) belongs to the family Rutaceae and is commonly known as orange jasmine. It is distributed over the greater part of India and the Andaman Islands to an altitude of 1500 m. Native to tropical Asia from India and Srilanka to Myanmar (Burma), southern China and Taiwan, Thailand and eastwords throughout the Malesian region to northeastern Australia and Caledonia. The leaves are stimulant and astringent; they are reportedly used in the form of an infusion to treat diarrhoea and dysentery in the Philipines. The powder leaves are applies to cuts to promote healing; there decoction is taken internally to treat dropsy. The leaves and root bark are sometime used to treat rheumatism, coughs and hysteria. Coumarins, murralongin, isomurralonginol isovalerate, murrangatin, minumicrolin (murpanidin), coumurrayin, toddalenone, aurapten, toddasin gardenin A, gardenin C, gardenin E and umhengerin was isolated from the leaves (Choudhary *et al*., 2002; Kinoshita and Shimada, 2002). It is reported to have anti-diabetic and antioxidant (Gautam *et al*., 2012), anti-nociceptive and anti-inflammatory (Wu *et al*., 2010), anti-diarrhoeal (Rahman *et al*., 2010), oxytocic (Yun-Cheung *et al*., 1986) and anti-fertility (Xiao and Wang, 1991) properties.

As to the best of our knowledge, there is no reference about the safe dosage of *Murraya paniculata* Linn. in traditional medicine so it was considered worthwhile to do the acute (mortality, CNS/ANS toxicities) and sub-acute (biochemical, hematological and histopathological) toxicity studies in mice and rats respectively with 50% ethanolic extract of *M. paniculata* extract with the aim to obtain information and guidance for selecting a safe dosage.

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MATERIALS AND METHODS

Collection of Plant material: The leaves of *Murraya paniculata* (Family-Ruteceae) were collected from Botanical Garden of National Botanical Research Institute, Lucknow, India. The freshly collected leaves materials of *Murraya paniculata* were washed with distilled water and air-dried at 30 ± 2°C then dried it in tray drier under the control conditions and powdered.

Preparation of hydro-alcoholic extract: The powdered plant leaves (1000 g) was macerated with petroleum ether to remove fatty substances. 50% ethanolic extract of *M. paniculata* was prepared by adding the marc with of 50% ethanol for 3 days (3×3 L) and centrifugation at 10,000 rev/min. The extract was separated by filtration and concentrated on rotavapour (Buchi, USA) and then dried in lyophilizer (Labconco, USA) under reduced pressure (yield 9.5% w/w). *M. paniculata* extract was stored at -20°C until further use.

Animals used: Sprague-Dawley rats (140-160 g) were selected for study. They were kept in the departmental animal house at 26±2°C and relative humidity 44-56%, light and dark cycles of 10 and 14 h respectively for one week before and during the experiments. Animals were provided with standard rodent pellet diet and the food was withdrawn 18-24 h before the experiment, though water was allowed *ad libitum*. All studies were performed in accordance with the guide for the care and use of laboratory animals, as adopted and promulgated by the Institutional Animal Care Committee, CPCSEA, India (Reg. No. 222/2000/CPCSEA).

Acute toxicity study: The adult Swiss albino mice (25-30 g) of both sexes selected for acute oral toxicity study, as per OECD guideline-423. Four group of mice of both sexes equal in number (n = 6) which have been fasting overnight. The control animals received 1% Carboxymethyl Cellulose (CMC) suspension in distilled water and extract was administered in CMC suspension at doses 50, 500 and 2000 mg kg^{-1}. Mice were closely observed for the initial 4 h after the administrations and then once daily during the following days. Additional observations include change in skin and fur, eyes and mucous membranes and also somatomotor activity and behaviour pattern. Attentions were given to observations of tremors, colonic convulsions, salivation, diarrhoea, sleep and coma and death. The animals were under further investigation up to a period of 2 week (Sim *et al*., 2010).

Sub-acute toxicity study: The Swiss albino rats of either sex weighing between 140-160 g were used for this study. The animals were permitted free access to standard pelleted food and water *ad libitum*. The animals were grouped into four groups of 6 animals (either sex) each. OECD guidelines were followed during the study. The Group I rats served as control, Group II, III and IV received *M. paniculata* extract at doses of 100, 200 and 400 mg kg^{-1} respectively for 28 days. All rats were observed daily for any behavioral and physiological changes. Food and water intake were measured on the 0, 7, 14, 21 and 28 day of the experiment by using digital feeding and drinking analyser (Ugo Basile, 41700, Italy).

