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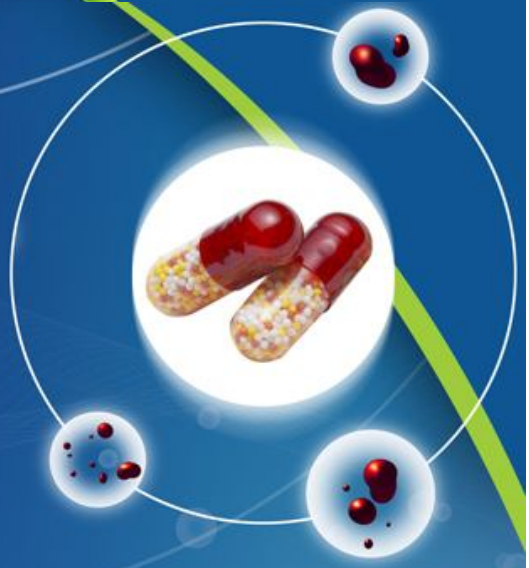

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Research Article

"HYPOGLYCEMIC EFFECTS OF FRUIT EXTRACTS OF MURRAYA KOENIGII (L) IN STREPTOZOTOCIN INDUCED DIABETIC RATS"

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ABSTRACT

Murraya koenigii leaves (Rutaceae) is used traditionally in Indian Ayurvedic system to treat diabetes. The aim of the present study was to evaluate the antidiabetic activity of ethanolic extract of Murraya koenigii fruits (MKF) in streptozotocin induced diabetic rats. Effect of oral administration of MKF (200 and 400 mg/kg) on the level of blood glucose, thiobarbituric acid reactive substances (TBARS), total cholesterol, triglycerides and glycogen were estimated. Glibenclamide was used as a standard drug. The elevated level of blood glucose, triglycerides and cholesterol were observed in diabetic rats while after treatment with MKF for 15 days normalized the values demonstrates the hypoglycemic activity. Decrease in glycogen in diabetic animal, suggests that the drug treatment promote the process of glycogenesis. Increase in the TBARS level in diabetic animals indicates to raise the level of lipid peroxidation which is a marker of oxidative stress while the treatment with MKF significantly ($p<0.05$) decreased the TBARS level indicated contributing protective antioxidant activity in diabetic animals. The ethanolic extract of M. koenigii possessed potent antioxidant properties which may be due to the presence of carbazole alkaloids. Thus the antidiabetic activity of M. koenigii leaves was probably due to the presence of its antioxidant property.

Key words: *Murraya koenigii fruits, antioxidant, antidiabetic, streptozotocin.*

INTRODUCTION

Diabetes is a group of metabolic diseases and is one of the oldest disorders known to mankind which is characterized by hyperglycemia. It has been presumed that diabetes results from defects in insulin secretion or insulin action, or both and/or from inherent stress in modern lifestyle. [1]. Although several synthetic drugs are available, attention is currently being focused on the use of plants and plant products in prevention or correction of various metabolic disorders associated with diabetes because of several side effects rise due to the use of synthetic drugs[2,3].

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Many herbal plants possess marked effect and have been used in traditional medicinal system for the treatment of diabetes [4-6].Several natural products such as alkaloids, flavonoids, terpenoids, saponins, polysaccharides and glycosides are isolated from medicinal plants and are being reported to possess anti-diabetic activities [7].

The plant *Murraya koenigii* (L.) Spreng, belonging to the family Rutaceae, is widely distributed in most part of the India. The leaves of this plant, called curry leaves, are commonly used for flavoring the Indian dishes. The leaves increase digestive secretions and relieve nausea, indigestion, and vomiting [8].

Phytochemical screening of *M. koenigii* leaves revealed the presence of some vitamins, carbazole alkaloid, terpenoids, phenolic

compounds [9] and mineral content such as calcium, iron, zinc and vanadium etc [10].

Previous studies on *M. koenigii* leaves were reported to have hypoglycaemic [11], antioxidant [12] activities. However no work has been reported for antidiabetic activity of *Murraya koenigii* except the work done on fruit juice of *Murraya koenigii* [13].

