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28 Days Repeated Dose Toxicity Study of Ethanolic Extract of Murraya Koenigii in Wistar Rats

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Abstract

The purpose of this study was to assess the safety use of *Murraya koenigii* in terms of repeated dose toxicity study in male and female rats. Three doses 300, 500 and 900 mg/kg of ethanolic extract of *Murraya koenigii* leaves were administered to groups of 10 animals (five male and five female) daily for 28 days respectively. Animals receiving the vehicle (0.5% CMC) served as a control. The biochemical parameters like RBC, WBC, glucose, hemoglobin, cholesterol, creatinine, bilurubin, SGPT and SGOT were analyzed in all groups of animals respectively. While in physiological parameters like daily food consumption, weekly body weight, visual, auditory and organ weights were analyzed respectively. At the end of study all the animals were sacrificed and tested for histopathology for any structural damage to major organs. Result of study showed that none of the animals from any dose levels showed test material related changes in RBC, WBC, creatinine, bilurubin, SGPT and SGOT parameter respectively. Similar results showed for some physiological parameter like daily food consumption, visual and auditory activity. There were no other changes except increased in hemoglobin, drastic and sudden weight loss, decrease in cholesterol and glucose of these animals. Animals from 500 mg/kg and 900 mg/kg showed loss of subcutaneous fat during last two week of treatment. At the same dose level there decreases in total cholesterol and glucose level. While the significant effect was observed with at dose of 900 mg/kg. The Histopathological study showed mild congestion in all treatments groups while haemorrhage and lymphocyte infiltrate observed at 900 mg/kg groups of male and female rats. From results it conclude that administration of ethanolic extract *Murraya koenigii* leaves for 28 days increases hemoglobin level, decreases the body weight, subcutaneous fat and blood glucose level, as observed in medium and high dose group. *Murraya koenigii* do not show any structural damage to major organs except lymphocyte infiltration and haemorrage.

Keywords: *Murraya koenigii*; Repeated dose toxicity study; Antiobesity; Antidiabetics; Dietary supplements

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Introduction

Murraya koenigii (L.) family rutaceae is an aromatic more or less deciduous shrub or a small tree up to 6m. in height found throughout India and is commonly known as Meethi neem and karry tree, is used traditionally as antiemetic, antidiarrhoeal, febrifuge and blood purifier. The whole plant is considered a tonic and stomachic. The leaves are used extensively as a flavoring agent in curries and chutneys. Almost every part of this plant has a strong characteristic odour. The people of the plains, particularly of southern India, use the leaves of this plant as a spice in different curry preparations [1,2]. In the present study, *Murraya koenigii* (L) was chosen since it is one of the most widely acclaimed remedies for the treatment of diabetes and gastrointestinal dysmotility as per findings of our previous work [3-6]. *Murraya koenigii* are used as flavorings, condiment and folk medicine for the treatment of various metabolic and infectious diseases. The leaves, bark, root and fruits are used intensively in indigenous system of medicine from ancient time, as a tonic for stomached, stimulant and creative [7]. Phytochemical screening of M. koenigii revealed the presence of some vitamins, carbazole alkaloid, terpenoids, phenolic compounds and mineral content such as calcium, iron, zinc and vanadium etc. in addition, carbazole alkaloid present in *M. koenigii* were reported to have antioxidant and antidiabetics activities [8-11]. Several biological activities of *M. koenigii* leaves have been reported for its anti-hypercholesterolemia [12,13] as well as its efficacy against colon carcinogenesis [14]. It also reported for anti-microbial, antioxidant [15-18]. Despite these pharmacological activities with *Murraya koenigii* no one report to found its toxicological evaluation.

Table 1: Influence MKL treatments on biochemical parameters Results are expressed in mean of 5 animals per group ± S.D; *Significantly different compared to control at p<0.05 by using student unpaired T test.

