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Safety assessment of the water extract of *Munronia pinnata* in wistar rats

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Abstract
Munronia pinnata (Meliaceae) is an important medicinal plant in Sri Lanka and it hasbeen broadly used for skin diseases, diabetes, fever and dysentery in the form of concoction and powder due to its bitterness. In Sri Lanka thishas been applied for hundreds of years asunorthodox medicine. This study was focused to assess the possible toxicity of the water extracts of *M. pinnata* (MPW) in healthy and diabetic Wistar rats. Acute and chronic administration of MPW produced no neither mortality nor changes in general behaviors compared with Control groups. The results showed no statistically significant changes in hematological and biochemical values of Test groups compared to the control groups of both healthy and diabetic animals. These results disclosed that the water extract of *M. pinnata* do not cause any undesirable effects as evaluated by the attempted studied.

Keywords: *Munronia pinnata*, hematological and biochemical assays, toxicity study

1. Introduction
Munronia pinnata (Wall) Theob (Meliaceae) called "*Bin Kohomba*" is a precious medicinal plant in Sri Lanka. It has been widely used by traditional physicians as a substitute for *Swertia chirata* (Gentianaceae) in the preparations of *Sudarshan Churna* and *Daruparpatadi* kwatha [1] with no known literature evidence. Moreover, ithasbeen remarkably used for diabetes, skin diseases, dysentery, and fever in the form of concoction and powder due to its bitterness [2, 3]. However, according to Sri Lankan traditional practitioners, *M. pinnata* has been used in folk medicine for centuries. The previous studies of the water and ethanol extracts of the natural plant of *M. pinnata* have shown the dose-dependent hypoglycemic [5-7] and anti-inflammatory activity [8] in diabetic and healthy Wistar rats. Despite its historical usage in traditional medicinal practice, possible toxicity study of MP has not been investigated yet using scientifically controlled experiments. Hence, the present study was thus to evaluate the safety of the water extract of MP by determining the behavioral and biochemical effects following long-term administration in healthy Wistar rats

2. Materials and Methods
2.1. Collection and authentication of plant material
Munronia pinnata whole plants were collected from the medicinal plant nursery at Haldummulla, Department of Ayurveda, Sri Lanka, and maintained in the planthouse at the Department of Dravyaguna Vignana, Institute of Indigenous Medicine, Rajagiriya, University of Colombo. *M. pinnata* plant was taxonomically identified and authenticated by the National Herbarium, Department of National Botanic Gardens, Peradeniya, Sri Lanka where a voucher specimen was deposited (PDA/ MP 01). The air-dried herbs were coarsely powdered and used for the preparation of extractions. Ethical clearance for the study was obtained from the Ethics Review Committee of Faculty of Medical Sciences, University of Sri Jayewardenepura (474/09).

2.2. Preparation of extractions
The water extract of whole plants of *M. pinnata* was prepared according to the conventional method [2] used by traditional medical practitioners in Sri Lanka. Air-dried coarsely powdered [60.0 g (12 *kalan*)] of whole plants (MPW) of *M. pinnata* were mixed with 1920.0 mL (8 parts = *patha*) of water in an earthen vessel and boiled over moderate heat and reduced to 1/8th part (240.0 mL). This gives the conventional dose for an adult which is 240.0 mL per day. The prepared extract was freeze-dried and stored at 4^{0}C until used.

2.3. Animals
Healthy out-bred male Wistar rats (175.0 – 225.0 g) purchased from Medical Research Institute, Colombo, were used in this study. Rats were housed individually in rat cages in a well-ventilated room at an ambient temperature of 29 ± 2^0 C at the Animal House, University of Sri Jayewardenepura. The standard WHO recommended diet was given and water was supplied *ad libitum*. The experimental procedures and animal care were conducted according to international laws and guidelines [9, 10].

2.4. Induction of diabetes
Diabetes was induced in male Wistar rats by a single intravenous injection of (40.0 mg/kg) alloxan monohydrate dissolved in sterile normal saline [11, 12]. The animals were given 2.0 mL of 5% dextrose solution orally immediately after induction to overcome the drug-induced hypoglycemia and fasting serum glucose level was measured after seventy-two hours. Rats with serum glucose levels above 11.0 mmol/L were considered diabetic and selected for the experiment.

2.5. Assessment of toxic effects in Wistar rats
2.5.1. Acute toxicity
According to WHO, 2008 and OECD 423 guidelines, male and female healthy rats were divided randomly into six groups (6 rats per each group). They were orally treated for 14 days with distilled water (2.0mL) for the control group and water extracts of MP (MPW) with the doses ofdoses100.0, 200.0, 500.0, 700.0, and 1000.0 mg/kg/day for tested groups. All the behavioral patterns, body weight, and food intake were recorded weekly.

