www.sphinxsai.com

PharmTech

International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.1, No.4, pp 1589-1593, Oct-Dec 2009

HYPOGLYCEMIC EFFECTS OF FRUIT JUICE OF *MURRAYA KOENIGII (L)* IN ALLOXAN INDUCED DIABETIC MICE

Tembhurne S. V. , Sakarkar D. M. *
Department of Pharmacology, Sudhakarrao Naik Institute of Pharmacy, Pusad, Yavatmal. 445204 (M.S), India.
Email: dsakarkar@rediffmail.com Phone no. +91-07233247308

ABSTRACT: Oxidative stress has been defined as a disturbance in the balance the production of reactive oxygen species (ROS) and antioxidant defence system, which can lead to tissue injury. Antioxidant level in the tissue is an important factor for sensitivity of individual tissue to oxidative stress. It has been suggested that oxidative stress can play an important role in tissue damage associated with diabeties and complications. Oxidative stress in diabeties and increased of free radicals are generated which cause injury or destruction of pancreatic beta cells which can repaired or regenerated by using potent antioxidant. Thus based on reported antioxidant phytochemical constituents in fruit of Murraya koenigii the present study was aim at to investigate hypoglycemic effect of fruit juice of Murraya koenigii in mice. Based on the results of normoglycemic and OGTT model the hypoglycemic effect in alloxan induced diabetic mice was evaluated at dose level of 2.5 and 5.0 ml/kg. The results of antidiabetic study reveled that FJMK decreases blood glucose level significantly at 10 and 15 day of administration (fasted serum glucose). From results it concludes that FJMK has hypoglycemic effect. The FJMK may act as cytoprotective in alloxan induced diabetic mice because of presence of antioxidant phytochemical so it can be attributed to the intended use of fruit juice for antidiabetic dietary supplement or as a herbal drink for diabetic and in diabetic complications.

Key words: Murraya koenigii, antidiabetics, dietary supplement, herbal drink.

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 [2, 3]. Phytochemical screening of M. koenigii leaves 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 leaves were reported to have antioxidant and antidiabetic activities [4, 5, 6, 7, 8, 9, 10]. There also having several biological activities of M. koenigii leaves reported for its anti-hypercholesterolemic [11, 12] as well as its efficacy against colon carcinogensis [11]. It also reported for anti-microbial, antioxidant [5, 13, 14, 15, 16]. While there is not any scientific report found on fruit of Murraya koeinigii for it's any biological activities including antidiabetic which has reported for its leaves [4, 5, 6]. Therefore, there was a strong interest to find the possible antidiabetic activity of fruit of Murraya koenigii.

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: Tolbutamide as a standard (Aventis laboratory), 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. The studies were performed with the approval of Institutional Animal ethics committee (IAEC) of S.N.Institute of Pharmacy Pusad.

EXPERIMENTAL DESIGN

ANTIDIABETIC STUDY

Preliminary hypoglycemic study of FJMK

The hypoglycemic activity was performed in normal glycemic and glucose loaded mice (oral glucose tolerance test (OGTT) model). Swiss albino female mice weighing 25-35 gm were assigning to each contain 6 animals. Four groups of animals were used for normal glycemic model and OGTT models respectively [6, 17].

In normoglycemic group 1 was normal control, group 2 was vehicle control, group 3 and 4 received 2.5 ml and 5 ml of FJMK respectively. While in OGTT model group 1 was vehicle control, group 2 was standard Tolbutamide (300mg/kg p.o.) and group 3 and 4 received 2.5 ml and 5 ml of FJMK respectively.

In normoglycemic and OGTT model initial blood was taken by retro-orbital after 30 min of administration while immediately glucose (3.0 mg/kg p.o) was given to all groups of animal in OGTT model and subsequent blood samples were taken at time interval of 30, 60 and 120 min intervals respectively.

Antidiabetic study of FJMK in alloxan induced diabetic model

Induction of experimental diabetes

Diabetes was induced in mice by a single injection of alloxan monohydrate (150 mg /kg i.p.) in 0.9% of NaCl. After 48 h, the animals showing serum glucose levels above 200 mg /dl (diabetic) were selected for the study [18]. All the animals were allowed free access to water and pellet diet. Blood samples were collected by retro-orbital puncture technique and the collected blood samples were analyzed for glucose levels by the glucose estimation kit and serum glucose levels were expressed in mg/dl.

