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Toxicological studies of *Turraeanthus mannii* (Meliaceae)

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Abstract

The present study investigated the acute (single oral administration) and subacute (during 8 weeks) toxicity of aqueous extract of stem bark of *Turraeanthus mannii* (TM) in mice, as this species has not been adequately studied for its safety. After oral administration, the water and food intake lowered significantly ($p<0.001$) at dose 15 g kg^{-1} and 20 g kg^{-1} and consequently had significant effect with $P < 0.01$ in body weight. There were no significant difference concerning triglyceride level, haematological parameters, relative weight of organs (liver, kidney, lungs, testis, ovaries) and usual leaver markers (ALT, AST, Gamma-GT). The LD_{50} of TM-extract in mice was 20.4 g kg^{-1}. The level of serum protein enhanced significantly at doses 15 g kg^{-1} and 20 g kg^{-1} with $p<0.01$, while serum creatinine increased at dose 15 g kg^{-1} and 20 g kg^{-1} with $p<0.05$. LDL cholesterol, HDL cholesterol and Total cholesterol level of treated animals lowered at all doses with $p<0.05$. The histology of lungs, liver, kidney of treated animals did not presented morphological changes (data not shown). In subacute treatment, the body weight decreased at dose 4 g kg^{-1} and 10 g kg^{-1} with $p<0.05$ in both sex. Other toxicological parameters was almost the same as in acute treatment except the significant increase of triglyceride at dose 4 g kg^{-1} with $p<0.05$, inflammation and leucocytes infiltration of lungs and liver. The results suggest that plant is not toxic, but high dose and long term use of TM-extract could cause damage to vital organs.

Keywords: *Turraeanthus mannii*, acute and subacute toxicity; histopathology

Introduction

A large majority of the world's population is using herbal medicines, one of the most important sources of active biological substances with therapeutic potential to cure human

diseases (Gill et al., 2010; Gill et al., 2011). According to World Health Organization about 80% of the world population has developed interest in phytotherapy for primary health care because of the increasing cost and non availability of synthetic drugs. The large and widespread of the continued therapeutic application of these medicinal plants in its different forms (decoction, infusion, macerate, cataplasm and enema) are always associated with several side effects. So there is a compelling need for thorough scientific safety evaluation of the medicinal plants (Ben-Arye et al., 2011) *Turraeanthus mannii* (TM) also known in the local language as «*Hípûl» to* Banen people and «*assana*» to Ewondo people and pygmies, is a leguminous woody species of humid forest ()Vivien and Faure, 1971) distributed throughout Angola, Benin, Ghana, Equatorial Guinea, Nigeria, Democratic Republic of Congo, Sierra Leone, Uganda, Ivory Cost and Cameroon where it's used as an economic timber (Letouzey 1972). TM is a large thorny tree that may reach 20m in height which can be easily recognized with its wide and rounded white crown. The trunk is thicker at the base and possesses yellow boughs a 1.5m long and yellow fruits which contain 2 to 6 yellow seeds. The bark is light grey and smooth when young, but becomes cracked with age. The tree is characterized by a long, deep taproot (Souane, 1983). The phytochemical studies of this plant revealed the presence of steroids, phenolic compounds as coumarins and lignanes, and terpenoids (J Vardamides et al., 2007). Stem bark or roots of TM are used in traditional medicine in Cameroon, Gabon and Ivory Cost to treat constipation, tumours, stomacal pain, yellow fever and food poisoning to capture fishes (Ekwalla and Tongo, 2003). In Democratic Republic of Congo decoction of stem bark is used for cough and headache while in Ghana, roots of the plants is apply as cataplasm for rheumatism (Bouquet, 1959). The present studies were carried out to evaluate the acute and sub-acute toxicity of TM in mice.

Materials and Methods

Plant material

The stem bark of TM was collected in the south west region of Cameroon in the locality of Limbe. The plant material was authenticated by Mr Nana Victor, botanist of the national herbarium of Cameroon where of a voucher specimen was deposited under number 18312/SRF/Cam.

Preparation of extract

The stem bark was cleaned, cut into small species, air-dried for 7 days and pounded into fine powder using plant mill. The powder was stored in an airtight container and kept in a cool dry place. A 2000 g of the powdered stem bark was suspended in 7 l of distilled water, heated and boiled for 30 min. After filtration by the Whatmann no. 3 papers, the resultant filtrate was lyophilised to give a dark brown extract. The crude yield of the lyophilised material was approximately 3.65% (w/w). The lyophilised extract was further diluted to obtain different concentrations in one 1 ml. Obtained solutions were stored at -4°C until required for use.