The *Murraya* species has richest source of carbazole alkaloids and is present in leaves, stem bark and roots of *Murraya koenigii*. Carbazole alkaloids reported to have anti-tumor, anti-viral, anti-inflammatory, anti-convulsant, diuretic and anti-oxidant activities [14].

Therefore, the present study was undertaken to evaluate anti-diabetic and anti-hyperlipidemic effects of carbazole alkaloid present in fruits of *Murraya koenigii* in streptozotocin-induced diabetic rats.

MATERIALS AND METHODS

Plant materials

The fruits of *Murraya koenigii* were collected from the region of Yavatmal district, Maharashtra, India during the month of June to September 2012. The fruits were authenticated by Dr. N. M. Dongarwar, Head of the Department; Botany Department, RTM Nagpur University, Nagpur.A voucher specimen (No. 9916) was deposited at Herbarium, Department of Botany, RTM Nagpur University Nagpur, India. The fruits were crushed in a mechanical grinder and used for further analysis.

Chemicals

Glibenclamide as a standard (Glenmark Pharmaceutical Mumbai), Glucose estimation kit (Hi-Media Lab, Mumbai), Streptozotocin (Sigma Aldreach, USA), Total cholesterol kit, Glycogen estimation kit, (Hi-Media Lab,Mumbai), Thiobarbituric acid. All the reagents and chemicals used in present study were of analytical grade

Experimental Animals

Adult male Wistar Rats (weighing 150-180 g) were used for the investigation. Before the starting of the experiment, the animals were acclimatized to the laboratory conditions for a period of 1 weeks. They were maintained at an ambient temperature (25 ± 2 ^{0}C) and relative humidity (40-60%), with 12/12 h of light/dark cycle. The animals were maintained on balance diet and water *ad libitum*. Institutional Animal Ethical Committee (IAEC) approved the study and all the experiments were carried out by following the guidelines of CPCSEA, India.

Phytochemical Analysis:

The extract was subjected to various phytochemical tests to determine the nature of constituents of the extract and two carbazole alkaloids isolated from extracts, were analysed by IR, MASS and NMR spectroscopic methods.

Extraction and isolation

Murraya Koenigii fruits (1kg) were dried under shade and crushed in electric blender to form coarse powder and subjected to extraction by using Soxhlet's extractor at room temperature using ethanol (95%) as a solvent. The percent yield of ethanolic extract was 2.9% w/w. The extract was concentrated by evaporation at room temperature and brown-green colored viscous residue was used for pharmacological studies.

Isolation

The ethanolic extract was evaporated under reduced pressure to give a residue (20 g), which was loaded onto a silica gel column (60−120 mesh), and the column was eluted with a gradient of a n-hexane-ethyl acetate (0−100%), which afforded several fractions, which were pooled based on their analytical TLC results. The fractions obtained with the mixture of n-hexane−ethyl acetate (92:8 v/v) were further chromatographed with a gradient elution using a mixture of n-hexane−$CHCl_3$ (88:12 and 86:14 v/v) to afford two fractions, labeled as MKF1 and MKF2. Fraction MKF1 was subjected to preparative TLC (PTLC) using n-hexane− chloroform (85:15 v/v) as the mobile phase in order to purify compound 1 (13.6 mg, Rf = 0.76, 0.00068%). PTLC of MKF2 using n-hexane−chloroform (80:20 v/v) as mobile phase yielded compound 2 (15 mg, Rf=0.54, 0.00075 %).

General experiments procedure

Melting point was determined in open capillary and was uncorrected. The UV spectrum was recorded using Agilent Cary 630 UV visible spectrophotometer in spectral grade alcohol (Merck); and the wavelength of

the spectrum was determined in the range of 200 to 400 nm. Column chromatography were prepared by using Silica Gel 60F254, 70-230 mesh and Analytical thin layer chromatography (TLC) was performed on commercially precoated aluminium supported silica gel 60F254 TLC sheets
(Anchrom Laboraries, Mumbai). The infrared spectrums (IR) were recorded on Agilent Cary 60 ATR spectrophotometer. Nuclear Magnetic Resonance spectra (1H NMR) were recorded using Bruker ADVANCE 300 DIGITAL MHz NMR spectrometer at 300 MHz. Deuterated chloroform (CDCl3) was used as the internal standard. Chemical shifts were recorded in CDCl3 and the values are in δ (ppm) on the basis of the δ residual of CDCl3.Coupling constants, *J*, are in hertz. Chemical shifts (δ) were reported in ppm and coupling constants (*J*) in Hz. Mass spectra (MS) were determined by using the LC-mass spectrometry on Waters model Qudrapole.