Biochemical analysis: After 28 days of extract administration, the rats were fasted overnight prior to blood collection by retro-orbital technique on 29th day of the study. The serum was carefully aspirated with a Pasteur pipette into sample bottles for the various biochemical assays. Assay kits (Span diagnostic reagent kit and Agappe diagnostic kit) were employed for Aspartate Transaminase (AST), Alanine Transminase (ALT), Alkaline Phosphate (ALP), creatinine, blood glucose, total protein, total cholesterol and Bilirubin analysis were determined in the serum following the procedure described in the kits.

Hematological parameter: Red Blood Cells (RBC), Haemoglobin (HB) and White Blood Cells (WBC) were estimated with the help of hematology analyzer (Medonic CA620, Boule, Sweden). The RBC and WBC were expressed as million/mm3 of blood and HB as g dL^{-1} of blood.

Organs weight and histology: The rats were quickly dissected and the liver, kidneys, stomach, spleen, lung and heart were excised and weighed. The specimens for histopathology were fixed in 10% neutral, buffered formalin for 18 h at 4°C. In each specimen of liver, kidney, heart, lung, spleen and stomach, 3-4 μm in thickness were obtained and stained with Hemotoxylin and eosin stains following the standard laboratory procedures. The stained sections were examined under microscope for any cellular damage or change in morphology of that particular tissue.

Statistical analysis: All the values were expressed as mean ± Standard Error of Mean (SEM) for six rats. Statistical analysis was carried out by using PRISM software package (version 3.0). Statistical significance of differences between the control and experimental groups was assessed by Oneway ANOVA followed by Newman-Keuls Multiple Comparision Test. The value of probability less than 5% ($p<0.05$) was considered statically significant.

RESULTS

Acute toxicity study: Mice administered with *M. paniculata* extract upto 2000 mg kg^{-1} did not show any kind of abnormal behavior, during initial 4h after drug administration. No mortality was observed during 14 days after treatment with *M. paniculata* extract in either sex.

Table 1: Effect on Body weight and isolated organs weight after 28 days oral administration of *M. Paniculata* extract

	Body weight (g)					Isolated organs weight		
Treatment/dose	Initial	1 week	2 week	3 week	4 week	Liver (g)	Kidney (g)	Heart (g)
Control group	149.7±11.7	157.5±11.9	166.2±12.3	173.4±13.3	182.8±14.8	6.93±0.39	1.09±0.02	0.59±0.04
M. Paniculata 100 mg kg^{-1}	152.9±12.4	161.3±12.3	169.1±12.8	177.3±13.5	186.1±14.6	6.96±0.22	1.08±0.02	0.57±0.05
M. Paniculata 200 mg kg^{-1}	154.0±13.1	163.2±13.2	171.4±13.5	179.1±14.2	189.5±15.7	7.04±0.31	1.07±0.01	0.53±0.06
M. Paniculata 400 mg kg^{-1}	149.5±11.6	156.9±12.0	165.8±12.6	173.1±13.4	183.4±14.5	7.16±0.41	1.10±0.20	0.61±0.08

Values are expressed as Mean ± SEM of 6 rats in each group

Table 2: Effect on food intake and water intake after 28 days oral administration of *M. Paniculata* extract

	Food intake (g/d)					Water intake (ml/d)				
Treatment/dose	Initial	1 week	2 week	3 week	4 week	Initial	1 week	2 week	3 week	4 week
Control group	9.8±1.81	11.5±2.54	13.2±2.42	14.1±2.84	15.2±2.72	24.3±1.2	25.4±1.7	26.2±1.4	27.0±1.6	28.2±1.4
M. Paniculata 100 mg kg^{-1}	10.1±3.32	12.2±3.41	14.1±3.63	15.1±2.53	16.0±3.23	24.1±0.9	25.3±1.5	26.5±1.6	27.3±1.3	28.5±1.6
M. Paniculata 200 mg kg^{-1}	9.7±2.21	11.4±2.55	13.5±2.61	14.4±3.11	15.5±2.32	25.0±1.4	26.1±1.9	27.0±1.8	27.9±1.5	29.1±1.8
M. Paniculata 400 mg kg^{-1}	10.3±3.32	12.6±3.63	14.4±3.84	15.3±2.72	16.2±3.43	24.7±1.5	25.6±1.6	26.4±1.7	27.6±1.2	28.7±1.5

