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Acute and Subacute Toxicity of *Persicaria minor* in Wistar Rats

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ABSTRACT

Persicaria minor a widely used herb in South-east Asia, was reported to have high antioxidant contents and therefore can be potentially used to reverse the effects of lipid peroxidation caused by free radicals. In this study, the toxicity profile of this herb was evaluated based on haematological and biochemical analyses. In acute toxicity study, male Wistar rats were given a single dose of either 300, 1000 or 2000 mg kg^{-1} b.wt. of aqueous extract of *Persicaria minor* leaves via oral gavage. Rats were observed for mortality within 24 h. In subacute toxicity study, the rats were treated with the three different doses of the extract daily for fourteen days. At the end of the study period, blood was taken intraorbitally for haematology and biochemistry. Results showed that there were no signs of toxicity, behavioural changes or death observed in both studies. However, feeding of extract at the dose of 2000 mg kg^{-1} b.wt. for 14 days significantly reduced the haemoglobin, calcium and sodium while increasing the potassium levels. In conclusion, the extract from the leaves of *Persicaria minor* administered orally did not cause any acute or subacute toxicity to male rats, with the exception of the extremely large dose whereby it may lead to electrolyte imbalance, anaemia and hypocalcaemia.

Key words: Biochemical and haematological analyses, *Persicaria minor*, toxicity, antioxidant

INTRODUCTION

The use of traditional and complementary medicine is becoming widely popular both in the developing as well as in the developed nation. In traditional Malay, Chinese and ayurvedic medicine, crude plant preparations from local herbs are often used for their supposed medicinal values (Jantan, 2006). Herbal products are considered crude drugs, which contain a melange of phytochemicals such as terpenoids, alkaloids, glycosides, flavonoids and tannins together with the primary compounds of carbohydrates, lignins, nucleic acids, lipids and protein (Jantan, 1998). By and large, the pharmacology and clinical application of the herbal products are still not fully understood, thus warranting scientific studies in order to identify their potential effects and toxicities.

One of the locally used herbs in Malaysia is the *Persicaria minor*. Locally called daun kesum or the laksa leaves, this herb was previously known as *Polygonum minus*. It is a perennial, herbaceous creeper growing up to 1.5 m in height with arrow-shaped leaves. It had been used

traditionally for treating dandruff, diarrhoea, fungal infections and indigestion. The aromatic compounds in *Persicaria minor* are used commercially as flavour and fragrance agents in beverages, food products, confectionery, toothpaste, cosmetics and medicinal preparations (Vimala and Adenan, 1999).

It was reported that *Persicaria minor* had the highest phenolic content and antioxidant among the herbs studied, i.e. *Cosmos caudatus* (ulam raja), *Oenanthe javanica* (selom), *Centella asiatica* (pegaga) and *Murrraya koenigii* (curry leaves) (Faujan *et al*., 2007). Natural antioxidants found in plants can counteract the Reactive Oxygen Species (ROS) which are responsible for lipid peroxidation in causing cellular injury and ageing process (Devasagayam *et al*., 2004; Fang *et al*., 2002). ROS have been implicated in many pathological processes like cancer, diabetes, atherosclerosis, osteoporosis and Alzheimer's disease (Maniam *et al*., 2008; Spector, 2000). Therefore, *Persicaria minor* may be useful in diseases in which lipid peroxidation and oxidative stress play a role in the pathogenesis. Nonetheless, the safety of *Persicaria minor* has never been evaluated.

Toxicity tests can be divided into acute, subacute and chronic toxicity studies based on the length of time the animals are exposed to the test compound. Additional toxicology tests are usually carried out in order to investigate any adverse effects on the reproductive system and whether the compounds are teratogenic, mutagenic or cancer-causing agents. Acute toxicity describes the adverse effects of a chemical substance which appeared after giving one or more doses of the test substance in 24 h. Subacute toxicity is conducted for two weeks duration whereby daily doses are given and the animals are observed for any signs of toxicities before they are sacrificed at day 15 whereby blood and tissue samples are collected for further analyses (Katzung, 2011).

The aim of the present study was to assess acute and subacute toxic effect related to different doses of *Persicaria minor* on oral administration.

