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Original Article

Influence of *Murraya koenigii* on experimental model of diabetes and progression of neuropathic pain

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Abstract

The aim of the present study was to evaluate the effect of ethanolic extract of *Murraya koenigii* leaves (MKL) on blood glucose level and in prevention or management of diabetic neuropathy. In the present study the diabetic neuropathy was developed 9 weeks after single injection of streptozotocin (STZ, 70 mg/kg i.v.) in rat. The treatment with MKL (300 and 500 mg/kg p.o.) was started after stabilization of blood glucose level (13 days after STZ) and evaluated for determination of glycemic level, glycated haemoglobin, grip strength, pain sensitivity and threshold. The result showed that the treatment with MKL possessed hypoglycemic effect in diabetic treated animals. The results also indicated that the decreases in the grip strength in diabetic animals represented the induction of neuropathy 9 weeks after STZ treatment. Prior treatments with MKL increased the grip strength of diabetic rats. The results of pain sensitivity indicated the loss of pain perception in diabetic animals because of nerve damage. While prior treatment with MKL upto 9 week in diabetic animals resulted in the increase in the licking time and withdrawal latency in hot plate and tail flick tests, respectively, which indicates the presence of pain perception and prevention of nerve damage due to protective effect of MKL in progression of diabetic neuropathic pain. Therefore, the present study concludes that the chronic treatment with MKL significantly decreased the glycemic level as well as it protected the animals against development of diabetic neuropathy.

Keywords: Diabetes mellitus; Diabetic neuropathy; Pain perception; *Murraya koenigii*

INTRODUCTION

Diabetic neuropathy is a peripheral nerve disorder caused by diabetes which leads to a significant morbidity rate (1-3). The risk of developing Diabetic Peripheral Neuropathy (DPN) increases with duration of the disease, the degree of glycemic control and other contributing factors, such as hypertension, dyslipidaemia, smoking, body mass index and hyperinsulinaemia (4). The symptoms of diabetic neuropathy are often slight at first but can occasionally flare up suddenly, and affect specific nerves so that an affected individual will develop double vision, drooping eyelids, or weakness and atrophy of the muscles. Nerve damage caused by diabetes generally occurs over a period of years and may lead to problems with the digestive tract and sexual organs, which can cause indigestion, diarrhea or constipation, dizziness, bladder infections, and impotence (2,5).

The main risk factor for diabetic neuropathy is hyperglycemia. It is important to note that people with diabetes are more likely to develop symptoms related to peripheral neuropathy as the excess glucose in the blood results in a condition known as glucojasinogen. This condition is affiliated with erectile dysfunction and epigastric tenderness which in turn results in lack of blood flow to the peripheral intrapectine nerves which govern the movement of the arms and legs (4,6,7).

The pathogenesis of DPN is believed to be multifactorial with hyperglycaemia being the primary risk factor. Suggested theories that postulate the aetiopathogenesis of diabetic neuropathy include abnormalities of protein glycation, sorbitol accumulation, polyol pathway flux, protein kinase C activation,

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advanced glycation end-products, receptor for advanced glycation end-products, a decrease in neuronal nitric oxide synthase protein and microvascular hypoxia, resulting in oxidative stress (4,8).

Since diabetic neuropathy is not clearly understood, it is hard to make a definitive course of treatment (9). Many pharmacological options are available to treat DPN but still it is difficult for patients to obtain complete relief because of poor glycemic control. Prevention through strict glycemic control remains the mainstay of therapeutic intervention because effective disease modifying therapies are yet not available.

Thus the novel approaches in antidiabetic therapy are aimed not only to decrease high blood glucose levels, but also to eradicate long-term diabetic complications which may cause a diminished life expectancy and/or a poor quality of life. Diabetes is associated with high medical costs. To decrease the high medical cost and expenditure the renewed interest has been focus on herbs and are increasingly gaining acceptance among various countries because of its safety profile (10,11).

The main approach of the present study was regulation of blood glucose level as well as prevention of progression and management of neuropathic pain perception in chronic diabetics by treatment with ethanolic extract of *Murraya koenigii* leaves, which is commonly known as Karry tree or Meethi neem in India. Our previous finding on fruit juice of *Murraya koenigii* indicated their anti-diabetic activity (12). The leaves are used extensively as a flavoring agent in curries and chutneys. Almost every part of this plant has a strong characteristic odour and is used traditionally as antiemetic, antidiarrhoeal, febrifuge and blood purifier. The people of the plains, particularly of southern India, use the leaves of this plant as a spice in different curry preparations (13,14).