ANTIDIABETIC STUDY

Antidiabetic activity in streptozotocin induced diabetic rats: A freshly prepared solution of streptozotocin (60 mg/kg) in 0.1M cold citrate buffer pH 4.5 was injected intra-peritoneally in overnight fasted rats. 15 days after streptozotocin, control and survival diabetic rats were randomly divided in five groups, each consisting of six animals: Group one as a normal vehicle control received 0.5% sodium CMC with twin 20 (0.2%v/v). Group 2 as a diabetic control and received vehicle only. Group 3 and 4 diabetic animals received 200 and 400 mg/kg of MKF extract respectively. Group 5 diabetic animals received glibenclamide (10 mg/kg, p.o.) After 15 days of above treatment scheduled animal's blood was withdrawn by retro-orbital plexus (fasted animals) for determination of serum glucose, triglycerides, glycogen and total cholesterol [15]. Liver was isolated from respective group of animals for determination of thiobarbituric acid reactive substances and glycogen content [15,16].

Determination of thiobarbituric acid reactive substance (TBARS)

After sacrificing, the liver were isolated from respective groups of animals and homogenized in 0.15 M KCl respectively. Homogenates were centrifuged and supernatant was used as a source of polyunsaturated fatty acid for determination of extent of TBARS. The content of TBARS indicates as index of lipid peroxidation or oxidative stress in the form of liver damage in diabetic animals [17].

Statistical analysis:

All the experimental results were expressed as the mean ± standard deviation. Student unpaired t-test was used to detect further difference between groups respectively, values of p
< 0.05 were considered significant.

HPTLC fingerprint profiles:

For HPTLC fingerprint profile, stock solution (1mg/ml) of each extracts was prepared in methanol. 2µg/ml,5µg/ml, 10µg/ml and 15µg/ml of sample extracts were spotted on pre-coated Silica gel G60 F254 TLC plates using CAMAG Linomat V automatic sample spotter and the plates were developed in solvent systems to resolve polar and non-polar components of the fractions. The plates were scanned using TLC Scanner 3 (CAMAG) at 254nm. Shown in figure 1.

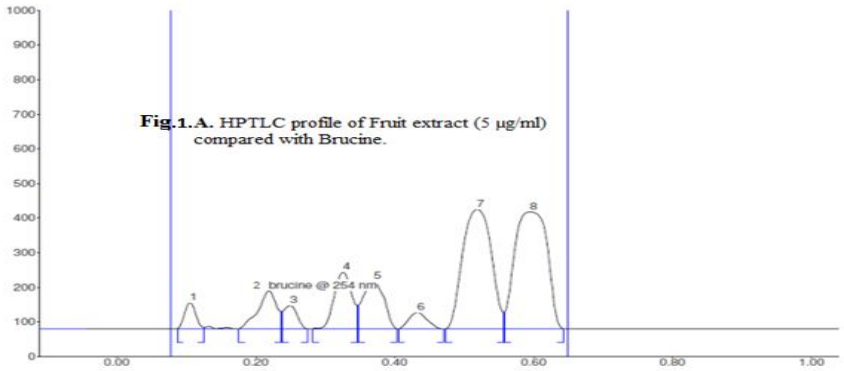

Fig.1.A. HPTLC profile of Fruit extract (5 µg/ml) compared with Brucine.