MATERIALS AND METHODS

Plant materials: The fresh herbs were obtained from a local wet market in Bandar Baru Bangi, Selangor, Malaysia. The herbs were washed under running tap water to clear out dirt and the excess water was drained. The cleaned herbs then were air-dried using an electric table fan. After drying, the herbs were shredded to fine particles and blended with distilled water with the use of an electric blender. The ratio of the water to herb used in the processing was 3:1. The aqueous mixture of *Persicaria minor* was then filtered with Whatman No.1 filter paper and the filtrates were evaporated under a vacuum evaporator at a temperature of 50°C. The viscous mass of the crude herbal extracts were stored at 0-4°C until further experiment. Once thawed, the extract was kept in a dark glass bottle wrapped in aluminium foil and stored in refrigerator.

Rats: Experimental animals used in this study were Wistar rats obtained from the Universiti Kebangsaan Malaysia (UKM) Animal House. Male rats aged 3 months and weighed between 200-250 g were used. The animals were treated after acclimatization period of seven days to room temperature and relative humidity of 28.5°C and 50%, respectively. They were housed in standard cages and put under 12 h-light/dark cycle and fed a standard rat chow diet with tap water given *ad libitum*. Food and water were not withheld before oral administration of the extracts to rats. Animals were maintained and handled according to the recommendations from the UKM animal ethics committee which had approved the study design of the experiment (Approval code: PP/FAR/2009/NORLIZA/24-FEBRUARY/247). Baseline values for clotting time, bleeding time as well as other haematological and biochemical parameters were obtained before the treatment began.

Study design: This study was conducted based on OECD Guideline 420 (OECD, 2001) whereby fixed dose procedure was used in order to assess the acute and subacute toxicities of chemicals. The fixed doses of *Persicaria minor* used in this study were 300, 1000 and 2000 mg kg^{-1} b.wt. The doses were selected after carrying out a sighting study whereby a single animal was used for each dosage level and the animals were observed for any evidence of toxicity or death. The purpose of the sighting study was to allow selection of appropriate starting dose for the main study. This procedure differs from the traditional method of assessing toxicity as the latter uses death of animals as endpoint. Instead, it relies on the observation of clear signs of toxicity at one of a series of fixed dose levels.

Acute toxicity study protocol: Twenty male Wistar rats were randomly assigned into four groups (n = 5). The first group served as control and was given distilled water at 1 mL kg^{-1} b.wt. The remaining groups were given a single dose of either 300, 1000 or 2000 mg kg^{-1} b.wt. of the test extract, respectively. The number of deaths would be recorded after 24 h of administration.

Subacute toxicity study protocol: Since there was no death observed after 24 h of administration, the study was continued with the subacute toxicity study. Each group was treated orally with *Persicaria minor* extract either at a dose of 300, 1000 or 2000 mg kg^{-1} b.wt. once daily for a duration of 14 days. The control group was given distilled water 1 mL kg^{-1} b.wt. instead of the extract. Rats were weighed daily. In the mean time, mortality as well as abnormal clinical manifestations such as piloerection, salivation and lacrimation, were monitored daily. On day 15, all rats were fasted for 16-18 h and measurements for bleeding and clotting times were taken prior to sacrifice. The heparinised blood samples were taken for determining complete blood count, red blood cell count, platelet count and red cell indices. The serum from non-heparinized blood was carefully collected for blood chemistry and enzyme analysis. All rats were sacrificed after the blood collection. The blood samples were sent to an independent lab for biochemical and haematological analyses.

Statistical analysis: Data were expressed as Mean±SEM and analyzed using ANOVA followed by Tukey's *post hoc* test. Results were considered significant at $p<0.05$.

RESULTS

Mortality: There was no mortality observed after 24 h and the following 14 days of administration of the *Persicaria minor* extract in all rats from both control and treated groups.

Body weight: There was an increase in daily body weight from day 0 until day 14 which was corresponding to normal growth in rats (Table 1).

Baseline parameters: Blood was taken at the beginning of the study before treatment commenced. There was no significant different in all baseline haematological and biochemical parameters between the control and treated groups (Table 2).

Blood parameters after 14 days: As shown in Fig. 1, administration of *Persicaria minor* water extracts at a dose of 2000 mg kg^{-1} b.wt. showed a significant reduction in haemoglobin level (12.4±0.9 g dL^{-1}) compared to control (14.2±1.1 g dL^{-1}). The rats in this group also had a

Table 1: Mean body weights of control and treated rats at the beginning and end of study period

	Body weight (g)			
Day	Control	PM300	PM1000	PM2000
1	200.20±12.674	213.80±14.610	213.40±9.745	202.40±11.466
14	245.00±13.740	262.00±19.105	267.40±7.270	246.00±10.597