MATERIALS AND METHODS

Plant

The fresh leaves of *Murraya koenigii* were collected from its natural habitat at Sakoli village in Nagpur region, Maharashtra, India. The plant was authenticated by 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 ethanolic extracts of Murraya koenigii leaves (MKL)

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 g powder with 200 ml solvent). The extraction was performed for 18 h. The extract was concentrated by evaporation at room temperature and used in present study. The percent yield for ethanolic extract of MKL was found to 7.4% w/w.

Administration of the extract

Suspension of ethanolic extract was prepared in 0.5% carboxymethyl cellulose using tween 20 (0.2% v/v) as a suspending agent. The extracts of MKL were administered in a dose of 300 and 500 mg/kg, which were selected as per our preliminary studies for its hypoglycemic effect. Control groups were given only 0.5% carboxymethyl cellulose with tween 20 (0.2% v/v).

Experimental animals

All the experiments were carried out in male Wister rat (180-220 g). The animals had free access to food and water, and they were housed in a natural light–dark cycle. The animals were acclimatized to the laboratory conditions for at least one week before experiments. The experiments were carried out between 9.00 am to 18.00 pm. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) and the laboratory animals were taken care according to the guidelines of CPCSEA, Ministry of Forests and Environment, Government of India (registration number 729/02/a/ CPCSEA).

Drugs and reagents

Streptozotocin (STZ) {Gift sample from Nicholas Piramal, Mumbai (Sigma Aldrich,

USA)} prepared in cold citrate buffer (pH 4.5, 0.1 M). Glibenclamide (Gift sample from Glenmark Laboratory Mumbai) were prepared similar to plant extract. All the drugs were injected in a constant volume of 1 ml/kg of body weight after stabilization of blood glucose level. Glucose kit obtained from Bio-Lab Mumbai was used for the estimation of blood glucose level. All the reagents and chemicals used in the present study were of analytical grade.

Experimental design

Experimental model of diabetes was induced by i.v. injection of streptozotocin (70 mg/kg) in Wister rats (180-220 g) and the treatment with MKL (300 and 500 mg/kg p.o.) and standard glibenclamide (10 mg/kg p.o.) was started after stabilization of the blood glucose level 13 days after streptozotocin injection. Effects of MKL on the level of blood glucose (overnight fasted), glycosylated haemoglobin and diabetic neuropathic pain were evaluated 9 weeks after injection of streptozotocin. Induction of diabetic neuropathy was evaluated by the grip strength (15,16) and the neuropathic pain perception in terms of pain threshold and pain sensitivity was assessed by tail flick and hot plate methods, respectively (16-18).

Measurement of diabetic neuropathy by behavioral studies

A grip strength determination was used for evaluating neuromuscular strength (15,16). The grip strength of animals was measured by simply hanging animals with their fore limbs on fine metal wire which was held at two end of a pole. The time taken from holding the metal wire and to fall on the surface was considered for the muscle strength determination. The animals with damaged or weak muscle or nerves fall soon on the floor. The force achieved (in terms of time) by each animal for staying in hanging stage was recorded.

The effect of diabetic neuropathy on the pain sensitivity and pain threshold was evaluated by hot plate and tail flick method (16-18). The rats were placed on the hot plate (55-58 °C) and the time until either licking or jumping occurs was recorded by a stop watch. A cut off time of 10 s was kept to avoid damage to the paw of the animals.

The evaluation of pain threshold in diabetic rats was determined by withdraw latency in tail flick test. Tail of each diabetic rat was exposed to radiant heat by placing the hot water in the glass. The intensity of radiant heat (55-58 °C) was adjusted to obtained withdrawal latency of not longer than 6 s in both diabetic and non-diabetic rats. The tail flick latency is the time interval taken by rat to flick its tail after exposure to a source of radiant heat. Cut time was fixed at 10 s.

Statistical analysis

Blood glucose level was analyzed by ANOVA followed by Dunnet test. Data of grip strength, pain sensitivity, pain threshold and the glycosylated haemoglobin were analyzed by student unpaired t test. The significant difference was compared at $P< 0.05$ and $P< 0.01$. (Graph pad Prism, version 5.0)

RESULTS

Effect of MKL on blood glucose regulation

Results of chronic treatments with MKL (300 and 500 mg/kg p.o) indicates decreasing the blood glucose levels at 30th and 60th day while the more significant ($P< 0.01$) effect took place only at 60th day (Fig. 1). The hypoglycemic effect of MKL was comparable to standard glibenclamide as antidiabetic agent.

