www.sphinxsai.com

PharmTech

International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.1, No.4, pp 1568-1575, Oct-Dec 2009

BIOCHEMICAL AND PHYSIOLOGICAL RESPONSES OF FRUIT JUICE OF MURRAYA KOENIGII (L) IN 28 DAYS REPEATED DOSE TOXICITY STUDY

***Tembhurne S. V., Sakarkar D. M**
Department of Pharmacology, Sudhakarrao Naik Institute of Pharmacy, Pusad., Yavatmal. 445204 (M.S), India.
****Corres. Author:Email: stembhurne@gmail.com***
Phone number: 09922070123

ABSTRACT : The purpose of this study was to asses the effects of fruit juice of Murraya koenigii (FJMK) on biochemical and physiological parameters in terms of repeated dose toxicity study in mice. Three doses 2.5, 5.0, and 10ml/kg were administered to a groups of 12 animals (six male and six female) daily for 28 days respectively. Animals receiving the vehicle (water) served as a control. The biochemical parameters like RBC, WBC, glucose, haemoglobin, 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, locomotor activity, grip strength, visual, auditory and organ weights were analysed respectively. Result of study showed that none of the animals from 2.5, 5.0 and 10ml/kg showed test material related changes in RBC,WBC, haemoglobin, creatinine, bilurubin, SGPT and SGOT parameter respectively. Similar results showed for some physiological parameter like visual, auditory, grip strength and locomotor activity.
There were no other changes except the drastic and sudden weight loss, decrease in cholesterol and glucose of these animals. Animals from 5.0 and 10 ml/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. The significant effect was observed with at dose of 10 ml/kg. There was increase in food consumption of animals at same dose level (5.0 and 10 ml/kg) compare to control and 2.5 ml/kg dose of animals.
From results it conclude that administration of FJMK for 28 days decreases the body weight, subcutaneous fat and blood glucose level, as observed in medium and high dose group so it can be attributed to the intended use of fruit juice for antidiabetic and antiobesity dietary supplement.
Key word: Murraya koenigii, repeated dose toxicity study, antiobesity, antidiabetic, dietary supplements.

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 to be 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. M. 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 indegenious system of medicine from ancient time, as a tonic for stomache, stimulat and creative [3, 4, 5]. Phytochemical screening of M. koenigii reveled 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 antidiabetic activities [5, 6, 7, 8, 9].
Several biological activities of M. koenigii leaves have been reported for its anti-hypercholesterolemic [10, 11] as well as its efficacy against colon carcinogensis [11]. It also reported for anti-microbial, antioxidant [5, 12, 13, 14, 15]. While there is not any scientific report found on fruit of Murraya koeinigii for its any biological activities. Therefore, the present study was designed to characterize the biochemical and physiological parameter for its possible biological effects and safety at long time administration of fruit juice of Murraya koenigii in terms of 28 days repeated dose toxicity study.

MATERIAL AND METHOD

Plant: The fresh fruit of Murraya koenigii were collected in the month of August 2008 from its natural habitat at Sakoli village in Nagpur region, Maharastra, 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.

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.

Preparation of the Fruit juice of *Murraya koenigii* (FJMK): The fruits were dried under shade for 15 days the final weight was found to be 300 gm. Dried fruit undergone washing with distilled water and then crushing in electric blender and adding subsequent boiled water and final volume was made 500 ml and was kept for 10 days for maceration in air tight container. The liquid obtained by filtration was red brown color and bitter in taste used as fruit juice.

Experimental Animals: Swiss albino mice of either sex weighing 25-35 gm were used. The animals were fed with standard mice diet (Amrut feed, Sangali, Maharastra), 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 [16]. The studies were performed with the approval of Institutional Animal ethics committee (IAEC) of S.N.Institute of Pharmacy Pusad.

Biochemical parameters: Initially at 0 day blood was collected from retro-orbital plexus and biochemical studies performed for estimation of total RBC, total WBC, blood glucose and haemoglobin and at 28 days before the animals dissect blood was collected by using retro-orbital plexus and finally from posterior vena-cava under light ether. The haematological studies were performed for estimation of total RBC count, total WBC count[17, 18] 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, locomotor activity, grip strength, auditory and visual activities were measured at the end of study. Body-organ weights ratio were measured by sacrificing the animals[16, 19].