PM300, PM 1000, PM2000: Groups of rats treated with oral *Persicaria minor* extracts at a daily dose of 300, 1000 and 2000 mg kg^{-1} b.wt., respectively, All values are expressed as Mean±SEM (n = 5/group), No statistical significance: control vs. treated groups (ANOVA)

Table 2: Biochemical and haematological indices in the different groups of rats at the beginning of study period

Parameter	Control	PM300	PM1000	PM2000
Bleeding time (s)	302±198	322±179	298±185	196±183
Clotting time (s)	98±25	159±98	76.3±23	162±18
RBC level (x10^{12} L^{-1})	6.78±0.88	6.86±0.79	7.02±0.11	7.8±0.91
Haemoglobin (g dL^{-1})	12.32±1.52	11.12±1.31	10.87±1.43	9.79±1.45
Platelet (x10^{9} L^{-1})	712±198	523±177	297±181	573±190
TWC (x10^{9} L^{-1})	10.1±3.9	11.3±3.5	11.4±3.7	8.9±3.3
Total cholesterol (mmol L^{-1})	1.56±0.56	1.48±0.58	1.52±0.61	2.54±0.49
Total protein (g L^{-1})	55.2±3.2	66.7±3.12	67.1±2.98	61.9±2.77
Albumin (g dL^{-1})	37.5±0.98	36.1±0.72	35.9±0.81	35.5±0.74
Total bilirubin (g L^{-1})	1.2±0.15	1.2±0.14	1.1±0.14	1.2±0.12
ALP (IU L^{-1})	623±78	511±81	409±83	544±91
AST (IU L^{-1})	441±32	402±31	325±39	349±37
ALT (IU L^{-1})	79±8	75±7	62±9	70±8
Urea (mmol L^{-1})	8.1±0.18	8.8±0.2	12.7±0.25	11.5±0.15
Creatinine (mmol L^{-1})	47±2.7	50±2.8	57±2.5	53±2.1
Calcium (mmol L^{-1})	2.65±0.16	2.56±0.13	2.67±0.12	2.81±0.15
Phosphate (mmol L^{-1})	3.9±0.88	3.2±0.79	4.5±0.76	4.9±0.85
Uric acid (mmol L^{-1})	0.11±0.02	0.13±0.02	0.15±0.02	0.14±0.01
Sodium (mmol L^{-1})	144.2±1.96	144±2.1	144.8±1.9	148±1.89
Potassium (mmol L^{-1})	5.6±0.2	4.8±0.16	5.2±0.19	5.2±2.1

PM300, PM 1000, PM2000: Groups of rats treated with oral *Persicaria minor* extracts at a daily dose of 300, 1000 and 2000 mg kg^{-1} b.wt., respectively, All values are expressed as Mean±SEM (n = 5/group), No statistical significance: control vs. treated groups (ANOVA)

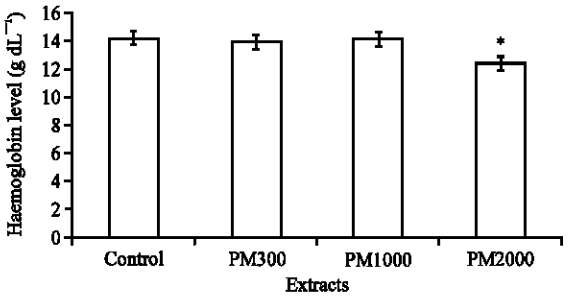

Fig. 1: Haemoglobin levels in rats treated with oral *Persicaria minor* extracts for 14 days, All values are expressed as Mean±SEM (n = 5/group), *p<0.05 compared with control group

significantly high level of serum potassium at 5.1±0.98 mmol L^{-1} compared to control group (4.3±0.89 mmol L^{-1}) (Fig. 2). Conversely, all the rats fed with *Persicaria minor* extract had a

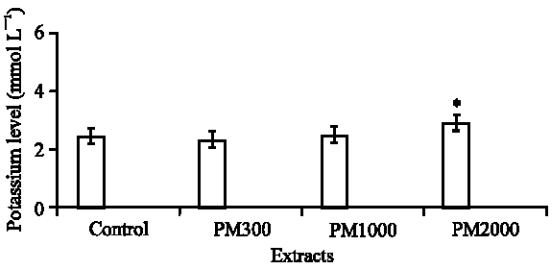

Fig. 2: Potassium levels in rats treated with oral *Persicaria minor* extracts for 14 days, All values are expressed as Mean±SEM (n = 5/group), *p<0.05 compared with control group