Glycosylated haemoglobin

The results indicates significant ($P< 0.05$) decrease in the level of glycosylated haemoglobin after chronic treatments with MKL which was comparable to the standard glibenclamide (Table 1).

Effect on grip strength

The results indicates that STZ-induced diabetes significantly ($P< 0.05$) reduced the grip strength in rats as compared to healthy rats, whereas chronic treatment with MKL upto 9 weeks increased the grip strength significantly ($P< 0.05$) in diabetic rats which was more effective than standard glibenclamide (Fig. 2).

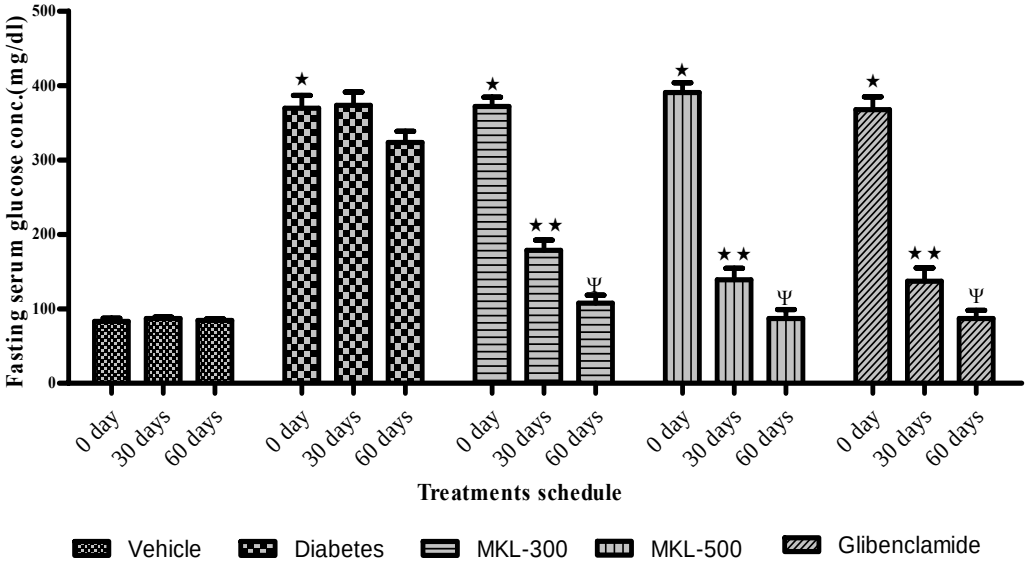

Fig. 1. Effect of chronic treatments of MKL on blood glucose regulation in diabetic rats. Data expressed as mean ± SD; n=5. The data are statistically ($P< 0.05$) significant (ANOVA followed by Dunnet test). ★indicates significant ($P< 0.001$) induction of diabeties compare to vehicle control at 0 day. ★★indicates significant ($P< 0.05$) hypoglycemic effect of drugs in diabetic animals compared to 0 day reading of diabetic control group. **Ψ** indicates significant ($P< 0.01$) hypoglycemic effect of drugs in diabetic animals compared to 0 day reading of diabetic control group.

Table 1. Level of glycosylated haemoglobin after treatment of MKL in STZ-induced diabetic rats.

Groups	Glycosylated haemoglobin (%Hb)
Vehicle control	8.340 ± 0.73
Diabetic control	16.43 ± 0.14*
MKL-300	12.88 ± 1.02**
MKL-500	11.02 ± 1.21**
Glibenclamide	10.98 ± 0.99**

Values are given in mean ± SD for groups of five rats in each. Values are statistically significant at $P< 0.05$ (student unpaired t test). *Diabetic control rat were compared with vehicle control. **Drug treated groups of rats were compared with diabetic control.

Effect on pain perception

The results indicate significant ($P< 0.05$) loss of pain perception in diabetic rats as indicated by decreased latency time in both hot plate and tail flick model. The chronic treatment with MKL indicated a significant ($P< 0.05$) increase in the latency time in both hot plate and tail flick models (Fig. 3 and 4) which was more effective than the standard glibenclamide.