Experimental procedure

Swiss albino female mice weighing 30-35 gm were assign to each group contains 6 animals respectively. Group 1 served as vehicle control; group 2 served as Alloxan induced diabetic control; group 3 and 4 diabetic mice given FJMK (2.5 and 5.0 ml/kg p.o.) for 15 days respectively; group 5 diabetic mice given standard tolbutamide (300mg/kg p.o) for 15 days.

Statistical analysis: All the experimental results were expressed as the mean ± standard deviation. Unpaired T-test was used to detect further difference between groups respectively, values of $p < 0.05$ were considered significant.

RESULT AND DISCUSSION

Diabeties mellitus is a chronic disease characterized by high blood glucose level due to absolute or relative deficiency of circulating insulin level or insulin resistance. Through different types of oral hypoglycemic agents are available along insulin for the treatment of diabeties, there is an increasing demand by patient to use the natural products with antidiabetic activity to overcome the side effects and toxicity of synthetic drugs. Herbal antidiabetic drugs are prescribed widely because of their effectiveness, less side effects and relatively low cost[19]. Thus the aim of the present work was to evaluate the antidiabetic activity of fruit juice of Murraya koenigii in alloxan diabetic mice.

The results of preliminary hypoglycemic effects in normoglycemic and OGTT model showed that FJMK at 5ml/kg was found to significantly ($p<0.05$) decrease the blood glucose level after 120 min. in both model respectively (figure 1 and 2). The results of the preliminary hypoglycemic study are in agreed with some report[4, 5, 6, 17] that proved the antidiabetic effects. The preliminary hypoglycemic study of FJMK also proved with antidiabetic effects of FJMK in alloxan induced diabetic mice which are presented in table 1. Analysis of results showed that the effect of FJMK on blood glucose level was significantly ($p< 0.01$) found to decreased at both doses 2.5 and 5.0ml/kg of 10 and 15 day of administration and its activity was comparable to standard Tolbutamide drug (table 1). The results of FJMK for its hypoglycemic activity are supported with previous studies which are reported on its leaves of Murraya koenigii[4, 5, 6, 11].

Oxidative stress has been defined as a disturbance in the balance the production of reactive oxygen species (ROS) and antioxidant defence system, which can lead to tissue injury[20]. The levels of reactive oxygen species are regulated by variety of cellular defence mechanisms consisting of enzymatic and non-enzymatic antioxidant systems[20]. Antioxidant level in the tissue is an important factor for sensitivity of individual tissue to oxidative stress[21]. It has been suggested that oxidative stress can play an important role in tissue damage associated with diabeties and complications[21, 22]. Oxidative stress in diabeties and increased of free radicals are generation by alloxan[20] which cause injury or destruction of pancreatic beta cells which can repaired or regenerated by using potent antioxidant. The phytochemical[7] data reveled that carbazole alkaloids namely koenimbine, murrayazoline, murrafoline and pyrayaquinone-A presents in fruit of Murraya koenigii which has reported to have antioxidant activities[23, 24, 25]. From reported phytochemical literatures

of Murraya koenigii it may conclude that antioxidant activity may be a possible mechanism responsible for protective effects of FJMK against beta cell damage and antioxidant defence system of plasma and pancreas in alloxan induced diabetic mice. The results of the FJMK are also supported with the effects of Murraya koenigii leaves extracts in which carbazole alkaloids[25, 26, 27, 28, 29, 30] responsible for the antioxidant activity[10, 16, 23, 24]. These observations conclude that the cytoprotective role of Murraya koenigii fruit in alloxan induced diabetic mice.