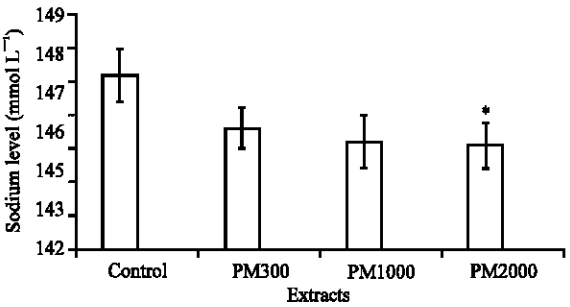

Fig. 3: Sodium levels in rats treated with oral *Persicaria minor* extracts for 14 days, All values are expressed as Mean±SEM (n = 5/group), *p<0.05 compared with control group

Table 3: Biochemical and haematological indices in the control and treated rats at the end of the 14-day study period

Parameter	Control	PM300	PM1000	PM2000
Bleeding time (sec)	487±110	462±115	423±118	426±114
Clotting time (sec)	125±111	261±108	76.3±115	162±103
RBC level (x10^{12} L^{-1})	7.3±0.12	7.5±0.11	7.6±0.11	7.8±0.09
Platelet (x10^{9} L^{-1})	819±180	716±179	754±180	1009±181
TWC (x10^{9} L^{-1})	14.5±3.5	15.1±3.8	14.4±3.5	16.8±3.4
Total cholesterol (mmol L^{-1})	1.42±0.2	1.33±0.18	1.48±0.17	1.35±0.2
Total protein (g L^{-1})	65±0.12	64.5±0.18	62.3±0.11	61.8±0.1
Albumin (g dL^{-1})	35.5±0.8	34.1±0.98	34.5±0.89	32.9±0.9
Total bilirubin (g L^{-1})	1.03±0.1	1.2±0.1	1.0±0.1	1.23±0.1
ALP (IU L^{-1})	407±10	483±11	398±11	415±12
AST (IU L^{-1})	253±22	300±24	257±23	315±25
Urea (mmol L^{-1})	8.1±0.1	7.9±0.2	8.3±0.15	7.5±0.11
Creatinine (mmol L^{-1})	51.8±2.4	55.9±2.3	56.8±2.5	60.5±2.3
Calcium (mmol L^{-1})	2.7±0.1	2.58±0.12	2.55±0.11	2.68±0.15
Phosphate (mmol L^{-1})	3.2±0.48	2.5±0.39	2.47±0.4	3.0±0.45
Uric acid (mmol L^{-1})	0.11±0.04	0.13±0.03	0.14±0.05	0.18±0.04

PM300, PM 1000, PM2000: Groups of rats treated with oral *Persicaria minor* extracts at a daily dose of 300, 1000 and 2000 mg kg^{-1} b.wt., respectively, All values are expressed as Mean±SEM (n = 5/group), No statistical significance: control vs. treated groups (ANOVA)

significant reduction in their serum sodium concentration (Fig. 3). The rest of the biochemical and haematological parameters including total cholesterol, platelet and creatinine were normal and did not differ significantly from the control rats (Table 3).

DISCUSSION

The present research investigated the acute and subacute effects of *Persicaria minor* exposure on rats based on OECD Guideline 420 (OECD, 2001). The procedure provides information on the hazardous properties of a substance that can cause acute toxicity. Eventually it enables the substance to be ranked and classified based on the Globally Harmonized System (GHS) of Classification and Labelling of Chemicals (UNEC, 2009). The fixed dose procedure differs from the classical method of acute toxicity testing in the way that it uses fewer animals and causes less suffering. This approach avoids using death of animals as an endpoint and rely instead on the observation of clear signs of toxicity such as changes in skin and fur, eyes and mucous membranes and also respiratory, circulatory, autonomic and central nervous systems, as well as somatomotor activity and behaviour pattern. In addition, observation of any tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma should also be done (Lipnick *et al*., 1995).

In the current study, three fixed doses were used with a dose of 2000 mg kg^{-1} b.wt. as the maximum dose. There was no mortality noted during the treatment period. The body weights of all the rats were increased throughout the two weeks of oral administration of the aqueous herb. The gain in body weight of all the animals indicates that the animals were growing normally and that the administration of the herb did not have any deleterious effects on the growth of the animals. Changes in body weight have been used as an indicator of adverse effects of drugs and chemicals (Tofovic and Jackson, 1999; Raza *et al*., 2002; Teo *et al*., 2002). Therefore, the present study revealed that oral administration of *Persicaria minor* extract with different doses did not pose any toxicity to rats.