Values are expressed as Mean ± SEM of 6 rats in each group

Table 3: Effect on hematological and biochemical parameters after 28 days oral administration of *M. Paniculata* extract

Treatment/dose	Control group	*M. Paniculata* 100 mg kg^{-1}	*M. Paniculata* 200 mg kg^{-1}	*M. Paniculata* 400 mg kg^{-1}
RBC (million/mm^3)	9.1±1.2	9.2±1.1	8.8±0.9	9.2±1.2
Hb (g/dL)	12.2±1.3	12.1±1.2	12.2± 1.1	12.2±0.9
WBC (million/mm^3)	8.9±1.3	8.7±1.1	8.6±1.2	9.0±1.0
Neutrophils %	23.46±6.2	22.38±8.3	21.58±6.4	24.56±7.8
Eosinophils %	2.73±1.1	2.43±1.4	2.28±1.2	2.74±1.1
Basophils %	0.18±0.05	0.19±0.08	0.16±0.07	0.17±0.06
Lymphocytes %	75.33±3.7	73.66±2.4	76.65±4.5	74.45±4.2
Monocytes %	3.24±1.5	4.06±1.3	3.14±1.6	2.66±1.4
AST (U/L)	196.69±1.36	197.99±1.09	194.86±1.77	198.78±2.08
ALT (U/L)	82.54±1.22	80.34±1.93	81.10±2.10	84.5±1.23
ALP (U/L)	231.62±1.09	233.16±1.67	232.62±1.15	231.8±0.90
Creatinine (mg/dL)	0.92±0.05	0.90±0.06	0.93±0.03	0.92±0.03
Albumin (g/dL)	2.68±0.07	2.72±0.07	2.73±0.08	2.74±0.06
Total protein (g/dL)	7.2±1.2	7.1±1.6	6.7 ±1.5	6.9±1.4
Glucose (mg/dL)	94.78±3.36	88.53±5.43	90.46±5.39	92.52±4.03
Total cholesterol (mg/dL)	121.6±2.6	123.2±3.4	119.9± 3.8	122.1±4.2
Bilirubin Total (mg/dL)	1.24±0.31	1.25±0.23	1.21±0.21	1.23±0.31
Bilirubin Direct (mg/dL)	0.72±0.01	0.69±0.01	0.71±0.02	0.70±0.01

Values are expressed as mean ± SEM of 6 rats in each group

Sub-acute toxicity study: In the sub-acute toxicity study in rats, the increase in body weight, food consumption and water intake in all test groups were not significantly different from control group with *M. paniculata* extract at various dose levels of 100, 200 and 400 mg kg^{-1} (Table 1 and 2).

Biochemical analysis: Biochemical parameters for liver and kidney function test like Aspartate Transaminase (AST), Alanine Transminase (ALT), Alkaline Phosphate (ALP), creatinine, albumin, blood glucose, total protein, total cholesterol and bilirubin did not show any difference with the above doses of *M. paniculata* extract studied compared to control group (Table 3).

Hematological studies: Hematological parameters like mean haemoglobin content, WBC, RBC and differential cell counts were not significantly different from control group with *M. paniculata* extract treated rats (Table 3).

Organs weight and histology: The organs like liver, kidney and heart isolated in various group did not reveal any abnormalities in their gross examinations and difference in their mean weights both in treated and control groups (Table 2). The histological studies with liver, stomach, spleen, kidney, heart and lungs did not reveal any pathological changes after treatment even with higher dose of 400 mg dose of *M. paniculata* extract when administered for 28 days (Table 1 and Fig. 1 and 2).