EXPERIMENTAL DESIGN [16, 19]

Swiss albino mice of both sexes weighing 30-35 were assign to each group contains 6 animals respectively. Four groups of animals were used. Group 1 received distilled water for 28 days and group II, III and IV received 2.5 ml/kg, 5.0 ml/kg and 10 ml/kg of FJMK respectively. Body weight of the animals was recorded at the beginning and thereafter every week of experiment. At beginning (0 days) the biochemical parameter e.g. total RBC, total WBC, haemoglobin and glucose were measured and at the end of experiment RBC, WBC, Glucose, haemoglobin, cholesterol, creatinine, bilurubin, SGPT and SGOT were analyzed in all groups of animals respectively. In physiological parameters like daily food consumption, weekly body weight, locomotor activity, grip strength, visual and auditory activity respectively. All the animals were sacricified and major organs like liver, lung, kidney, spleen, brain were removed and analysed the organ weight ratio of respective group of animals.

Statistical analysis: All the experimental results were expressed as the mean ± standard deviation. Unpaired T-test and one way analysis of variance (ANOVA) with subsequent Tukey's test were used to detect further difference between groups respectively, values of $p< 0.05$ were considered significant.

RESULTS

Biochemical parameters

Results of biochemical analysis do not showed any significant effects on red blood cells (RBCs) white blood cell (WBCs) count at any dose level. Same results were observed for total serum bilurubin, serum creatinine, SGOT and SGPT level respectively. While there were slight increased in the haemoglobin level at both middle and higher doses but statistically it was not significant ($p<0.05$) figure 1a, b.

The blood glucose level however showed to decrease significantly ($p<0.05$) in 5ml/kg and 10ml/kg FJMK female mice while in male mice significant decreases was observed only at 10ml/kg on 28 days compared to their initial values taken at 0 days (figure 2a,b).

The results of total serum cholesterol estimation showed a dose dependant decreased in level while significant ($p<0.05$) effect was observed at higher doses in both male and female mice respectively (figure 3a, b).

Effect on body weight

The effect of 28 days administration of FJMK was shown as a sudden and drastic weight loss in 5ml/kg and 10 ml/kg group of male mice after 2nd week of administration. Significant ($p<0.05$) decrease in body weight and loss of subcutaneous fat was observed at 10ml/kg group in last week compared to 2nd week reading. While FJMK do not found to produce any effect on body weight in female mice (table 1a, b).

Effect on food consumption

The result of food consumption showed to increased in all groups of male and female mice. At higher doses of FJMK, the food consumption significantly starts to increase from 3rd week in 5ml/kg and from 2nd week in 10ml/kg of male mice. In male mice treated with 10ml/kg showed to increased food consumption more significantly ($p<0.01$) compare to other group (table 2a, b).

Auditory and visual response

FJMK do not found to produce any significant effects on auditory and visual activity in female as well as male mice at any dose level (table 3).

Effect on locomotor and grip strength: FJMK at any dose level do not found to produce significant effects on locomotor and grip strength activity in female as well as male mice (table 3).

Body organ weight ratio: There were no any significant differences in body and organ weight ratio of respective group of animals (table 4a, b).

DISCUSSION

Several biological activities of M. koenigii leaves have been reported for its anti-hypercholesterolemic, hypoglycemic, colon carcinogensis, anti-microbial, antioxidant etc [9, 10, 11, 12, 13, 14, 15] while there are not found any scientific reports on fruit of Murraya koeinigii for any biological activity. Therefore the present study was design to investigate its biochemical and physiological effects and safety in relation to its long term administration in terms of 28 days repeated dose toxicity study.