DISCUSSION

Diabetic complications chiefly seen in the long term are persistently deleterious to a large extent. Major complications include nephropathy, neuropathy, retinopathy and heart disease, which affect thousands of diabetics every year (19,20). While some of these complications, which are closely related to a lack of compliance during antidiabetic therapy, are apparent even with an optimal therapeutic regimen. Thus novel approaches in antidiabetic therapy are aimed not only to decrease high blood glucose levels, but also to eradicate long-term diabetic complications which may cause a diminished life expectancy and/or a poor quality of life. In diabetes there is loss of pain perception and it is thought to be due to nerve damage and induction of peripheral neuropathy (21,22). Painful diabetic

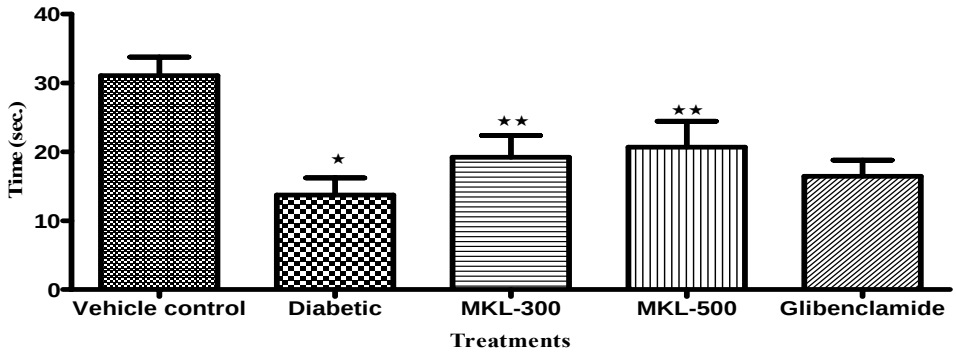

Fig. 2. Effect of MKL on grip strength after 9 weeks in diabetic rats. Data expressed as mean of three reading (s) for each animals in a group ± SD, n=5. ★Values were statistically significant ($P< 0.05$) compared to vehicle group. ★★Values were statistically significant ($P< 0.05$) compared to diabetic control group (student unpaired t test).

neuropathy significantly affects the quality of life; so far no ideal drug has been available for its management. In the absence of curative therapy, the main aim of the management is to provide symptomatic pain control along with good glycemic control. Thus the present study was aimed to evaluate the influence of chronic treatment of ethanolic extract of *Murraya koenigii* on the glycemic control as well as in progression of neuropathic pain in streptozotocin-induced diabetic rats.

Results of the chronic treatments with MKL indicated dose dependant decreased in the blood glucose level. More reduction was observed on 60th day which was closer to vehicle control which was comparable to standard antidiabetic glibenclamide.

During diabetes, the excess of blood glucose reacts with the haemoglobin to form HbA1c which has been found to be increased over a long period of time in diabetes mellitus (23). The results of the present study indicated an increase in the percentage of glycosylated haemoglobin in diabetic rats. The observed increase in the level of glycosylated haemoglobin in diabetic control rats might be due to the presence of excessive amounts of blood glucose. While chronic treatments with MKL caused a significant ($P< 0.05$) decrease in the level of glycosylated haemoglobin similar to standard glibenclamide which may possibly be due to decreased blood glucose level after the treatments (Table 1).

The main risk factor for diabetic neuropathy is hyperglycemia. It is important to note that people with diabetes are more likely to develop symptoms relating to peripheral neuropathy as the excess glucose in the blood results in a condition known as glucojasinogen (23). The risk of developing DPN increases with duration of the disease and degree of glycemic control. There is an evidence that glycation may itself induce the generation of oxygen derived free radicals in diabetic condition which may lead to development of diabetic neurological complications like neuropathic pain and depression (20,23,24). Thus, the drug which has antioxidant property may be used for the prevention of diabetic complications (24). In the present study, induction of diabetic neuropathy was evaluated by muscle and nerve strength by measuring grip strength after 9 weeks of STZ-induced diabetes. STZ significantly reduced the grip strength in the rats as compared to healthy animals, indicating muscle weakness and induction of neuropathy. The chronic treatment with MKL upto 9 weeks raised the grip strength significantly in diabetic rats which was even more effective than the standard glibenclamide (Fig. 2).