The general behavior of the animals was continuously monitored for 1h after dosing, periodically during the first 24 h (with special attention given during the first 4 h) and thereafter, continuously for 14 days [13]. Changes in the weights and also behavior is monitored daily. Blood samples were collected for hematological analysis. The serum levels of alanine transaminase (ALT), aspartate transaminase (AST), γ-glutamyl transferase (GGT), alkaline phosphatase (ALP), and creatinine were estimated using commercial reagent kits (Biolabo Reagent, France). Serum glucose concentration was measured by the glucose oxidase method using BIOLABO reagent kits. Whole blood was used to assay the white blood cell count (WBC), andred blood cell count (RBC) using a hemocytometer. Hemoglobin (Hb) was estimated using a commercial reagent kit (Pro Dia International UAE, Germany).

2.5.2. Chronic toxicity
Healthy and alloxan-induced diabetic rats were treated with distilled water (2.0mL) for the control group and 700.0 mg/kg/day dose of MPW for tested groups. All the parameters tested during the acute toxicity study were recorded weekly for three months. At the end of three months, all groups fasted overnight with free access to water and the following morning animals were euthanized with an overdose of diethyl ether. Blood samples were collected from cardiac puncture immediately after anesthesia for hematological and biochemical assays. Selected organs (liver, pancreas, spleen, and kidney) were dissected out for histopathological examination [14]. Blood parameters were measured as above. Results were compared with the control groups (healthy and diabetic).

2.6. Statistical analysis
Data are presented as the mean ± SEM. Analysis of variance (ANOVA) was tested in SPSS 16.0 and the results were further subjected to the student's *t*-test. A *P* value of less than 0.05 was considered significant. Significant differences between means of different groups were statistically analyzed by ANOVA followed by post hoc Tukey's HSD multiple comparison test.

3. Results
3.1. Acute effect of MPW
Rats fed with the given doses did not show any signs of toxicity during the initial 24hours and as well as during the entire period of 14 days of observation. No mortalities were observed. Physical observation of the treated rats throughout the study indicated that none of them showed signs of toxic effects such as changes in fur, eyes, and mucus membranes, change in behavior patterns, tremors, salivation, diarrhea, sleep, and coma.

Further, toxicity evaluation was carried out by observing body temperature, body weight gain, hematological and biochemical parameters. The mean body weights of the treated rats were not significantly different when compared to the control. The results as shown in Table 1, revealed that all the doses of MPW when administered orally caused no statistically significant changes (P > 0.05) in hematological and biochemical parameters of tested groups compared to the control group of healthy rats. All results were within reference values for the strain and age of the animals used [15]. These results revealed that tested MPW does not exert any acute adverse effects as assessed by the parameters studied.

Table 1: Blood /serum biochemical values of healthy rats on Day 15 of the single administration of different doses of MPW

Treatment group	Hemoglobin (g /L)	RBC 10^6/ µL	WBC 10^3/µL	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Creatinine (µmol/L)	GGT (µl/L)
MPaq g/kg								
100.0	15.71±0.34	8.12±0.17	8.74±0.23	43.9 ±2.7	37.30 ±2.4	163.3±8.9	61.9±4.2	1.64 ±0.1
200.0	16.20±0.44	7.89±0.11	8.32±0.22	45.7± 3.1	36.70±1.2	169.4±0.03	67.3±0.02	1.61±0.23
500.0	16.13±0.47	8.73±0.12	8.41±0.43	43.52.2	38.10±2.2	163.60.43	61.5±0.32	1.64±0.57
700.0	16.42±0.34	8.27±0.24	9.12±0.22	44.7±3.6	37.80±4.4	165.1±0.07	61.3±0.44	1.61±0.82
1000.0	16.13±0.41	8.37±0.05	9.01±0.31	42.6±7.1	35.90±3.2	163.4±0.45	61.7±0.61	1.69±0.04
DW	15.87±0.13	8.12±0.41	9.00±0.44	64.2±2.2	38.20±4.6	163.20.87	59.2±0.75	1.71±0.84

Values are expressed as mean ±SEM, n=6. $P < 0.05$ compared to control group.
MPW; water extracts of *M. pinnata*, DW; control group

3.2. Chronic effects of MPW in healthy and diabetic rats
The selected dose (700.0 gm/kg) of MPW produced neither mortality nor changes in general behavior compared with the control group when administered to healthy and diabetic rats for 90 days of treatment. The mean body weights and rectal temperature of healthy and diabetic rats with multiple-dose administration of the selected doseof *M. pinnata*are illustrated in Table 2.In the beginning, there was no significant

difference in the mean body weights between the selected extracts and the control groups of healthy and diabetic rats. In contrast, there was a statistically significant change ($p < 0.05$) in the body weights of the treated rats compared to control ones following 90 days of consecutive administrations of MPW extract.