Parameters	Sex	Control	MKL-300	MKL-500	MKL-900
Bilurubin(mg/dl)	Male rats	1.015 ± 0.12	1.03 ± 0.076	1.05±0.11	1.03 ± 0.17
	Female rats	1.02 ± 0.13	0.97 ± 0.112	1.02 ± 0.16	0.982 ± 0.13
Hb (gm/dl)	Male rats	12.46 ± 1.28	13.41 ± 0.84	13.65 ±0.934	14.76 ± 0.51*
	Female rats	11.49 ±0.71	12.22 ± 0.93	12.80 ±0.628	13.50 ±0.917*
creatinine	Male rats	1.061 ± 0.11	1.116 ±0.127	1.094 ± 0.19	1.10 ± 0.157
	Female rats	1.09 ± 0.12	1.15 ± 0.08	1.026 ± 0.09	1.187 ± 0.12
RBC	Male rats	12.29 ± 1.10	13.025 ±0.84	12.205 ±0.75	12.575 ± 1.29
	Female rats	12.00 ± 0.97	11.74 ± 0.77	12.99 ± 0.79	11.31 ± 1.11
WBC	Male rats	11.25 ± 1.64	10.81 ± 1.74	10.88 ± 1.54	11.65 ± 1.90
	Female rats	9.59 ± 0.85	10.26 ± 1.20	10.79 ± 0.87	11.12 ± 1.08
SGPT	Male rats	0.42 ± 0.074	0.43 ± 0.06	0.438 ±0.042	0.43 ± 0.048
	Female rats	0.39 ± 0.04	0.42 ± 0.072	0.41 ± 0.055	0.41 ± 0.058
SGOT	Male rats	0.58 ± 0.059	0.69 ± 0.042	0.67 ± 0.089	0.69 ± 0.091
	Female rats	0.699 ±0.098	0.693 ±0.106	0.654 ±0.070	0.6865 ±0.112

Table 2: Esults are expressed in mean of 5 animals per group ± S.D; * indicates significant (p<0.05) different compared to vehicle control readings.

Rat	Physiological response	Control	300 mg/kg	500 mg/kg	900 mg/kg
Male	**Auditory**	Pass	Pass	Pass	Pass
	Visual	Pass	Pass	Pass	Pass
	Locomotor activity(sec.)	92.20± 12.38	101.32 ±9.3	98.21 ±11.28	90.99 ± 10.21
	Grip strength (sec.)	162.09±11.32	161.08±7.89	153.97± 10.22	147.44± 4.21*
Female	**Auditory**	Pass	Pass	Pass	Pass
	Visual	Pass	Pass	Pass	Pass
	Locomotor activity(sec.)	102.7 ± 16.04	99.92 ± 13.22	105.42± 14.86	109.27 ±11.65
	Grip strength (sec.)	142.2± 27.96	141.2 ± 24.15	133.2 ±18.07	129.2 ±27.96*

Therefore, the present study was designed to evaluate its toxicological effects at long time administration of *Murraya koenigii* in terms of 28 days repeated dose toxicity study.

Materials and Methods

Plant

The fresh leaves of *Murraya koenigii* were collected in the month of August 2008 from its natural habitat at Sakoli village in Nagpur region, Maharashtra, India. The plant was authenticated by Dr. N. M. Dongarwar of Botany Department; RTM Nagpur University, Nagpur India. A voucher specimen (no. 9439) was deposited at Herbarium, Department of Botany, RTM Nagpur University Nagpur.

Preparation of extracts of *Murraya koenigii* leaves

The collected leaves of *Murraya koenigii* were dried under shade and undergone crushing in electric blender to form powdered and subjected to extraction by soxhlet's extractor using distilled ethanol as a solvent in ratio of 1:4 (50 gm powder with 200 ml solvent). The extraction was performed for 18 hours. The extract was concentrated by evaporation at room temperature and was used in present study. The percent yield for ethanolic extract of MKL was found to 7.4% w/w.

Material

Biochemical estimation kits e.g. RBC diluting fluid, WBC diluting fluid, Drabkin's reagent for haemoglobin estimation (AGAPPE Diagnostics), Serum creatinine estimation kit (Biolab Diagnostic Pvt. Ltd.), Total bilurubin estimation kit (Biolab Diagnostic Pvt. Ltd.), SGOT and SGPT estimation kit (Biolab Diagnostic Pvt. Ltd.) were used for biochemical estimation.