In present investigation, the fruit juice of Murraya koenigii did not produce any significant ($p<0.05$) changes in biochemical parameter like WBC count, RBC count, SGOT, SGPT, serum creatinine and serum bilurubin while in haemoglobin estimation (figure 1a,b) it increased in higher doses but it was not statistically significant ($p<0.05$) in both male and female mice respectively. Similarly in physiological parameters it did not produce any significant ($p<0.05$) changes in auditory, visual, locomotor, grip strength (table 3). Also at higher doses and repeated exposure it did not produce any mortality or morbidity in any animals. Thus from these finding it provides evidence for safety of FJMK. The results also supported with body organ weight ratio in both male as well as female mice (table 4a, b).

In some biochemical parameter like glucose and cholesterol level which found to decrease significantly ($p<0.05$). At higher doses of FJMK (10ml/kg) potentiate to decrease the glucose level in both female as well as male mice while at middle dose (5ml/kg) it significantly decreased the glucose level only in female mice. In cholesterol estimation only 10ml/kg dose of FJMK potentiate to decrease the level of cholesterol. The results of fruit juice of Murraya koenigii for decreased in glucose and cholesterol level can correlate and support with previous reported studies on leaves of M. koenigii [4, 5, 10, 11, 20].

The present study also showed that drastic and sudden weight loss observed at doses of 5.0 and 10ml/kg in male mice because of loss of subcutaneous fats and decease in total cholesterol level while FJMK fail to produce any changes in female mice at any dose level. There was also found to increase the food consumption by FJMK at the same dose level in male mice, the more significant effects observed at 10ml/kg in last week of study. The increased in demand for food consumption from 3rd week might be due to decrease in body weight with loss of subcutaneous fats and decrease in glucose level in mice which observed during 3rd and 4th week respectively.

CONCLUSION

Thus present study is thus indicates that at long term administration (28 days in terms of repeated dose toxicity study) of FJMK decreases the body weight, with loss of subcutaneous fat and blood glucose level, as observed in medium and high doses groups respectively, while there is need of more research on fruits of Murraya koenigii for its biological activities specially hypocholesteremic, antidiabetic and antiobesity for its intended use.

Table 1a: Body weight of Female mice (gm)

Control	2.5ml/kg	5.0ml/kg	10ml/kg	
31.433 ± 2.562	33.033 ± 3.58	31.333 ± 3.29	32.033 ± 4.433	**First week**
31.366 ± 2.86	32.6 ± 1.939	31.133 ± 1.573	35.75 ± 3.649	**Second week**
32.833 ± 1.533	34.766 ± 2.507	33.166 ± 2.296	34.72 ± 4.487	**Third week**
34.566 ± 2.54	35.233 ± 2.202	31.04 ± 1.800	32.52 ± 4.451	**Forth week**

Results are expressed in mean of 6 animals in a group ± S.D

Table 1b: Body weight of Male mice (gm)

Control	2.5ml/kg	5.0ml/kg	10ml/kg	
34.45 ± 3.255	33.6 ± 3.517	33.68± 3.10	33.533 ± 2.777	**First week**
36.1 ± 4.207	36.5 ± 2.634	36.34 ± 3.34	36.9 ± 3.32	**Second week**
38.9 ± 2.090	37.3 ± 2.26	35.52 ± 3.641	34.41 ± 2.17	**Third week**
41.85 ± 4.561*	35.5 ± 2.0950	34.8 ± 4.574	32.5± 1.85**	**Forth week**

Results are expressed in mean of 6 animals in a group ± S.D

*Significantly different compared to first week value at $p<0.05$ by using one way ANOVA fallowed by Tukey's test.

** Significantly different compared to second week value of respective group at $p<0.05$ by using one way ANOVA fallowed by Tukey's test.

Table 2a: Food consumption (gm)

Male mice				Week
Control	**2.5ml/kg**	**5.0ml/kg**	**10ml/kg**	
43.16 ± 3.191	41.36 ± 4.767	37.8± 3.384	41.72± 6.903	**1st**
45.3 ± 0.922	35.7 ± 5.302	39.8 ± 3.821	57.1 ± 0.436*	**2nd**
44 ± 6.149	44.04 ± 8.258	54.68 ± 11.475*	60.56 ± 10.150*	**3rd**
53.05 ± 4.410*	49.9 ± 4.396*	69.1 ± 5.856*	74.55 ± 0.833**	**4th**

Results are expressed in mean of 7 observations in a week per group ± S.D

*Significantly different compared to first week value with respective group at $p<0.05$ by using one way ANOVA fallowed by Tukey's test.