In diabetic neuropathy, the damage of nerve results in the loss of pain perception (21). In the present study, the pain threshold measured by hot plate and tail flick, indicated a decrease in the foot withdrawal and flicking latency (the time interval taken by the rats to withdraw their legs or flick their tail after exposure to source of radiant heat) in both hot plate and tail flick test, respectively after 9 weeks in STZ-induced diabetic rats (Fig. 3 and 4). These results indicated the loss of pain

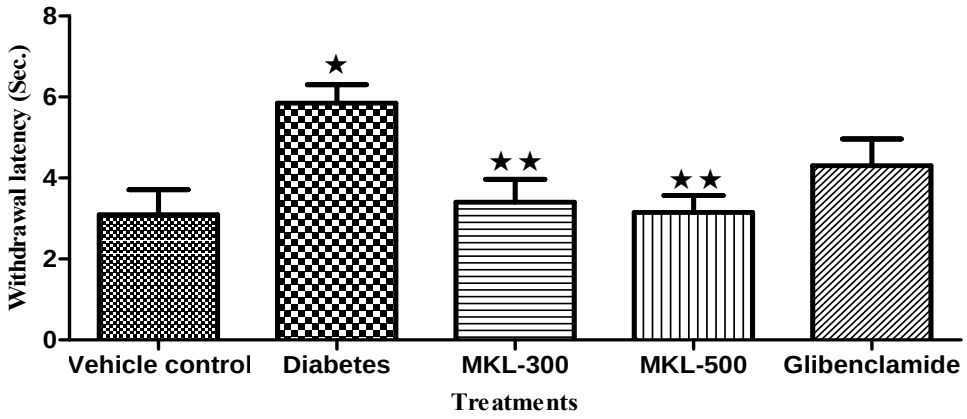

Fig. 3. Effect of MKL on Pain sensation using Hot plate method. Data expressed as mean ± SD, n=5. ★Values were statistically significant ($P< 0.05$) compared to vehicle group. ★★Values were statistically significant ($P< 0.05$) compared to diabetic control group (student unpaired t test).

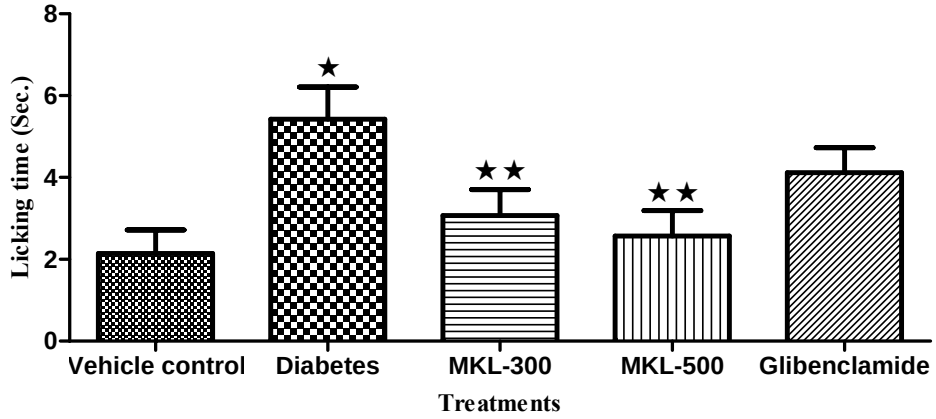

Fig. 4. Effect of MKL on Pain sensation using tail flick method. Data expressed as mean ± SD, n=5. ★Values were statistically significant ($P <0.05$) compared to vehicle group. ★★Values were statistically significant ($P <0.05$) compared to diabetic control group (student unpaired t test).

perception in diabetic rats. The loss of pain perception in diabetes may be due to induction of diabetic neuropathy after 9 weeks in the diabetic rats (21). The chronic treatment with MKL increased the latency time in both hot plate and tail flick models. The increase in latency time indicates the presence of pain perception in animals. This demonstrates that the extract of *Murraya koenigii* can prevent the progression of neuropathy in the diabetic animals. The prevention or management of diabetic neuropathic pain in the present study may be due to the control of the glycemic level in the diabetic rats.

The generation of oxygen derived free radicals in diabetic condition is the leading cause of the development of diabetic neurological complications like neuropathic pain and depression (20,23,24). Thus the drug which has antioxidant property may be used for the prevention of diabetic complications (24). Previous studies have confirmed the presence of antioxidant carbazole alkaloids in leaves of *Murraya koenigii* (25,26). Thus these constituents i.e. carbazole alkaloids may probably be responsible for the prevention of the neuropathic pain in the present study.

Further studies are required to elucidate the exact protective mechanism of *Murraya koenigii* in progression of neuropathic pain perception in diabetes.

CONCLUSION

In conclusion, our study demonstrated beneficial effects of *Murraya koenigii* in diabetes and its neuropathic pain. The plant has the potential of offering a better choice in the curative therapy in the progression of diabetic neuropathy which can be extracted by either preventing the nerve damage or controlling of symptomatic pain along with good glycemic control. Therfore, it could be helpful in treating the diabetic patient having the complication like diabetic neuropathy.