CONCLUSION

Excess production of active oxygen radicals causes oxygen stress in cell membrane which consequently induces toxic effects and disease. Self defence system against these oxidative damages is facilitated by antioxidant. Many new antioxidants have been isolated and identified from herbs and species. The daily intake of these foods might be one of the most promising sources against major diseases leading to leatherier life. Oral administrations of FJMK attenuate these problems in the alloxan induced diabetes in mice as a consequence of its potential antioxidant properties. This could be due to presence of biologically active ingredients carbazole alkaloids in fruits like koenimbine, murrayazoline, murrafoline and pyrayaquinone-A. While there require further studies to isolate and characterized the more other constituents from fruit of Murraya koenigii in controlling diabeties and its related complication.

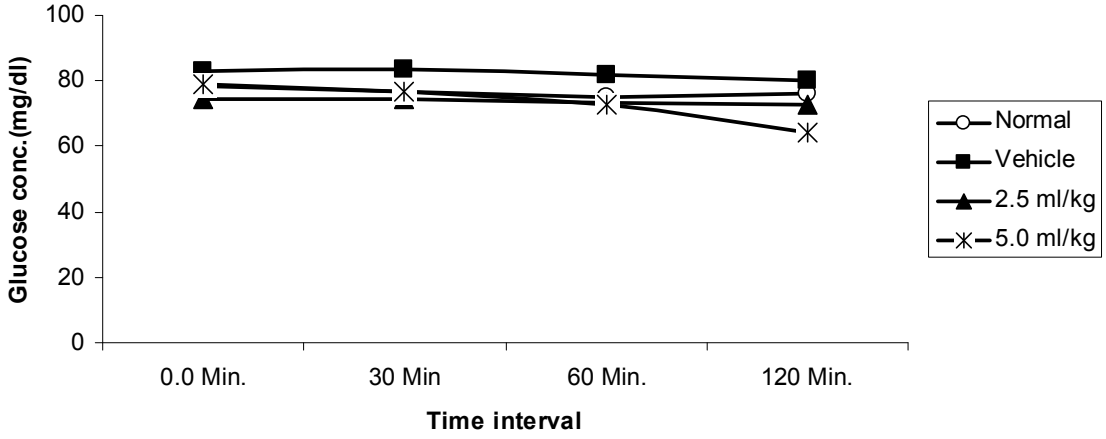
Table 1: Antidiabetic activity in Alloxan induced diabetic model

Groups	0 day	5 day	10 day	15 day
Vehicle control	89.76± 9.35	90.01 ± 7.024	87.82± 7.15	89.58± 7.06
Diabetic control	310.2± 22.05	324.75± 19.74	353.74± 22.08	390.34± 27.59
2.5 ml FJMK	311.12± 27.35	293.69± 34.06	259.07± 36.67*	210.44± 33.19*
5 ml FJMK	305.53 ± 27.09	290.6± 25.74	239.75± 36.24*	182.52± 24.165*
Tolbutamide	307.16 ± 30.38	279.62± 37.27	218.39± 27.84*	179.32± 22.10*

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

* Significant ($p<0.05$) difference compared with their initial 0 day reading of respective groups by using student unpaired T-test.

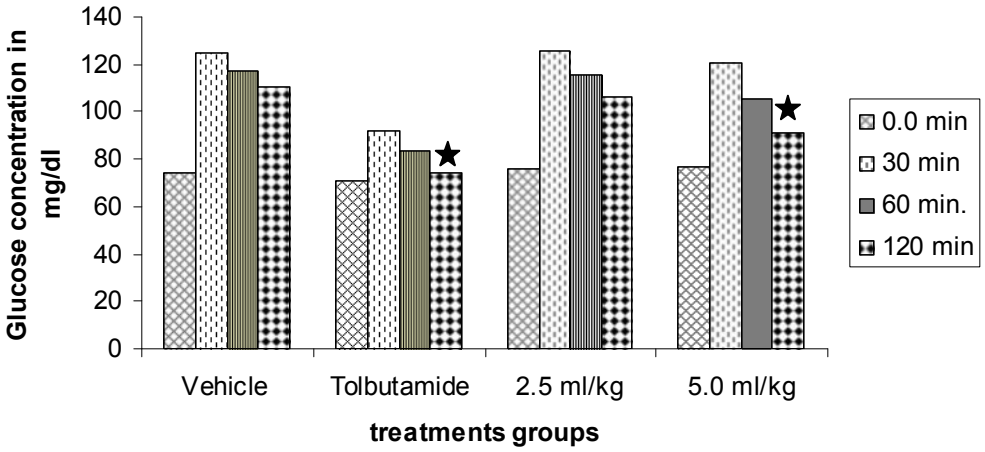
Fig 1: Effect of FJMK in Normoglycemic model