** Significantly different compared to first week value of respective group at $p<0.01$ by using one way ANOVA fallowed by Tukey's test.

Table 2b: Food consumption (gm)

Female mice				Week
Control	**2.5ml/kg**	**5.0ml/kg**	**10ml/kg**	
37.96± 3.397	38.56 ± 5.607	46.8± 2.363	40.72± 4.494	**1st**
29.1 ± 0.717	30.2 ± 7.683	26.65 ± 11.854	31.725 ± 8.77	**2nd**
42.52 ± 4.890	37.72 ± 7.728	39.62 ± 7.165	45.036± 25.287	**3rd**
50 ± 6.038*	50 ± 4.953*	54.72 ± 8.18*	75.38 ± 6.961**	**4th**

Results are expressed in mean of 7 observations in a week per group ± S.D

*Significantly different compared to first week value with respective group at $p<0.05$ by using one way ANOVA fallowed by Tukey's test.

** Significantly different compared to first week value of respective group at $p<0.01$ by using one way ANOVA fallowed by Tukey's test.

Table 3: Effect of FJMK on Physiological responses of mice

Mice	Physiological response	Control	2.5ml/kg	5ml/kg	10ml/kg
Male	**Auditory (Male)**	√	√	√	√
	Visual (Male)	√	√	√	√
	Locomotor activity(sec.)	132.33± 22.44	145.33 ±18.59	124.66 ±12.01	143.6 ± 27.50
	Grip strength (sec.)	100.83± 04.45	137.16±108.03	95.66± 111.82	156± 79.81
Female	**Auditory (Female)**	√	√	√	√
	Visual (Female)	√	√	√	√
	Locomotor activity(sec.)	143.2 ± 23.72	131.16 ± 23.71	145.2 ± 24.53	133.75 ±21.85
	Grip strength (sec.)	155.6± 41.743	151.2 ± 92.015	151.2 ±92.015	112.2 ± 27.96

Results are expressed in mean of 6 animals per group ± S.D
√ Indication for no significant effect

Table 4a: Body organ weight ratio (Male mice)

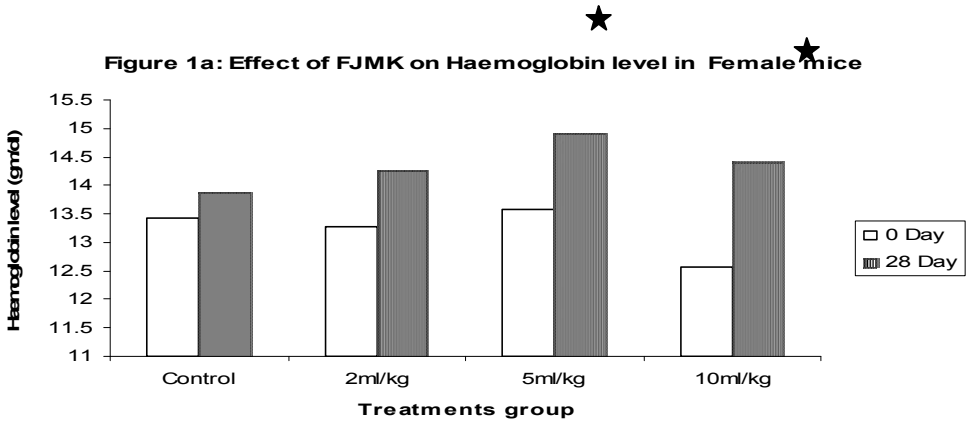
Group	Liver	Lung	Heart	Spleen	Brain	Kidney
Control	1 : 5.37	1 : 0.525	1 : 0.467	1 : 0.508	1 : 1.262	1 : 1.791
2.5ml/kg	1 : 4.64	1 : 0.547	1 : 0.46	1 : 0.456	1 : 1.47	1 : 1.532
5ml/kg	1 : 4.77	1 : 0.543	1 : 0.4137	1 : 0.4413	1 : 1.344	1 : 1.57
10ml/kg	1 : 5.76	1 : 0.5737	1 : 0.429	1 : 0.5121	1 : 1.438	1 : 1.98

Results are expressed as organ ratio of 6 animals per group.