Acute toxicity studies

About 50 Swiss mice of both sexes weighting between 14.5 - 25.50g were obtained randomly from the bred colony in the animal house of the faculty of science of the University

of Douala. The animals were allowed to acclimatize for 2 weeks. They were housed in 10 (five females and five males per each different plastic cage) in a controlled environment (ambient temperature, 27.0± 2.0°C and with a 12 h light/darkness cycle), and mice –chow and water were given ad-libitum except for a short fasting period before oral administration of single doses of the TM-extract. All experiments in mice were carried out in accordance with the recommendation of the guidelines for care and use of laboratory animals approved by the Institutional Animal Ethics Committee of the Faculty of Science-Section, University of Douala. The control group received distilled water as vehicle and each treated group received the aqueous TM- extract by gavages in a dose of 5, 10, 15, 20g kg^{-1} of raw material. Treated animals were deprived of food and water for 2 h to assess the general behaviour of mice and thereafter during a period of 48 h for dead animals (WHO, 1998). During a 48 h-period of observation, the body weight changes, food and water intake were recorded. After 7 days of observation for any signs of toxicity and deaths and the latency of death, blood was collected from the orbital sinus under ether anaesthesia for biochemical and haematological analysis. The biochemical parameters evaluated included creatinine, protein, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase, total cholesterol, triglycerides, HDL cholesterol and LDL cholesterol and were assessed using comercial kits. Red blood-cell count, hematocrit and leucocytes counts were determined concerning haematological parameters. After the blood collection, the animals were sacrificed by cervical displacement and selected organs like liver, kidney, lungs, (testis and ovaries for further research) were removed for macroscopic analysis. Portions of these organs collected from the control group and the TM-treated groups were fixed in Bouin medium and were embedded in paraffin, then subjected to haematoxylin–eosin staining. The pathological observations of all organs were performed on gross and microscopic bases. The LD_{50} value was determined according to the method of Deichmann and Leblanc (Molle, 1986).

Subacute toxicity studies

Female and male Swiss mice (Mus musculus) weighting between 14.5 - 25.50g were housed under the same conditions as described above for the acute toxicity. The animals were divided into one control group (distilled water) and three treated groups (4, 7, 10 g kg^{-1} of raw material as per the guidelines of World Health organization (WHO, 1992). Toxic manifestations, mortality, body weight changes, food and water intake were monitored daily during the 8-week period. At the end of the experiment, the biochemical, haematological and histopathological analysis was also assessed as described above for the acute toxicity.

Statistical analysis

Values are expressed as mean ± SEM Statistical analysis was performed using the Mann–Whitney test. P-values less than 0.05 were considered to be significant.

Results

Acute toxicity

In this study, the oral administration of the aqueous extract of TM-extract at all given doses (up to 20g kg^{-1}) did not produce significant changes in behaviour, breathing, coetaneo-

Table 1. Effect of *Turraeanthus mannii* (TM)-extract on organs weight 7 days after a single daily oral administration.

Dose (g kg^{-1})	Left kidney		Left Liver		Left lung		Left testis	Left ovary
	Males	Females	Males	Females	Males	Females	Males	Females
Control	0.28±0.04	0.27±0.11	0.97±0.04	1.11±0.3	0.2±0.005)	0.21±0.002	0.16±0.04	0.17±0.03
10	0.27±0.08	0.23±0.03	0.92±0.23	1.11±0.26	0.22±0.02	0.23±0.05	0.17±0.06	0.15±0.02
15	0.29±0.02	0.2±0.02	0.97±0.08	1.08±0.27	0.16±0.005	0.26±0.08	0.18±0.01	0.16±0.06
20	0.31±0.04	0.28±0.02	0.89±0.03	0.98±0.09	0.19±0.24	0.19±0.01	0.15±0.05	0.14±0.04

Value are mean (g/100 g per body weight) ± SEM; n = 4. No significant difference was observed in any parameter.

Table 2. Mean relative body weight in mice treated with *Turraeanthus mannii* (TM)-extract 7 days after a single daily oral administration.