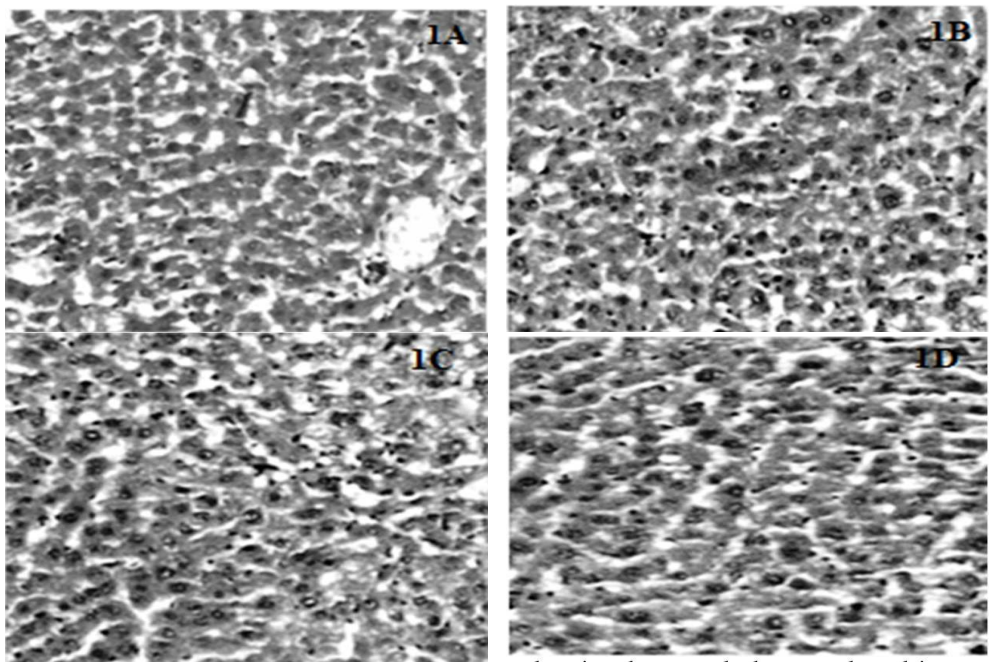

Fig. 1: Histopathological picture of liver of control and experimental group of animals. (1A) The section of liver from control animals revealed normal architecture and hepatic cells with granulated cytoplasm; (1B, 1C and 1D) liver from *M. paniculata* extract 100, 200 and 400 mg kg^{-1} treated exhibit normal architecture of hepatocytes and normal architecture

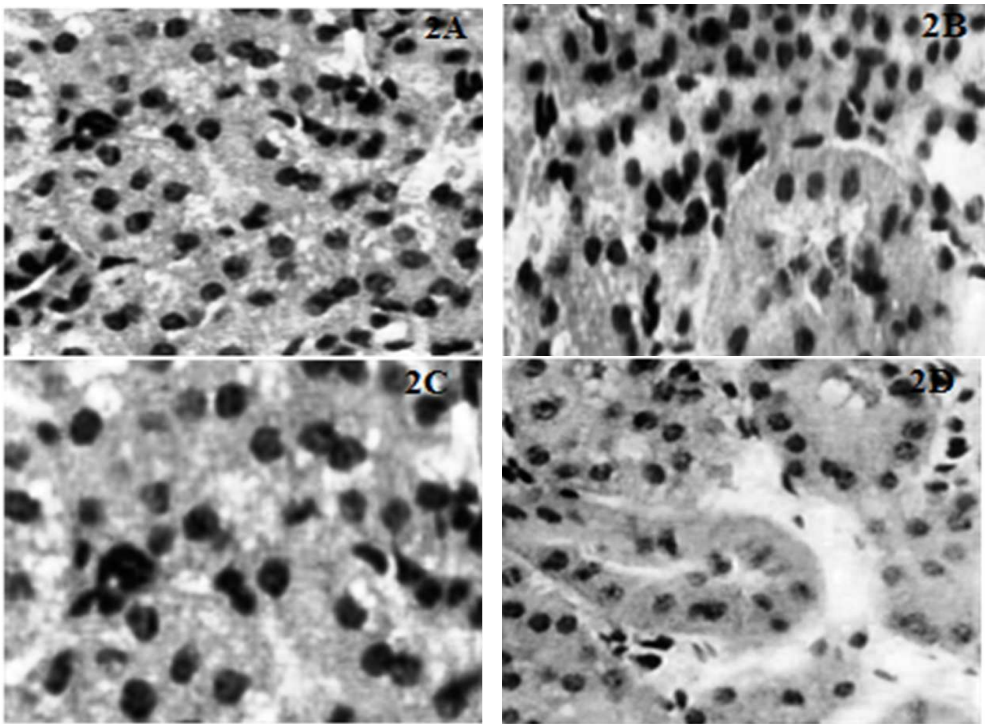

Fig. 2: Histopathological picture of kidney of control and experimental group of animals. (2A) The section of kidney from control animal showed normal size of glomeruli with normal tubules; (2B, 2C and 2D) kidney from *M. paniculata* extract 100, 200 and 400 mg kg^{-1} treated exhibit normal size of glomeruli with normal tubules