The OECD 420 does not allow the calculation of a precise LD50, but since death of a proportion of the animals is still the major endpoint of the study, it is therefore still possible to determine the exposure ranges where lethality is expected. From the data obtained in this study, the LD50 for *Persicaria minor* can be estimated to be above 2000 mg kg^{-1}, since there was no death of the animals recorded at the highest dose used. According to OECD guideline, an LD50 dose of 2000 mg kg^{-1} and above cannot be categorized under the GHS category and therefore is regarded to be safe (OECD, 2001).

Haematological analysis provides valuable information about blood and bone marrow, which is the blood-forming tissue. Total blood count and differential leucocytes count are used to diagnose anaemia, to identify acute and chronic illnesses, bleeding tendencies and white cell disorders (Miller and Starks, 2010). Analysis of blood parameters is relevant in toxicity studies as any damage to blood cells by any harmful chemicals can be detected (Olson *et al*., 2000). Oral administration of *Persicaria minor* extract did not have any adverse effects on the total white count. However, daily feeding of rats with the extract at a very high dose for 14 days showed a reduction in haemoglobin level. This finding is similar with the previous study performed on green tea extract which is rich in polyphenol (Kapetanovic *et al*., 2009). The reduction in haemoglobin level could probably be caused by improper absorption of either folic acid or iron due to the high polyphenol contents in *Persicaria minor*, as it was shown previously that polyphenols had an antifolate activity (Navarro-Peran *et al*., 2005). Polyphenol antioxidants in several herbal extracts were also proven to block absorption of iron in the intestines (Brown *et al*., 1990). Some polyphenols bind to iron and form a complex that cannot be transported into intestinal cells. Because they are not absorbed, these iron-containing complexes are excreted into the faeces. Further study of course is required in order to establish the exact mechanism on how *Persicaria minor* causes anaemia.

Biochemical tests can be used to diagnose any toxic effects of drugs and phytochemicals on the liver, heart and kidney (Obici *et al*., 2008). Biochemical measurements can also detect any acid-base imbalance in the respiratory and metabolic systems, abnormalities in lipid metabolism and various endocrine systems as well as other nutritional or metabolic disorders (Corns, 2003). The results of the liver function tests showed that the conjugating ability of the liver was intact and there was no hepatocellular damage induced by consumption of *Persicaria minor* extract, as revealed by the levels of bilirubin, serum ALP and AST. The synthetic ability of the liver was also normal as the levels of total proteins and albumin in the treated rats were of no different from the control group. The cholesterol synthesis was also normal.

In toxicity studies, renal function is likely to be affected when high doses of test compounds are being given since kidneys serve as the main organ of elimination for many drugs and their metabolites (Van Vleet and Schnellmann, 2003; Greaves, 2007). Determinations of serum electrolytes, creatinine and urea are critical as they are the important markers of kidney function (Arneson and Brickell, 2007). Electrolytes, such as calcium, sodium and potassium, are important minerals in the body. The body uses electrolytes to help regulate nerve and muscle function, maintaining acid-base balance necessary for normal cell functions and also maintaining fluid balance in cells, tissues and organs (O'Hara and Richardson, 2008). Electrolyte abnormalities thus affect many organs and tissues, with the potential for significant morbidity and mortality (Goh, 2004). In this study, there were no significant differences in serum levels of creatinine and urea in all of the treated groups compared to controls. As for the serum electrolytes, the aqueous extract of *Persicaria minor* was noted to reduce the serum sodium in all the three groups of treated rats. Conversely, the serum potassium level was increased but only in the group of rats receiving the highest dose of the extract. It seems that the herb, especially in. The renal regulation of sodium is closely tied to that of potassium via the renin-angiotensin-aldosterone system whereby active reabsorption of sodium in renal tubules is accompanied by secretion of potassium in the distal tubule of the kidneys (Edwards, 2001). Therefore, it is not surprising that a very high dose of *Persicaria minor* could precipitate electrolyte imbalance by causing hyponatraemia and hyperkalaemia. However, renal function is not totally affected by this herb as calcium, urea and creatinine in the treated groups were not significantly different from those of the untreated rats.

CONCLUSION

It is therefore concluded that the oral administration of the *Persicaria minor* extracts of 300, 1000 and 2000 mg kg^{-1} per b.wt. for 14 consecutive days to male Wistar rats did not cause any mortality or short-term toxicological effects. At a dose of 2000 mg kg^{-1} per b.wt. for 14 consecutive days, the oral intake of the extract induces anaemia and electrolyte imbalance. Long-term studies with graded doses of the extract are required in order to rule out any chronic effects.

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