Dose (g kg^{-1})	Day before treatment (D_0)		Day before treatment (D_7)	
	Males	Females	Males	Females
Control	22.28 ± 3.2	17.80 ± 3.3	23.22 ± 1.5	19.68 ± 3.2
10	24.71 ± 2.3	21.93 ± 1.8	21.03 ± 2.9	18.73 ± 2.7
15	22.19 ± 4.8	21.03 ± 1.6	16.49 ± 1.4*	16.27 ± 1.8*
20	25.01 ± 2.1	23.99 ± 1.9	17.01± 1.7**	16.31 ± 0.15**
25	-	-	-	-

Value are mean ± SEM; n = 4. *P < 0.05. **p< 0.01 *vs. control group*

us effects, sensory nervous system responses, and relative weight of organs (liver, kidney, lungs, testis, ovaries) (Table1) during the experimental period (48 h), however we noticed a significant decrease of body weight gain at dose 15 g kg-1 with p<0.05 and at dose20 g kg-1 with p<0.01(Table 2). In the other hands, the water and food intake was also affected significantly at dose 15 and 20 g kg^{-1} with p<0.001 (Fig 1). The acute oral toxicity (LD_{50}) of TM-extract in mice was 20.4 g kg^{-1}. Although there were no significant difference concerning haematological parameters (table 4), the biochemical analyses presented reliable significant differences between the treated and control animals. So in comparison with the control group (Table 3) , the level of serum protein enhanced significantly at doses 15 g kg^{-1} and 20

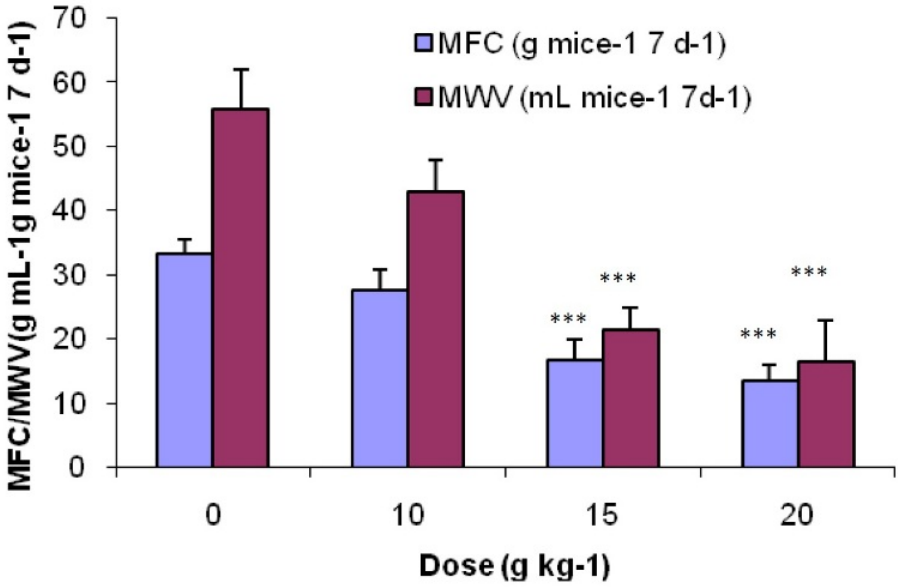

Figure 1. Effect of *Turraeanthus mannii* extract on feed and water consumption in mice.

Table 3. Biochemical parameters of mice treated with *Turraeanthus mannii* (TM)-extract 7 days after a single daily oral administration.

Parameter	Dose (g kg^{-1})			
	Control	10	15	30
LDL cholesterol (mm)	2.72 ± 0.3	1.61± 0.18*	1.63± 0.14*	1.23 ± 0.15*
HDL cholesterol (mm)	2.69 ± 0.2	1.57± 0.12*	1.55 ± 0.13*	1.82 ± 0.1*
TC/HDL	2.1 ± 1.02	2.18 ± 0.6	2.2 ± 0.9	1.93 ± 1.07
Total cholesterol (TC) (mm)	5.66 ± 0.7	3.47 ± 0.6*	3.41 ± 0.3*	3.3 ± 0.4*
Triglyceride (TG) (mm)	0.51 ± 0.5	0.49 ± 0.4	0.45 ± 0.9	0.52 ± 0.7
AST (U/L)	53.71 ± 15.6	50.67 ± 9.7	51.69 ± 12.1	47.03 ± 11.02
Gamma-glutamyl-transferase (U/L)	2.41 ± 1.6	2.87 ± 1.5	2.18 ± 1.3	2.38 ± 2.5
ALT (U/L)	4.44 ± 0.42	3.98 ± 0.88	4.78 ± 0.66	5.16 ± 0.32
Serum protein(µm)	388.61±40,29	447.62±28,84	519,16±21,98**	576,9±41,13**
Serum creatinine (µm)	19.64±12.42	32.74±9,76	59.10±9.81*	64.19±14.73*

Value are mean ± SEM; n = 4. *P < 0.05; **P < 0.01 *vs.* control group, ALT, Alanine aminotransferase; AST, Asparate aminotransferase

Table 4. Haematological parameters of mice treated with *Turraeanthus mannii* (TM)-extract in acute treatment.