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

***Significantly different compared to 0.00 min reading at, p<0.05 student T test.**

Fig 2: Hypoglycemic activity in OGTT model



Results are expressed in mean of 6 animals in a group ± S.D
*** Significant ($p<0.05$) difference compared with glucose loaded reading at 30 min interval in a respective group 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. 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.
7. Iyer, D., & Uma, D. P., (2008), Plant Review: Phyto-pharmacology of *Murraya koenigii* (L.). *Pharmacognosy Reviews.*, 2,180**.**
8. Khosa, R. L., (1975) Chemical studies on *Murraya paniculata* leaves. *J Res Indian Med.,* 10, 75.
9. Gupta, G. L., & Nigam, S. S., (1970), Chemical examination of the leaves of *Murraya koenigii*. *Planta Med.,* 19, 83.
10. 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.
11. 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.
12. 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.
13. Singh, L., & Sharma, M., (1978), Antifungal properties of some plant extracts. *Geobios.,* 5, 49.
14. 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.
15. 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.
16. 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.
17. Akhtar, M., & Iqbal, J., (1991), Evaluation of the hypoglycemic effect of *Achyranthes aspera* in

normal and alloxan diabetic rabbits. *Journal of Ethnopharmacology.,* 31, 49-56.

18. Kameswararao, B., Kesavulu, M., Giri, R. & Appa, Rao. C., (1999), Antidiabetic and hypolipidemic effect of *Momordica cymbalaria* Hook fruit powder in alloxan-diabetic rats. Journal of Ethnopharmacology., 67(1), 103-109.
19. Jahodar, L., (1993), Plants with hypoglycemic effects. Cesk. Farm., 42, 251-259.
20. Halliwell, B., & Gutteridge, J.M.C., (1989), Free radicals in biology and medicine 2nd Edn., Clarendon. Press. Publ., Oxford.
21. Baynes, J.W., (1991), Perspective in diabeties. Role of oxidative stress in development complications in diabeties. Diabeties., 40, 405-412.
22. Baynes, J.W., & Thorpe, S., (1999), Perspective in diabeties. Role of oxidative stress in diabetic complication perspective on an old paradigm. Diabeties., 48, 1-9.
23. Arulselvan, P., & Subramanian, S.P., (2007), Beneficial effects of *Murraya koenigii* leaves on antioxidant defense system and ultra structural changes of pancreatic beta-cells in experimental diabetes in rats. *Chem Biol Interact*., 165(2), 155-164.
24. Arulselvan, & Subramanian, S., (2008), Ultrastructural and biochemical abnormalities in the liver of streptozotocin-diabetic rats: protective effects of Murraya koenigii. Journal of Pharmacology and Toxicology., 3(3),190-202.
25. Arulselvon, G.P., Senthilkumar, D., Sathish Kumar., & Subramanian, S., (2006), Antidiabetic effect of *Murraya koenigii* leaves on streptozotocin induced diabetic rats. *Pharmazine.,* 61(10), 874-877.
26. Sanyal, P.K., Basak, A., Barua A.K., Bose, P.K., (1975), Chemical examination of leaves of*Murraya paniculata*. *J. Indian Chem. Soc*., 52, 1213 (1975).
27. Kureel, S.P., Kapil, R.S., & Popli S.P., (1969), New alkaloids from *Murraya koenigii. Experentia.,* **25,** 790.
28. Chakraborty, D.P., Roy, S., Bhattacharya, S.P., Biswas, A.K., & Bhattacharya, P., (1978), Structure and synthesis of mukonine, a new carbazole alkaloid from *Murraya koenigii. Phytochemistry.,* **17,** 834.
29. Khosa, R.L., (1975), Chemical studies on *Murraya paniculata* leaves*. J. Res. Indian Med.,* **10 (1),** 75.

*****

.