DISCUSSION

The administration of herbal preparations without any standard dosage, coupled with a scarcity of adequate scientific studies on their safety, has raised concerns regarding their toxicity (Saad *et al.*, 2006). To determine the safety of drugs and plant products for human use, toxicological evaluations are carried out on various experimental animals to predict toxicity and to provide guidelines for selecting a 'safe' dosage in humans. The highest overall concordance of toxicity in animals with humans is with hematological, gastrointestinal and cardiovascular adverse effects (Olson *et al.*, 2000), while certain adverse effects in humans, especially hypersensitivity and idiosyncratic reactions, are poorly correlated with toxicity observed in animals. Furthermore, it is quite difficult to ascertain certain adverse effects in animals, such as headache, abdominal pain, dizziness and visual disturbances. In addition, interspecies differences in the pharmacokinetic parameters make it difficult to translate some adverse effects from animals to humans. In our study mice administered with *M. paniculata* extract upto 2000 mg kg^{-1} did not show any kind of abnormal behavior, during initial 4h and no mortality was observed during 14 days. Acute toxicity studies with a range of doses have to be conducted first to select a proper dosage for chronic and sub-acute studies; the doses selected for chronic and sub-acute toxicity studies should be at and above the suggested human dose.

Our sub-acute studies in rats did not show any increase in the body weight, food consumption and water intake as well as in hematological, Liver and kidney function test with 400 mg kg^{-1} *M. paniculata* extract when administered for 28 days. The hematopoietic system is one of the most sensitive targets for toxic compounds and an important index of physiological and pathological status in man and animal (Mukinda and Syce, 2007). Analysis of blood parameters is relevant to risk evaluation as the changes in the hematological system have a higher predictive value for human toxicity, when the data are translated from animal studies (Olson *et al.*, 2000). Sub-chronic exposure of rat to the lower doses of the *M. paniculata* extract produced small and transient changes in some biochemical and hematological parameters without affecting the liver or kidneys; these changes showed a tendency to recover at the end of the sub-acute exposure. In our study, the biochemical parameters like AST (aspartate aminotransferase), ALT (alanine amino transferase) and creatinine did not show any treatment-related increase even at the 400 mg kg^{-1} dose compared to the control group. Indeed, the transaminases (AST and ALT) are well-known enzymes used as good indicators of liver function (Hilaly *et al.*, 2004) and as biomarkers predicting possible toxicity (Rahman *et al.*, 2001). Generally, any damage to the parenchymal liver cells results in elevations of both transaminases in the blood (Wolf, 1972). In addition, AST found in the serum is of both mitochondrial and cytoplasmic origin and any rise can be taken as a first sign of cell damage that leads to the outflow of the enzymes into the serum (James and Eagles, 2010). Ordinarily, liver cell damage is characterized by a rise in serum enzymes like AST, ALT, ALP, etc. (Brautbar and Williams, 2002). Thus, no significant increases observed in ALT and AST activities strongly suggest that the subacute administration of *M. paniculata* extract did not alter the hepatocytes and consequently the metabolism in the rats. Kaplan (1995) have mentioned the liver to be the site of cholesterol disposal or degradation and its major site of synthesis. In the same perspective, it controls glucose synthesis and generates free glucose from hepatic glycogen stores (Kaplan, 1995). Since, no significant changes were observed in glucose and cholesterol levels this study; it suggests that *M. paniculata* extract had no effect on the lipid and carbohydrate metabolism in rats. Further, *M. paniculata* extract neither showed any significant difference in the weight of the organs, organ-to-body weight ratio or color of organs nor affected the histopathological changes indicated the safety of the extract and plant as such.

CONCLUSION

This study provides valuable data on the acute and sub-acute oral toxicity profile of *M. paniculata* that could be very useful in its future study (*in vivo* and clinical study) with this plant. The 50% ethanolic extract of *M. paniculata* seemed to be non-toxic as was seen after acute and sub-acute oral administrations. Further, teratogenic, mutagenic and carcinogenic and cell lines studies with this plant are needed to complete the safety profile of this plant.

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