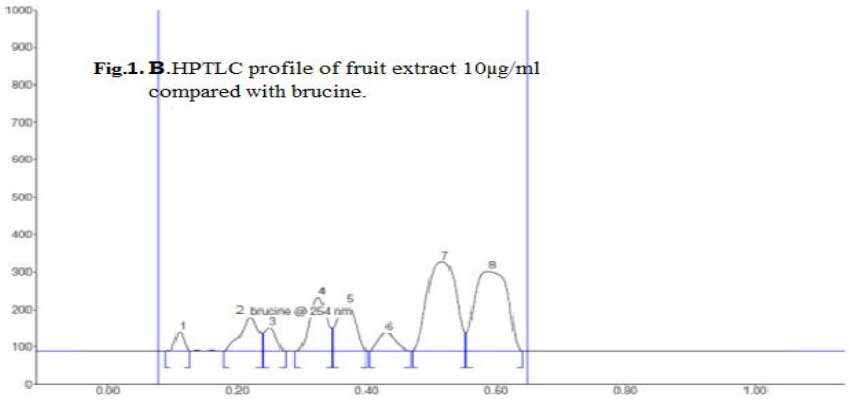

Fig.1. B.HPTLC profile of fruit extract 10µg/ml compared with brucine.

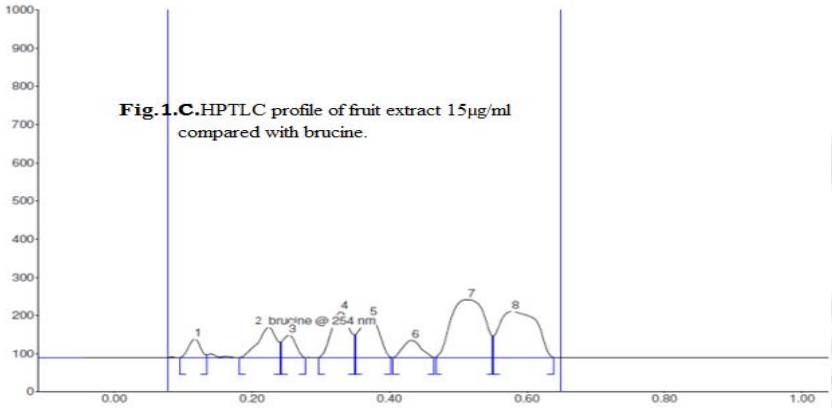

Fig.1.C.HPTLC profile of fruit extract 15µg/ml compared with brucine.

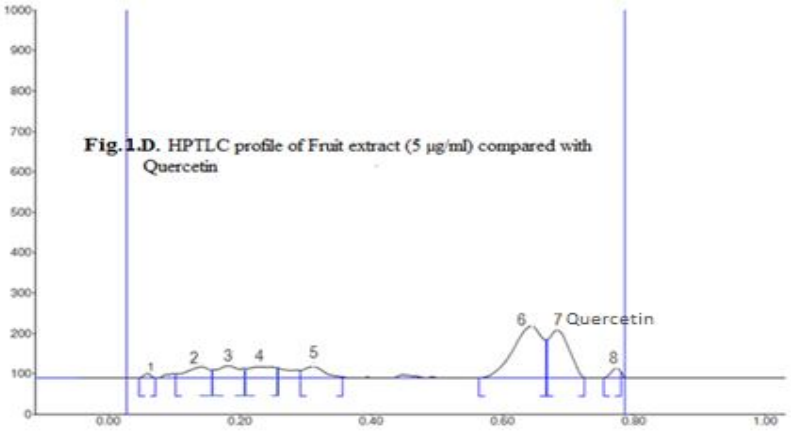

Fig.1.D. HPTLC profile of Fruit extract (5 µg/ml) compared with Quercetin

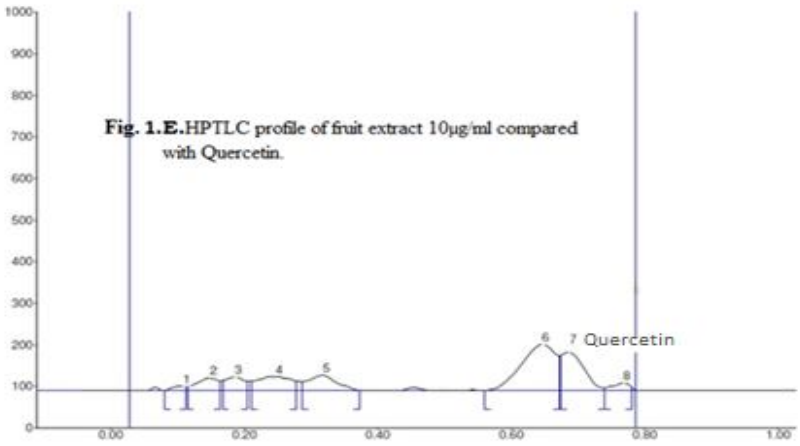

Fig. 1.E.HPTLC profile of fruit extract 10µg/ml compared with Quercetin.