Table 4b: Body organ weight ratio (Female mice)

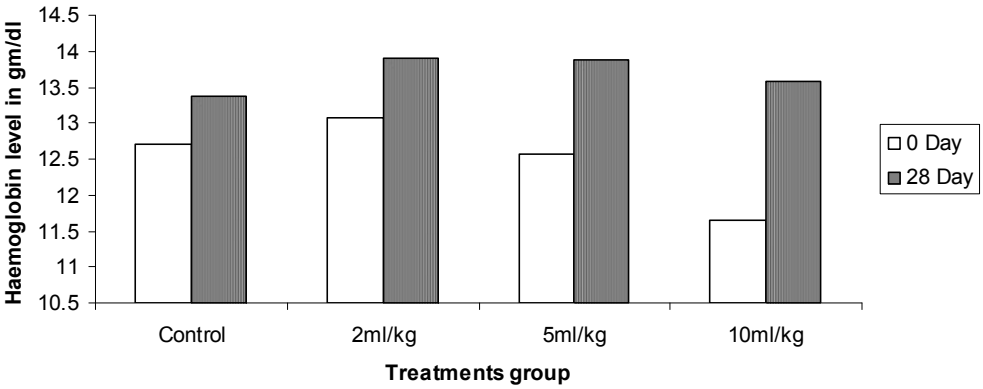
Group	Liver	Lung	Heart	Spleen	Brain	Kidney
Control	1 : 5.85	1 : 0.6258	1 : 0.37	1 : 0.3766	1 : 1.613	1 : 1.68
2.5ml/kg	1 : 4.668	1 : 0.605	1 : 0.333	1 : 0.397	1 : 1.321	1 : 1.278
5ml/kg	1 : 5.077	1 : 0.5757	1 : 0.35	1 : 0.413	1 : 1.436	1 : 1.391
10ml/kg	1 : 4.41	1 : 0.5523	1 : 0.366	1 : 0.358	1 : 1.302	1 : 1.249

Results are expressed as organ ratio of 6 animals per group.



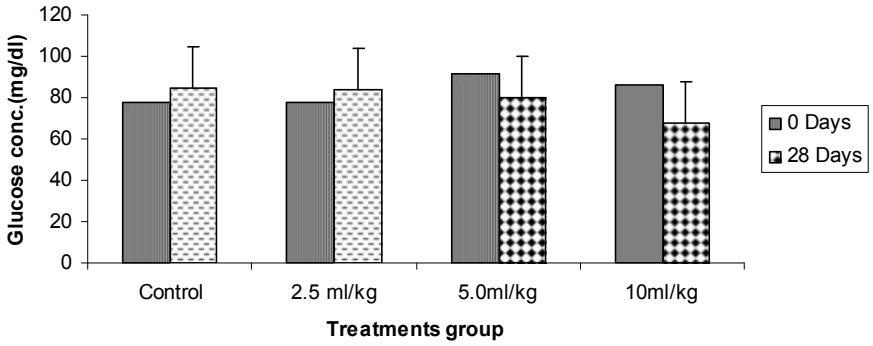
Results are expressed in mean (gm/dl) of 6 animals per group ± S.D