REFERENCES

1. Dejgaard A. Pathophysiology and treatment of diabetic neuropathy. Diabetes Med. 1998;15:97-112.
2. Mark AR. Neuropathies associated with diabetes. Med Clin North Am. 1993;27:111-124.
3. Rai S, Gupta A, Rai M, Birari KV. Diabetic peripheral neuropathy-emerging pharmacologic options. Bombay Hospital J. 2008;50:595-606.
4. Boulton AJM, Malik RA, Arezzo CJ, Sosenko JM. Diabetic somatic neuropathies. Diabetes Care. 2004;27:1458-1486.
5. Ardid D, Guilbaund G. Antinocieptive effects of acute and chronic injection of tricyclic anti-depressants drugs in a new model of mono neuropathy in rats. Pain. 1992;49:279-287.
6. Duby JJ, Campbell K, Setter SM. Diabetic neuropathy: an intensive review. Am J Health Syst Pharm. 2004;61:160-176.
7. Apfel SC, Kessler JA. Neurotrophic factors in the therapy of peripheral neuropathy. Baillieres Clin Neurol. 1995;4:593-606.
8. Ziegler D. Antioxidant treatment in diabetic polneuropathy-update. 16th Annual Neurodiab Meeting. Ystad, Sweden; 2006.
9. Zareba G. Pregabalin: a new agent for the treatment of neuropathic pain. Drugs Today. 2005;41:509-516.
10. Sofowora A. Medicinal plants and traditional medicine in Africa. 2nd ed. New York: John Wiley publishers; 1984.
11. Gill LS. Ethnomedical uses of plants in Nigeria. 1st ed. Benin City: Uniben Press; 1992.
12. Tembhurne SV, Sakarkar DM. Hypoglycemic effects of fruit juice of *Murraya koenigii* (L) in alloxan induced diabetic mice. Int J Pharm Tech Res. 2009;1:1589-1593.
13. Anonymous. The wealth of India, a dictionary of Indian raw materials and i*ndustrial* products. New Delhi: Council of Scientific and Industrial Research; 1998.
14. Prajapati ND, Purohit SS, Sharma AK, Kumar T. A Handbook of Medicinal Plants. Jodhpur: Agrobios Publisher; 2003.
15. Ali A, Pillai KK, Vohora D, Ahmad FJ. Evidence of the antiepileptic potential of amiloride with neuro-pharmacolgical benefits in some rodent models of epilepsy and behaviour. Epilep Behav. 2004;5:523-530.
16. Jawaid T, Shakya AK, Kamal M, Hussain S. Amitriptyline and sertraline in diabetic neuropathy: a comparative view. Int J Health Res. 2008;1:73-78.
17. D'Amour WL and Smith DL. A method for determining loss of pain sensation. J Pharmacol Exp Ther. 1941;72:74-79.
18. Sawynok J, Reid AR, Esser MJ. Periphreal antinociceptive actions of desipramine and fluo-xetine in an inflammatory and neuropathic pain test in the rat. Pain. 1999;82:149-155.
19. Leedom I, Meehan WT, Procci W. Symptoms of depression in patients with type II diabetes mellitus. Psychosomatics. 1991;32:280-286.
20. Oztork Y, Altan VM, Ari N. Diabetic complications in experimental models. Tr J Med Sci. 1998;22:331-341.
21. Raz I, Hasdi D, Melmed RN. Effect of hyperglycemia on pain perception and on efficacy of morphine analgesia in rats. Diabeties. 1988;37:1253.
22. Simon GS, Dewey WL. Effect of streptozotocin induced diabeties on the anti-nociceptive potency of morphine. J Pharmacol Exp Ther. 1981;218:318.
23. Ochoa JL. Pain mechanism in neuropathy. Curr Opin Neurol. 1994;7:407-414.
24. Baynes JW, Thorpe S. Role of oxidative stress in diabeties complications: a new perspective on an old paradigm. Diabeties. 1999;48:1-9.
25. Chakrabarty M, Nath A, Khasnobis S. Carbazole alkaloids from *Murraya koenigii*. Phytochemistry. 1997;46:751-755.
26. Tachibana Y, Kikuzaki H, Lajis NH, Nakatani N. Comparision of antioxidant properties of carbazole alkaloids from *Murraya koenigii* leaves. J Agric Food Chem. 2003;51:6461-6467.