Parameter	Dose (g kg^{-1})			
	Control	10	15	30
Red blood cell (mm3)	10.082 ± 0.5	9.072 ± 0.4	9.78 ± 0.3	9.82 ± 0.2
Haematocrit (%)	49.057 ± 4.4	46.023 ± 2.4	49.1 ± 1.4	50.025 ± 2.7
Leucocyte (·106 ml-1)	9.42 ± 2.2	10.60 ± 2.4	9.187 ± 2.7	8.85 ± 2.3

Value are mean ± SEM; n = 4. No significant difference was observed in any parameter.

g kg^{-1} with $p<0.01$, while serum creatinine increased at dose 15 g kg^{-1} and 20 g kg^{-1} with $p<0.05$. LDL cholesterol, HDL cholesterol and Total cholesterol level of treated animals lowered at all doses with $p<0.05$, while no significant difference were noticed about triglycerides. No concomitant alteration in the activity of alanine aminotransferase, aspartate aminotr ansferase and gamma-glutammyl transferase was found. The histology of lungs, liver, kidney of treated animals did not presented morphological changes except leucocytes infiltrations, vascular congestion and cells clarification (data not shown).

Sub acute toxicity

The lethal effect of oral administration of TM-extract for 8 consecutive weeks begins after one week of treatment so that eighteen mice (60%) have died at the end of the experiment. There were no significant difference concerning relative weight of organs (liver, kidney, lungs, testis, ovaries) (Table 5), water, food intake and body weight gain during the first 4th weeks, however body weight gain significantly decreased from the 5th week till the end of treatment at dose 4 g kg^{-1} and 10 g kg^{-1} with $p<0.05$ in both sex (Table 6). Neither significant difference was observed concerning haematological parameters (Table 7) and

Table 5. Effect of *Turraeanthus mannii* (TM)-extract on relative organs weight for 8 consecutive weeks.

Dose (g kg^{-1})	Left kidney		Left Liver		Left lung		Left testis	Left ovary
	Males	Females	Males	Females	Males	Females	Males	Females
Control	0.35±0.07	0.24±0.06	1.41±0.22	1.01±0.19	0.23±0.02	0.24±0.09	0.2±0.09	0.16±0.04
4	0.25±0.02	0.29±0.05	1.28±1.19	1.16±0.06	0.18±0.05	0.21±0.02	0.21±0.03	0.15±0.03
7	0.19±0.09	0.27±0.03	0.79±0.12	1.01±0.3	0.16±0.03	0.18±0.01	0.19±0.07	0.13±0.08
10	0.3±0.03	0.25±0.05	1.01±0.08	1.1±0.09	0.19±0.04	0.19±0.09	0.22±0.06	0.14±0.09

Value are mean (g/100 g per body weight) ± SEM; n = 5. No significant difference was observed in any parameter.

Table 6. Mean relative body weight in mice treated with *Turraeanthus mannii* (TM)-extract for 8 consecutive weeks.

Dose (g kg^{-1})	Period of treatment (week)							
	First	Second	Third	Fourth	Fifth	Sixth	Seventh	Eighth
Male								
Control	21.8±0.9	21.3±0.7	20.9±0.6	20.7±0.7	20.9±0.3	21.7±0.8	19.33±0.3	20.12±0.5
4	21.1±0.5	21.2±0.3	20.7±0.6	20.3±0.8	18.1±0.2*	18.2±0.5*	17.01±0.7*	17.8±0.6*
7	22.3±0.2	22.6±0.4	21.8±0.3	21.4±0.7	21.1±0.56	21.2±0.75	20.9±0.89	21.04±0.9
10	20.9±0.8	20.7±0.5	19.7±0.3	19.1±0.6	17.4±0.9*	17.2±0.6*	17.08±0.2*	15.48±0.8**
Female								
Control	23.5±0.37	22.9±0.81	22.7±0.50	23.1±0.25	22.8±0.41	22.74±0.7	23.2±0.9	23.6±0.6
4	22.6±0.13	22.6±0.19	22.4±0.17	22.7±0.10	23.01±0.4	22.81±0.2	22.5±0.12	22.4±0.2
7	22.0±0.1	22.1±0.2	22.01±0.1	21.4±0.23	20.8±0.3*	20.5±0.7*	20.6±0.5*	20.3±0.4*
10	22.2±0.8	22.04±0.6	21.8±0.9	21.6±0.71	20.1±0.5*	19.8±0.7*	18.7±0.7*	16.2±0.6**