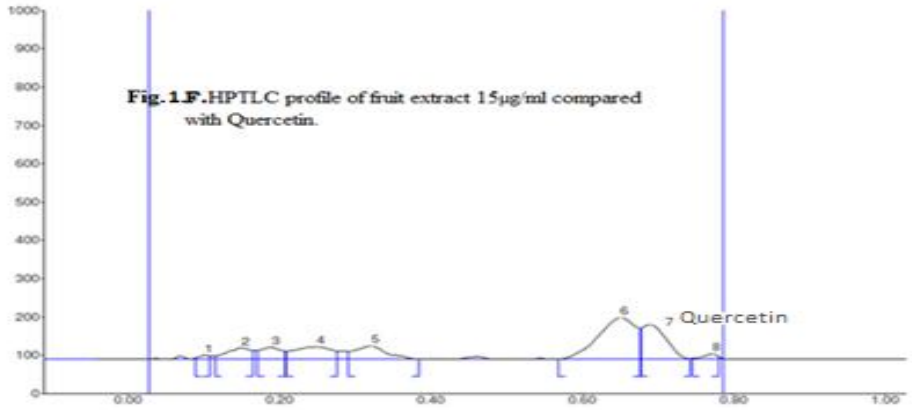

Fig. 1.F.HPTLC profile of fruit extract 15µg/ml compared with Quercetin.

Figure 1: HPTLC analysis of Fruit extracts

RESULTS

Phytochemical evaluation.

Various phytochemical tests performed on extracts of *Murraya koenigii* fruits shows positive results for alkaloids, flavonoid and phenolic contents. Table 1 illustrates the presence of various phytoconstituents.

Table 1: Phytochemical evaluation of fruit extracts of *Murraya koneigii*

Sr. No.	Compound	Test	Inference
1	Flavonoids	Shinoda test	+
2	Alkaloid	Lead acetate	+
		Wagner	—
3	Phenolic content	Dragendroffs	+
		Ferric chloride test	+
		Libermann's test	+

Isolated components

Compound 1 : White powder *IR* v_{max}(cm^{-1}) 3309,2920,2851,1647,1610,1496,1377,1346,1251,1198,1142,1056,979,845,746,679.*MS* m/z (% intensity) 331(M^+,basepeak),319.3,248.1, 182.1,170.4. *1H NMR:* 1.25 (3H, s, 3-CH3), 1.45, (each 3H, br s, 4-CH3), 1.73- 1.79 (2H, m, H-1), 2.12-2.21 (2H, m, H-2), 2.33 (3H, s, 5-CH3 *J* 3.10), 5.11 (1H, t, H-3, *J* 1.11), 5.64,5.68 (1H, d, *J* 1.099 Hz H-2), 6.63,6.67 (1H, d, *J* 1.09 Hz, H-1), 7.14-7.17 (1H, br,t, *J* 1.42 Hz, H-8), 7.30-7.32 (1H, br t, *J 0*.921 Hz, H-9), 7.36 (1H, br d, *J 1.0* Hz, H-10), 7.661 (1H, s, H-6), 7.861 (1H, br s, NH), 7.89-7.92 (1H, br d, *J 1.079* Hz, H-7),

Compound 2 : White powder *IR* v_{max} (cm^{-1}) 3402,2915,2848,1645,1582,1456,1206,1139,1139,1108,1055,808,718. *MS* m/z(% intensity) 116.1(M^+ base peak),293.7,278.8,266.9,244.4,212 .