There was no statistical change in the organ weights of the treated rats compared to that of the control (Table 3). In addition, there were no histopathological findings that could be credited to the *M. pinnata*, and no differences were observed in the organ morphology across the dose groups compared to the control group. The results of the hematological and biochemical results express (Table 4) that the oral administration of MPW for 14 days and 90 days respectively showed no statistically significant changes ($p > 0.05$) in treated groups compared to the control groups of both healthy and diabetic animals. The blood glucose level in the diabetic MPW treated group was exhibited a significant ($p > 0.05$) hypoglycemic effect (28% reduction) compared to the negative control group at the end of the 90 days of consecutive administration of MPW extract. When observing multiple comparisons using ANOVA -Turkey Post Hoc Tests, there was no statistically significant difference between MPW and the tested standard anti-diabetic drug (glibenclamide 10.0mg/kg body weight/day).

Table 2: Effect of MPW on Rectal temperature and bodyweights of healthy and diabetic rats after 90 days

Treatment groups	Day 0		Day 15th		Day 91st	
	Bodyweight (g)	Rectal temperature ^{0}C	Bodyweight (g)	Rectal temperature ^{0}C	Bodyweight (g)	Rectal Temperature ^{0}C
MPW(Healthy)	212.80± 3.31	36.23±0.21	215.17±4.62	36.31±0.16	219.66± 4.58	36.16± 0.28
MPW(Diabetic)	213.47±4.21	36.23±0.13	215.43±3.32	36.28±0.33	216.17±4.00*	36.41 ±0.13
DW	213.21±4.21	36.36±0.18	219.17±3.21	36.45±.22	225.83±3.62	36.39± 0.23

Values are expressed as mean ± SEM; n=6. *P< 0.05 compared to Control, MPW water extracts of *M. pinnata*, DW; distilled water (control group)

Table 3: Effect of MPW on relative organ weight (g) per 100.0 g bodyweights of healthy and diabetic rats following 90 days of multiple-dose administration

Treatment groups	Relative organ weight (g/100g)			
	Liver	Pancreas	Spleen	Kidney
MPW(Healthy)	4.34±0.08	0.254±0.03	0.65±0.02	0.355±0.01
MPW(Diabetic)	3.56±0.03	0.253±0.03	0.591±0.03	0.244±0.02
DW	5.45±0.22	0.232±0.02	0.66±0.04	0.325±0.02

Values are expressed as mean ± SEM (n=6). *p <0.05 compared to Control. MPW water extracts of *M. pinnata*, DW; distilled water (control group)

Table 4: Hematological and serum biochemical Serum parameters of healthy and diabetic rats following 90 days of consecutive administration of MPW

Treatment group	Hemoglobin (g/L)	RBC 10^6/ µL	WBC 10^3/µL	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Creatinine (µmol/L)	GGT (µl/L)
MPW(Healthy)	15.5±0.3	8.32± 0.73	7.84±0.47	43.9 ±2.7	37.3 ± 2.4	163.3±8.9	61.9±4.2	1.94 ±0.1
MPW(Diabetic)	15.1±1.2	8.31±0.33	7.12±0.31	44.6±1.5	37.4±2.3	242.8±1.9	74.9±1.4	2.04±0.2
DW	15.9±0.3	8.51± 0.46	7.85±0.41	44.1±6.7	38.2±8.3	163.5±1.4	60.0±7.2	1.98±3.2

Values are expressed as mean ± SEM (n=6). *p <0.05 compared to Control. MPW water extract of *M. pinnata*, DW: distilled water

4. Discussion

As indicated by the results, all groups treated with the MPW produced neither mortality nor changes in general behaviors when compared with control groups. Effective doses of MPW exerting significant hypoglycemic [7] and anti-inflammatory [8] activities were calculated from previous studies. The results are shown in Table 1 and 4 indicate that oral administration of the water extracts of *M. pinnata* for two weeks as well asthree months caused no statistically significant changes ($P \geq 0.05$) in hematological and biochemical values of test groups compared to the control groups of both healthy and diabetic animals. Results obtained were within reference values for Wistar rats [15]. This is an importing finding as hypoglycemic and anti-inflammatory drugs, have to be administered over a long period of time in all systems of medical practice. A possible suggestion is that the MPW might pay a vital role in improving the diabetic status in terms of serum glucose concentration by restoring the structural and functional properties of β-cells of the pancreas, a primary target organ of alloxan.

5. Conclusion

The findings of the present study indicate that there is no possible toxicity when MPW following chronic administration of the water extract of *Munronia pinnata*.

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