Value are mean ± SEM; n = 5. *P < 0.05. **p< 0.01 *vs. control group*

Table 7. Biochemical parameters of mice treated with *Turraeanthus mannii* (TM)-extract for 8 consectives weeks.

Parameter	Dose (g kg^{-1})			
	Control	4	7	10
LDL cholesterol (mm)	2.87 ± 0.43	1.45 ± 0.18*	1.06 ± 0.16*	0.94 ± 0.12*
HDL cholesterol (mm)	2.81 ± 0.27	1.65 ± 0.17*	1.67 ± 0.15*	1.9 ± 0.14*
TC/HDL	2.58 ± 2.36	271 ± 1.23	2.92 ± 1.48	2.32 ± 0.28
Total cholesterol (TC) (mm)	7.25 ± 1.38	4.72 ± 0.9	5.05 ± 0.52	4.41 ± 0.86
Triglyceride (TG) (mm)	2.02 ± 0.51	3.42 ± 0.38*	2.50 ± 1.2	2.75 ± 0.78
AST (U/l)	49.52 ± 11.9	46.47 ± 9.55	47.3 ± 9.01	43.8 ± 9.82
Gamma-glutamyl-transferase (U/L)	2.29 ± 1.71	2.89 ± 1.57	2.17 ± 1.19	2.44 ± 2.61
ALT (U/L)	13.76 ± 17.87	3.44 ± 8.42	5.16 ± 10.32	4.18 ± 9.47
Serum protein (µm)	201±22,31	270,46±27,66	231,27±11,52	219,75±23,05
Serum creatinine (µm)	43.21±5.19	47.15±7.45	44.20±8.84	53.04±17.68

Value are mean ± SEM; n = 6. *P < 0.05 *vs. control group,* ALT, Alanine aminotransferase; AST, Asparate aminotransferase

Table 8. Haematological parameters of mice treated with *Turraeanthus mannii* (TM)-extract in sub acute treatment.

Parameter	Dose (g kg-$^{-1}$)			
	Control	10	15	30
Red blood cell (mm3)	8.072 ± 0.38	7.062 ± 0.217	7.68 ± 0.183	7.71 ± 0.124
Haematocrit (%)	47.043 ± 2.41	45.017 ± 1.04	46.9 ± 1.16	48.016 ± 1.67
Leucocyte (·106 mL-1)	7.36 ± 1.15	8.54 ± 1.05	7.187 ± 1.46	7.87 ± 1.22

Value are mean ± SEM; n = 4. No significant difference was observed in any parameter.

transaminases activities nor serum protein and creatinine when compare treated and control groups, however LDL cholesterol and HDL cholesterol significantly decreased at all doses with $p<0.05$ while the level of triglyceride significantly increased at dose 4 g kg^{-1} (Table 8). The histology of kidney of treated animals did not presented any morphological changes at all doses, but we noticed a generalized inflammation associated with leucocytes infiltrations of lungs and liver indicating that alveoli and hepatocytes were altered (data not shown).

Discussion

Nowadays, Many scientific research based on the study of medicinal plants are generally focused to elucidate their pharmacological effects and to determine their chemical compounds, whereas there are many limitation regarding the safety and the efficacy of these preparations. In the present study TM well known plant in the bakingili village of Cameroon used as weapon to capture fish was investigated to assess its safety and tolerability profile in short and long term treatment. It is found that a single oral administration of TM extract did not lead to any behavioural change and relative weight of target organs after 48h of observation. Nevertheless body weight gain was affected particularly at dose 20 g kg^{-1}. Our results are similar to those observed by Emerson et al (1983) when studying toxicological effect of the root of *Plumbago rosea*. They found no death at doses less than 10 g kg^{-1}.This, however, suggest that the extract has no acute toxicity in those organs. The acute oral toxicity (LD_{50}) of TM-extract in mice was 20.4 g kg^{-1}. According to the chemical labelling and classification of acute systemic toxicity based on oral LD_{50} values recommended by the OECD organization (Walum, 1998), substances with LD_{50} less than 2000 mg kg^{-1} body weight given orally are considered safe. Therefore, the obtained high LD_{50} (>2000 mg kg-1body weight) of the TM-aqueous extract in the present study, showed that the extract could be considered relatively safe, particularly when administered orally where absorption may not be complete due to different inherent factors that could limit absorption in the gastro intestinal tract (Jaouad et al., 2004). The reduction in the body weight of the treated mice specially at dose 15 and 20 g kg^{-1} may be due to the decreased of food and water intake in the same doses indicating that the diet was not well accepted by the animals or the extract likely lead to the slowing down of nutrients metabolism (Verbois et al., 2000).