Experimental animals

All the experiments were carried out in male and female Wister rat (100 gm-110 gm). The animals were fed with standard mice diet (Amrut feed, Sangali, Maharashtra), had free access to water under well ventilated condition of 12h light cycle. The animals were adapted to laboratory condition for 7 days prior to the experiments. Investigations using experimental animals were conducted in accordance to the Organization for Economic Cooperation and Development guidelines no. 407 (OECD, 1993). The studies were performed with the approval of Institutional Animal ethics committee (IAEC) of S.N. Institute of Pharmacy Pusad.

Experimental design

Wister rats of both sexes weighing 100 gm-110 gm were assign to each group contains 5 animals for each sex respectively. Four groups of animals were used for each sex. Group 1 received 0.5% CMC for 28 days and group II, III and IV received orally 300 mg/kg, 500 mg/kg and 900 mg/kg of MKL extract respectively. Body weight of the animals was recorded at the beginning and thereafter every week of experiment.

Table 3:

Organs	Sex	Control	MKL-300	MKL-500	MKL-900
Liver	Male rats	2.28±0.214	2.76±0.436	2.93± 0.353	3.13± 0.332
	Female rats	2.9±0.312	2.82±0.278	2.98±0.398	3.14±0.408
Lung	Male rats	1.07± 0.209	0.936±0.094	1.02±0.17	1.85±0.11
	Female rats	0.989±0.076	1.19±0.172	1.21±0.191	1.114±0.18
Heart	Male rats	0.358±0.041	0.304±0.035	0.32±0.0281	0.37±0.033
	Female rats	0.274±0.018	0.298±0.023	0.301±0.011	0.289±0.03
Spleen	Male rats	0.185±0.028	0.195±0.019	0.185±0.021	0.163±0.015
	Female rats	0.145±0.022	0.187±0.03	0.173±0.03	0.167±0.023
Brain	Male rats	0.92±0.103	0.94±0.14	1.04±0.21	1.08±0.19
	Female rats	0.94±0.09	0.91±0.12	0.99±0.24	1.03±0.104
Kidney	Male rats	0.558± 0.043	0.581±0.07	0.599±0.16	0.632±0.18
	Female rats	0.455±0.09	0.423±0.065	0.434±0.071	0.476±0.055

Results expressed as mean of relative organ weight of 5 animals per group± S.D. The values are not statistically significant compared to vehicle control (Student unpaired T test).

Biochemical parameters

At the end of study (28 days) the animals dissect blood was collected by using retro-orbital plexus and finally from posterior vena-cava under light ether. The hematological studies were performed for estimation of total RBC count, total WBC count [19,20]. Haemoglobin, SGOT, SGPT, total bilurubin, serum creatinine, total cholesterol in the serum were measured by using commercial kits.

Physiological parameters

Daily food consumption, weekly body weight, locomotors activity, grip strength, auditory and visual activities were measured at the end of study [21,22]. The animals were sacrificed after the experimentation and organs liver, lung, kidney, spleen, brain and heart were carefully disserted and their absolute weights were determined. The relative organ weight of each animal was then calculated as follows [23]. After weighing of the organs weight, the organs preserved in 10% formalin solution and were examined for any structural damage by histopathological study.

Statistical analysis

All the experimental results expressed as the mean ± standard deviation. Student unpaired T-test used to detect further difference between groups respectively, values of $p< 0.05$ considered as significant (Graph Pad Prism Version 5.0).

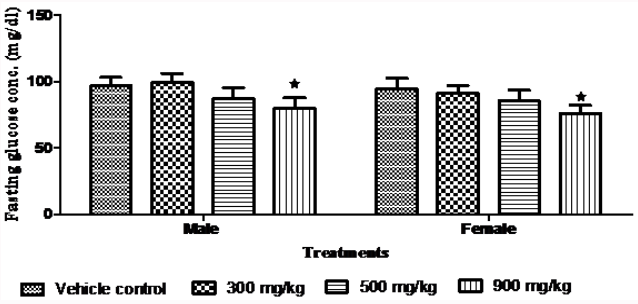

Figure 1: Effect of 28 days treatments of MKL on blood glucose level Results are expressed in mean of 5 animals per group ± S.D; *Significantly different compared to control at p<0.05 by using student unpaired T test.