Figure 1b: Effect of FJMK on Haemoglobin level in male mice



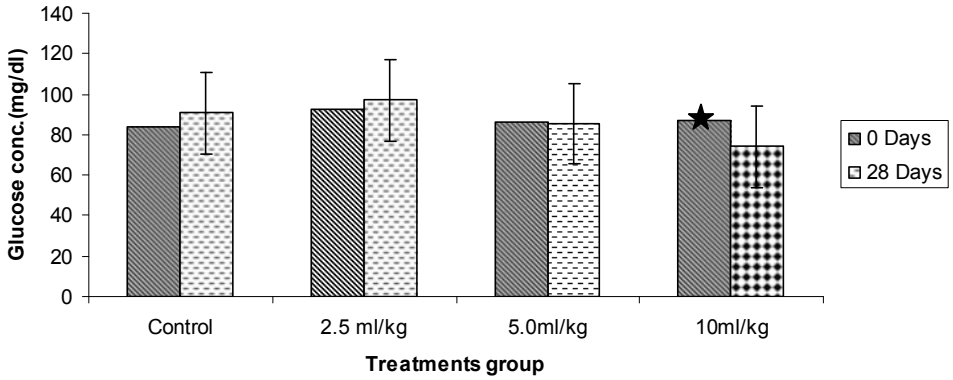
Results are expressed in mean (gm/dl) of 6 animals per group ± S.D

Figure 2a: Effect of FJMK on blood glucose level in Female mice



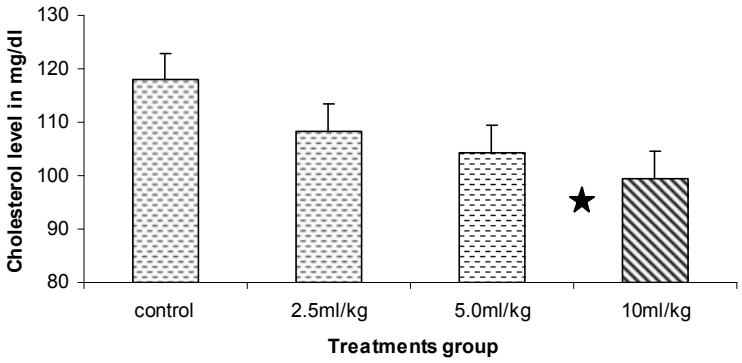
Results are expressed in mean (mg/dl) of 6 animals per group ± S.D
*Significantly different compared to 0 day value of respective group at $p<0.05$ by using student unpaired T-test.

Fig 2b: Effect of FJMK on blood glucose level in Male mice



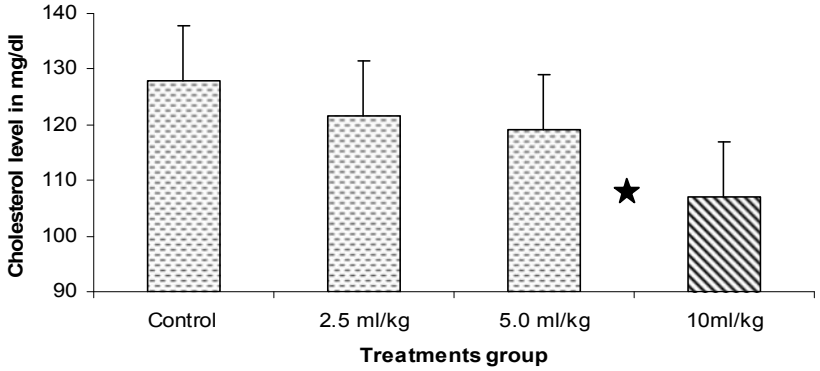
Results are expressed in mean (mg/dl) of 6 animals per group ± S.D
*Significantly different compared to 0 day value of respective group at $p<0.05$ by using student unpaired T-test.