The present study also showed that serum protein was significantly increased at dose 15 and 20 g kg^{-1}. It is known that albumin, fibrinogen and 80% of globulin are plasmatic proteins synthesized in the liver (Guyton, 1982). In the normal condition the level of serum protein depends on the amino acids present in the blood (Dohm et al., 1985). The increase of serum protein at doses 15 and 20 g kg^{-1} may be due to the stimulation of hepatic cells. The effect of TM extract in protein metabolism shows that the extract can stimulate cell growth, although no body weight gain of animals was observed at those doses.

The lipid profile of mice after TM treatment was significantly affected as far as the plasma levels of T-C, LDL-C and HDL-C has lowered when compared to control. The studied alterations in some cholesterols value could be related to modified plasma nitric oxide levels and the presence of terpenoids in the extract. It is well studied that Nitric oxide (NO) is known as a signaling molecule involved in elicitor-induced defence responses of plants (Mai et al., 2011). In the other hands, the patients with unregulated blood glucose levels have abnormal lipid and lipoprotein metabolism and decreased nitric oxide end-products, with relationships between nitric oxide products and dyslipidaemia, especially between low HDL cholesterol levels and increased oxidative stress (Vanizor et al., 2007). Moreover several lines of evidence indicate that low HDL cholesterol per se impaired endothelial function through a decrease in NO (Yukihito et al., 2009). Furthermore HDL activates endothelial NO synthase by enhancing Akt and MAP kinases, leading to an increase in NO, suppresses endothelial cell apoptosis by activation of the Akt pathway and inhibition of caspase-3 and -9, inhibits the oxidation of LDL-C (Mineo et al., 2003). Now it has been

found that Turraeanthus mannii have terpenoıds (Vardamides et al., 2007) which can decreese HDL-C levels (Pouteau et al., 2003) and markedly inhibit LDL oxidation via a mechanism involving scavenging of free radicals (Fuhrman et al., 1997). Therefore, under low cholesterol condition particularly HDL-C, atherosclerotic processes develop progressively, resulting in cardiovascular outcomes. This explain why local people in Cameroon use TM to kill fishes. In fact, powder of stem bark of TM is poured into the river. Once dissolved in the water, the formed solution, soaked up by fishes lead to the hardening of blood vessel of gills. The dissolved oxygen of water can no more be absorbed through gills, by the way fishes died by asphyxia.

The present findings revealed changes in the serum concentration of creatinine. As the macroscopic appearance and weight of the kidney was not altered, hence, the possibility of nephropathology could not be confirmed. The increased level of serum creatinine could be related to filtration's disturbances of blood occurred in Bowman's capsule due to formation of thick plaques into the inner wall of blood vessels . These plaques are resulting from cholesterol alterations.During the experimental period, there were neither treatment-related effects on the haematological parameters nor the activity change of usual liver markers as alanine aminotransferase, aspartate aminotransferase and gama-glutammyl transferase. These results suggest that TM aqueous extract is not a hepatotoxic at all doses

Results of the TM extract after 8 consecutive weeks of treatment was almost similar to acute treatment concerning body weight gain, relative weight of target organs, biochemical, haematological and histopatological parameters apart from elevation of triglycerides, pneumocytes and hepatocytes degeneration indicating that, high dose (10000 mg/body weight) with long term consumption of TM aqueous extract could cause injury of lungs and liver , but without any concomitant modifications in the usual liver markers (ALT, AST) and breathing system.Data of the present study shows strong evidence of the non toxic effect of the aqueous extract of TM. Hence the utilization of TM extract is safe and cannot compromise its application in traditional medicine.

Conflict of interest

The authors declare that there is no conflict of interest in the writing of the manuscript and there is also no financial competing interest in regards to the funding source.

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