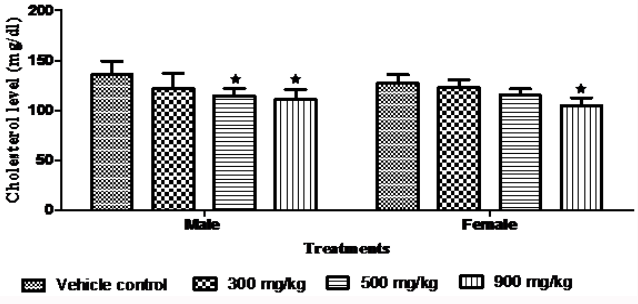

Figure 2: Effect of 28 days treatments of MKL on total blood cholesterol level Results are expressed in mean of 5 animals per group ± S.D; *Significantly different compared to control at p<0.05 by using student unpaired T test.

Results and Discussion

The plants have acquired crucial importance in folk medicine, because of its therapeutic or toxic properties [24]. Use of any drug for treatments is often base on long-term clinical experience. Medicinal plants have plays an enormous importance in public health, especially in low-income population, whose access to modern medicines is limited. There is limited scientific evidence regarding the safety and efficacy to support the continued therapeutic application of these herbal remedies [4]. Considering the importance of *Murraya koenigii,* which known for its rich medicinal properties, the present study was undertaken to evaluate toxicity profile of *Murraya koenigii* on haematological as well as physiological paramaters. To the best of our knowledge, there is no any record in the literature for toxicity profile of *Murraya koenigii*. The repeated dose toxicity data may be required to predict the safety and effects of long-term exposure to a particular medicinal plant. Therefore, our present study was design to investigate toxicological effects of *Murraya koenigii* in relation to its long-term administration in 28 days repeated dose toxicity study. The acute treatment of ethanolic extract of *Murraya koenigii* found to show safe up to 1000 mg/kg dose level but when it exceed there found to shows the mortality in animals. Thus in the present study we selected the highest dose i.e. 900 mg/kg for assessing the toxic effect at repeated dose study. The LD_{50} of extract found to show at 3000 mg/kg. $1/10^{th}$ of the LD_{50} was considered as an effective dose i.e. 300 mg/kg. So considering 300 mg/kg as effective dose, we selected this dose for assessing the toxic effects in present investigation. In the present investigation the results of 28 days at repeated dose administration do not produce any significant effects on red blood cells (RBCs) and white blood cell (WBCs) count at any dose level. Same results

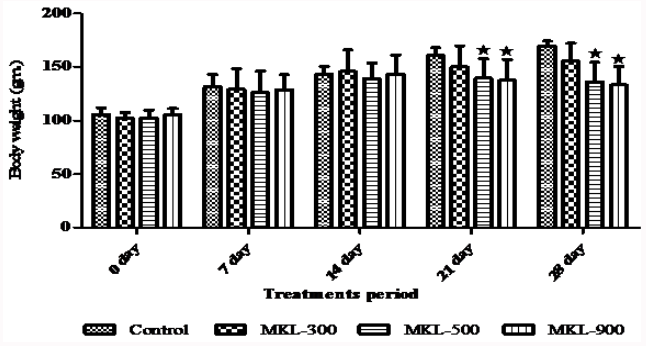

Figure 3a: Effect of MKL on Body weight in male rats Results are expressed in mean of 5 animals per group ± S.D. *Significantly different compared to control of respective period at p<0.05 by using student unpaired T test.

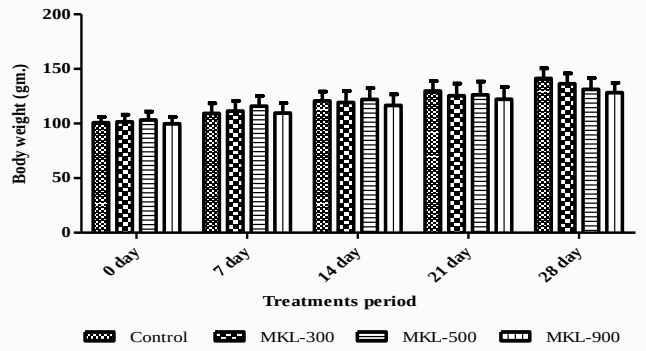

Figure 3b: Effect of MKL on Body weight in Female rats Results are expressed in mean of 5 animals per group ± S.D; Values are not found statistically significantly compared to control of respective period at p<0.05 by using student unpaired T test.