1H NMR: 1.253 (3H, s, 3-CH3), 1.48, (each 3H, br s, 4-CH3), 2.32 (3H, s, 5-CH3), 3.90(s,—OCH_3) 5.67,5.70 (1H, d, *J* 1.00 Hz H-2), 6.59,6.62 (1H, d, *J* 1.01 Hz, H-1),6.91(d, *J* 1.05 Hz) 7.40,7.41(1H,s,) 7.62 (1H, s,) 7.69 (1H, br s, NH),

Effect on body weight

The body weight was slightly increased in normal control rats compared to initial body weight whereas streptozotocin-induced diabetic rats showed significant ($p<0.05$) weight loss after 30 days as compared with initially weight of diabetic rats. However, body weight of diabetic rats was restored significantly ($p<0.05$) by treating with glibenclamide (10 mg/kg) and FEMK (at a dose of 200 mg/kg and 400 mg/kg, p.o.) (Table 2).

Table 2: Body weights of streptozotocin-induced diabetic rats after treatment with FEMK .

Groups	Initial body weight	Final body weight
Normal control	165.5 ± 8.49	185.8 ± 9.31
Diabetic control	168. 3 ± 7.64	139.1 ± `10.57*
200 mg/kg FEMK	164.2 ± 10.63	153.3 ± 9.71*
400 mg/kg FEMK	162.1 ± 9.74	155.2 ± 7.69*
10mg/kg Glibenclamide	169.0 ± 8.53	158.1 ± 9.69*
Data expressed as means ± s.d; n = 5. The data are statistically ($p < 0.05$) significant (students unpaired T test). * indicates significantly ($p < 0.05$) different compare to vehicle control group.		

Effect on blood glucose, triglycerides and cholesterol level

Streptozotocin treatment resulted in elevation of fasting blood glucose,triglycerides and total cholesterol, as compared to the normal control rats as noted at end of the study (Table 3). When diabetic rats treated with FEMK (200mg/kg and 400 mg/kg, p.o.) for 15 days showed significant ($P < 0.05$) reduction in fasting blood sugar, triglycerides and cholesterol. However, the standard drug glibenclamide (10 mg/kg, p.o.) exhibited potent anti-diabetic activity with maximum reduction of fasting blood sugar level.

Table 3. Effect on blood glucose, triglycerides and cholesterol level of streptozotocin-induced diabetic rats after treatment with FEMK

Parameters	Days	Normal control	Diabetic control	FEMK (200 mg/kg)	FEMK (400 mg/kg)	Glibenclamide
Blood glucose	0	89.70 ± 6.36	323.62± 22.86 ¥	317.53 ± 19.17 ¥	308.17 ± 21.75 ¥	308.17 ± 21.75 ¥
	15	87.23± 7.08	339.41± 18.90	192.83± 23.87*	138.18 ± 18.43 *	138.18 ± 18.43 **
Cholesterol	0	102.53± 6.74	143.97± 11.46¥	151.54± 13.25¥	138.36± 10.64¥	147.53±14.35¥
	15	98.36±4.98	168.81±15.72 *	123.05±9.98*	103.39±10.48 *	133.45± 11.27
Triglycerides	0	80.63±6.39	147.44±11.30¥	139.86±12.43¥	151.97±13.78¥	148.64±09.83¥
	15	84.06± 5.90	163.29±14.24	121.37±10.02	125.63±*	117.54± 09.97*
Data expressed as means ± s.d; n = 5. The data are statistically ($p < 0.05$) significant (students unpaired T test). ¥ indicates significantly ($p < 0.001$) different compare to vehicle control group at 0 reading. * indicates significantly ($p < 0.05$) different compared to 0 hr reading of respective group. ** indicates significantly ($p < 0.01$) different compared to 0 hr reading of respective group.						

Effect on TBARS and glycogen level

Streptozotocin treatment resulted in elevation of TBARS and depletion of glycogen in liver homogenate, as compared to the normal control rats as noted at end of the study (Table 3). When diabetic rats treated with FEMK (200mg/kg and 400 mg/kg, p.o.) for 15 days showed significant ($P < 0.05$) decrease in TBARS level and increase in glycogen, while the TBARS values was not found to significant in glibenclamaide treated rats.(Table 4)

Table 4. Effect of FEMK in TBARS in diabetic rats

Groups	TBARS value (abs.)	Glycogen level (abs.)
Normal control	0.2451± 0.0156	0.8731±0.0793
Diabetic control	0.8702 ± 0.1054 **	0.3463±0.1658**
200 mg/kg FEMK	0.5693± 0.0249 *	0.5180±0.1915*
400 mg/kg FEMK	0.3891 ± 0.1295 *	0.5985±0.2008*
10 mg/kg of Glibenclamide	0.7056 ± 0.1008	0.6817±0.1872*
Data expressed as means ± s.d; n = 5. The data are statistically ($p < 0.05$) significant (students unpaired T test). * indicates significantly ($p < 0.001$) different compare to diabetic control group.** indicates significantly ($p < 0.05$) different compare to vehicle control group.		