Figure 3a: Effect of FJMK on Cholesterol level in Male mice



Results are expressed in mean of 6 animals per group ± S.D
*Significantly different compared to control at p<0.05 by using student unpaired T test

Figure 3b: Effect of FJMK on cholesterol level in Female mice



Results are expressed in mean of 6 animals per group ± S.D
*Significantly different compared to control at p<0.05 by using student unpaired T test

REFERENCES

1. Anonymous., (1998), *The wealth of India*, (Council of Scientific and Industrial Research, New Delhi). pp. 446-448.
2. Prajapati, N. D., Purohit, S. S., Sharma, A. K., & Kumar, T., (2003), *A Handbook of Medicinal Plants* (Agrobios, Jodhpur), pp. 352-353.
3. Anonymous., (1987), *Medicinal Plants of India,* (Indian council of medicinal research, Cambridge printing works, New Delhi), pp. 289-295.
4. Yadav, S., Vats, V., Dhunnoo, Y., Grover, J.K., (2002), Hypoglycemic and antihyperglycemic activity of *Murraya koenigii* leaves in diabetic rats. *J Ethnopharmacol.,* 82, 111.
5. Vinuthan, M. K., Girish, Kumar. V., Ravindra, J. P., & Narayana, K., (2004), Effect of extracts of *Murraya koenigii* leaves on the levels of blood glucose and plasma insulin in alloxan induced diabetic rats. *Indian J Physiol Pharmacol*., 48, 348.
6. Iyer, D., & Uma, D. P., (2008), Plant Review Phyto-pharmacology of *Murraya koenigii* (L.). *Pharmacognosy Reviews.*, 2,180**.**
7. Khosa, R. L., (1975) Chemical studies on *Murraya paniculata* leaves. *J Res Indian Med.,* 10, 75.
8. Gupta, G. L., & Nigam, S. S., (1970), Chemical examination of the leaves of *Murraya koenigii*. *Planta Med.,* 19, 83.
9. Yukari, Tachibana., Hiroe, Kikuzaki., Nordin, H.J. L., & Nobuji, Nakatani., (2001), Antioxidative activity of carbazoles from *Murraya koenigii* leaves. *J Agric Food Chem.,* 49, 5589.
10. Iyer, U.M., & Mani, U.V.,(1990), Studies on the effect of curry leaves supplementation (*Murraya koenigii*) on lipid profile, glycated proteins and amino acids in non-insulin dependent diabetic patients. *Plants and Food in Human Nutrition.,* 40, 275.
11. Khan, B. A., Abraham, A., & Leelamma, S., (1996), *Murraya koenigii and Brassica juncea*

alterations on lipid profile in 1-2 dimethylhydrazine induced colon carcinogenesis. *Investigational New Drugs*, 14, 365.

12. Singh, L., & Sharma, M., (1978), Antifungal properties of some plant extracts. *Geobios.,* 5, 49.
13. Goutam, M.P., & Purohit, R.M., (1974), Antimicrobial activity of essential oils of the leaves of *Murraya koenigii* Spreng. *Indian Journal of Pharmaceuticals.,* 36, 11.
14. Deshmukh, S. K., Jain, P. C., & Agarwal, S. C.,(1986), Antimicrobial activity of the essential oil of the leaves of *Murraya koenigii* (Linn) Spreng (Indian curry leaf). *Fitoterapia.*, 57, 295.
15. Baliga, M.S., Jagetia, G.C., Rao, S.K., Babu, K.,(2003), Evaluation of nitric oxide scavenging activity of certain spices in vitro: a preliminary study. *Nahrung.*, 47(4)**,** 261.
16. Organization for Economic Cooperation and Development (OECD), *Guidelines for Testing Chemicals: Repeated Dose 28-Day Oral Toxicity Study in Rodents, no. 407,* OECD, Paris, 1993.
17. *Ghai, C. L., (1995), A textbook of Practical Physiology* (Jaypee Brothe, New Delhi, India), *pp., 119.*
18. *John, M.B., (1998), Laboratory medicine Haematology,* 4th edition *(*C.V.Mosbey co.st. Louis Publishers*), pp. 1198.*
19. Jagtap, A., Tembhurne, S., Shirke, S., Patel, M.,(2006), Toxicological evaluation of *Caralluma fimbriata* extract in Wistar Rats. *The American Abstract of Pharmaceutical Sciences.*, 8 S2, W4083.
20. Achyut, N. K., Gupta, R. K., & Watal, G.,(2005), Hypoglycemic effects of *Murraya koenigii* on normal and alloxan-diabetic rabbits. *Journal of Ethnopharmacology.,* 97, 247.