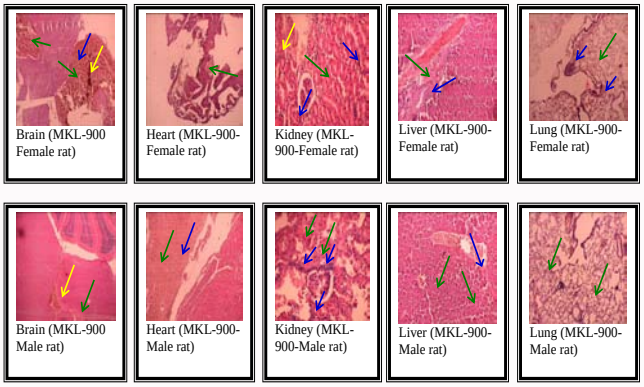

Figure 4: Histopathological observations of subacute (28 days) toxicity study of MKL extract: The results of histopathological study indicates **Yellow arrow:** Haemorrage; **Blue arrow:** Lymphocyte infiltrates; **Green arrow:** Congestion; spleens are found to normal. All the histopathological observations were comparing with vehicle control results.

were observed for total serum bilurubin, serum creatinine, SGOT and SGPT level respectively. While there were increased in the hemoglobin level at both middle and higher doses but statistically ($p<0.05$) significant was found in 900 mg/kg (Table 1) in male and female rats respectively. The other 28 days repeated dose treatment of MKL significantly ($p<0.05$) decreases blood glucose and cholesterol level at highest dose 900 mg/kg in both male and female rats while at 500 mg/kg MKL treatments significantly decreases the blood cholesterol level only in male rats (Figure 1 and 2). These results demonstrates the some adverse effects of *Murraya koenigii* on blood glucose as well as on cholesterol level suggest to use as hypoglycemic and hypocholesteremic agent. The results of *Murraya koenigii* for its decreased in glucose and cholesterol level can correlate and support with previous reported studies on leaves of *M. koenigii* for its antidiabetics as well as hypocholesterolemic activities. The present study also showed that drastic and sudden weight loss at doses of 500 and 900 mg/kg in male rats after 2nd week of MKL administration because of loss of subcutaneous fats and decreased in total cholesterol level. Significant ($p<0.05$) decrease in body weight and loss of subcutaneous fat was observed at 900 mg/kg group in last week compared to 2nd week reading. While in female rats there was slightly decreased in body weight but statistically not significant compared to 2nd week (Figure 3a and 3b). The result of food consumption showed increased in all groups of male and female rats including vehicle control indicates no significant effect of MKL on food consumption in any groups. In other physiological parameters such as auditory and visual activity MKL do not produce any significant ($p<0.05$) effects (Table 2). At repeated exposure up to 28 days there was absent of any sign of mortality or morbidity in any animals at any dose levels except congestion, haemorrhage and lymphocyte infiltration in higher doses (900 mg/kg) in both male and female rats (Figure 4). While there were absence of congestion, hemorrhages and lymphocyte infiltration in 300 and 500 mg/kg treatments in both male and female rats indicates safety at both permissible doses. The safety report of MKL treatments also supported with body organ weight ratio in both male as well as female rats (Table 3). From the results, our study concludes that at long-term administration (28 days in terms of repeated dose toxicity study) of ethanolic extract of *Murraya koenigii* do not produce any toxic effects on hematological and physiological parameters. While it increase the hemoglobin level, decreases the body weight, with loss of subcutaneous fat, blood glucose and cholesterol level, as observed in medium and high doses groups respectively indicates its therapeutic application in correcting hemoglobin level in anemia, controlling glycemic in diabetes and reducing cholesterol level. The study also concludes the safety consumption up to 500 mg/kg dose level without producing any structural damage to organs.

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