DISCUSSION

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 [18].

Diabetes causes increased oxidative stress, which is thought to play an important role in the pathogenesis of various diabetic complications. The antioxidant actions are keys to preventing or reversing diabetes and its complications. Thus the aim of the present work was to evaluate the antidiabetic activity of ethanolic extract of *Murraya koenigii* fruits in terms of its effects on glycemic status and on the oxidative stress in STZ induced diabetic rats.

Rats treated with STZ develop almost identical diabetic states and exhibit symptoms like hyperglycemia, glucosuria, polyuria, polyphagia, polydipsia and weight loss. The ability of STZ to produce such diabetes has previously been reported in numerous studies [15]. Similar result of STZ was obtained in our present study. The results of the present study demonstrated the antidiabetic activity of *M. koenigii*. The extract was found to decrease the blood glucose, triglycerides as well as cholesterol level after15 days of treatment with FEMK. The reduction in glucose level was highest at the dose of 400 mg/kg which was comparable to standard Glibenclamide (Table no.02).

Glycogen is a primary intracellular storable form of glucose in various tissues especially liver and heart muscle. The results of this study indicate that there was significant increase in glycogen content in liver of extract and Glibenclamide treated diabetic animals compared to that of diabetic control itself. This result indicates extract promotes the peripheral conversion of glucose into glycogen and that is stored in the liver. The level of the glycogen in the tissues is indirect reflection of blood glucose level as the antidiabetic drug stimulates the glycogen synthatase and thereby converts the glucose into glycogen in liver and heart.

There is an evidence that glycation itself may induce the formation of oxygen-derived free radicals in diabetic condition [19]. Thus it suggested that oxidative stress can play an important role in tissue damage associated with diabeties and complications. Therefore, the measurement of oxidative stress is one of the important criteria to explain the possible protective mechanism in diabetes. Lipid peroxidation of unsaturated fatty acids is commonly used as an index of increased oxidative stress and subsequent cytotoxicity [20].

In the present study, elevated level of lipid peroxidation in the diabetic animals is either due to due to enhanced production of reactive oxygen species. In present study, lipid peroxidation was measured in terms of TBARS [16,17] and results indicated to significant increase in liver TBARS content in diabetic rats compared with control rats. However oral administration of MKF extract reduces the level of TBARS indicating a decrease rate of lipid peroxidation. These finding suggest that the MKF extract prevents the formation of reactive oxygen species and induction of lipid peroxidation by STZ in diabetic rats.

The presence of carbazole alkaloids may be responsible for antidiabetic activities which are isolated from extracts of *Murraya koenigii* fruits. Their presence is verified by IR, MASS & NMR analysis. Thus based on our present finding as well as reported phytochemical literatures [21-23]. demonstrated that the presence of carbazole alkaloids of *M. koenigii* might be involved in stabilization of glycemic level. Literature also suggest direct or indirect antioxidant nature of the MKF extract, which could be due to the free radical scavenging of carbazole alkaloids present in the *M. koenigii* [23] fruit acting as a strong free radical scavenger, thereby improving the antioxidant nature in STZ diabetic rats.

In present study the HPTLC fingerprinting profile of fruit extract of *Murraya koneigii* was generated in solvent system in order to ascertain the total number of chemical moieties which will also help in designing the method of isolation and characterization of the bioactive components

In conclusion, our study demonstrated beneficial effects of *M. koenigii* in diabetics rats. It decreases blood glucose level dose dependently and also has potential to decrease the level of TBARS by inhibiting the lipid peroxidation formation so this findings would be helpful in diabetic patient for prevention of diabetic complications related to level of oxidative stress.

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