ejpmr, 2022,9(10), 374-378

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

EVALUATION OF ANTI-DIABETIC ACTIVITY OF SELECTED PLANT IN DRUG INDUCED DIABETIC RATS

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Article Received on 25/07/2022 **Article Revised on 15/08/2022** **Article Accepted on 05/09/2022**

ABSTRACT

The medicinal plant *Murrayakoenigii*shown to have a widevariety of pharmacological activities (hypoglycemic and hypolipidemic).Objective of this study is the present study was designed to evaluate of Antidiabetic activity of *Murrayakoenigii*in drug induceddiabetic rats.Oral administration of ethanolextracts of Murraya leaf (300 and 500mg/kg body weight) for 30 days resulted in significant decrease of bloodglucose from 76.96±3.47 to 62.35 ± 2.07 and decrease in the activities of enzymes of liver. Diabetic rats treated with murraya(55 ml/kg/bw) showed an improvement in the spleenhistology and treated with Murraya*(*50 ml/kg/bw) shows a result similar to that of non-diabetic control. Theresults showed not only significant anti-hyperglycaemic effect of Murraya extracts in experimental model ofdiabetes mellitus but also indicated a dose dependant activity of the extracts.

KEYWORDS: Diabetes, Hypoglycaemic, *MurrayaKoenigii*.

1. INTRODUCTION

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects ininsulin secretion or insulin action, or both.[1,2] Broad research on diabetes leads to a number of synthetic oral hypoglycemic agents like biguanides, sulphonylureas and thiazolidinedione'sbeing used to treat diabetes. Butall have side effects associated with their uses.[3,4] On other hand, traditional medicinal plants with their variousbiological constituents have been used effectively by the communities since long time to treat diabetes.[5,6] Severalnatural products such as alkaloids, flavonoids, terpenoids, saponins, polysaccharides and glycosides are isolatedfrom medicinal plants and are being reportedto possess anti-diabetic activities.[7,8] Inaddition, herbal drugs areextensively used to treat various diseases due to their effectiveness, minimal side effects and relatively low cost. Therefore, it is important to isolate the bioactive molecules from traditional anti-diabetic plants.[9,10] Management of this disease may include lifestyle modifications, diet, exercise, long – term use of oralhypoglycaemic agents or insulin therapy. Since ancient times, plants have been an exemplary source ofmedicine. The search for plants with hypoglycaemic property is an area that draws attention of research workersglobally reviewed 45 of such plants and their products that have been used in the Indian traditional system ofmedicine.[11,12] Management of diabetes without any side effect is still a challenge to themedical community. There is continuous search for alternative drugs.[13,14] Even though herbal medicines have longbeen used effectively in treating diseases in Asian communities and throughout the world, it is prudent to lookfor more herbal medicines for diabetes.From ancient times, some of these herbal preparations have been used in the treatment of diabetes.[15,16] Many traditional plants were used for treatment of diabetes. The active compounds of medicinal plants play an important role in the management of diabetes mellitus especially in developing countries. Moreover, during thepast few years some of the new bioactive drugs isolated from plants showed antidiabetic activity with moreefficacy than oral hypoglycemic agents used in clinical therapy.[17,18,19]

Murrayakoenigii(Rutaceae) commonly known as "Curry Patta" (Hindi) is widely used as a spice andcondiment in India and other tropical countries.[20,21] Various parts of *Murrayakoenigii*have been used in traditionalor folk medicine for the treatment of rheumatism, traumatic injury and snake bite and it has been reported tohave antioxidant, anti-diabetic and anti-dysenteric activities. Curry leaf is used traditionally as a stimulant, antidysenteric and for management of diabetes mellitus.[22,23]

2. MATERIAL AND METHODS

2.1 Plant material and extraction

MurrayaKoenigii Linn(LEAF) will be collectedfresh from the forest areas in Udaipur, Rajasthan, India, and will be dry in the shade and then powdered. The plant will be identified by Professor and Head of the Department of pharmacology, pacific college of pharmacy. The powderedmaterials will be kept in an air-tightcontainer in a refrigerator until the time of use. Aqueous and alcoholic extracts of MKL will prepared according to the standard extract procedure. The yield of extracts will be approximately 8.5% and 7%, respectively.

2.2 Toxicity studies

The acute oral toxicity was carried out as per the guidelines set by Organization for Economic cooperation and Development(OECD), revised draft guidelines423, received from Committee for the Purpose of Control and Supervision of Experiments onAnimals (CPCSEA), Ministry of Social justice and Empowerment, Government of India. The principle is based on a stepwise procedure with the use of a minimum number of animals per step to obtain sufficient information on the acute toxicity of thetest substance to enable its classification. The substance is tested using a stepwise procedure,each step using three animals of either sex.

2.3 Antidiabetic study of EMKL in streptozotocin induced diabetic model

2.3.1 Induction of experimental diabetes: After fasting, diabetes was induced by single intravenous (i.v.) injection of STZ (55 mg/kg) dissolved in 0.1 M cold sodium citrate buffer, pH 4.5. The control rats received the vehicle alone. The animals were allowed to drink 5% glucose solution overnight for prevention of mortality due to hypoglycemic convulsing reaction. After 13 days of STZ administration the remaining survival animals which showed blood glucose concentration more than 250 mg/dl considered as diabetics and were used for experimentation.

2.3.2 Determination of thiobarbituric acid reactive substance (TBARS)

After sacrificing, the liver was isolated from respective groups of animals and homogenized in 0.15 M KCL respectively. Homogenates were centrifuged andsupernatant 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.

2.4 Administration of Extract

Suspension of ethanolic extract was prepared in 0.5% carboxymethyl cellulose using Tween 20 (0.2% v/v) as a suspending agent. The extract was administered in a dose of 300 and 500 mg/kg respectively to streptozotocin induced diabetic Wister rats. Control groups were given only 0.5% carboxymethyl cellulose with Tween 20 (0.2% v/v).

2.5 Number of animals and dose levels

Group 1- Control: This group was used for studying the baseline values of the parameters studied.
Group 2- Diabetic control: This group consisted of streptozotocin induced diabetic rats.
Group 3- Diabetic rats treated with (200 mg/kg b.w.) aqueous extract of MKL.
Group 4- Diabetic rats treated with (400 mg/kg b.w.) aqueous extract of MKL.
Group 5- Diabetic rats treated with (200mg/kg. b.w) alcoholic extract of MKL.
Group 6- Diabetic rats treated with (400 mg/kg. b.w.) alcoholic extract of MKL.
Group 7- Diabetic rats treated with glibenclamide 10mg/kg.

The drug was administered orally for 10 days and 30 days in different groups of animal, with each group containing 3 animals. The efficacy of this drug will be compared with the treated diabetic rats.

3. RESULT AND DISCUSSION

STZ-induced experimental diabetes is a valuable model for induction of diabetes. Further, the STZ diabetic animals may exhibit most of the diabetic complications, namely, myocardial cardiovascular, gastrointestinal, nervous, vas deferens, kidney, and urinary bladder dysfunctions through oxidative. 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. Thus the aim of the present work was to evaluate the antidiabetic activity of MKL in terms of its effects on glycemic status and on the oxidative stress in STZ induced diabetic rats. The results of the present study demonstrated the antidiabetic activity of M. koenigii.

Table 1: Hypoglycaemic effect on MKL innormoglycermic and in oral glucose tolerance test.

Treatments	0 min	30 min	60 min	120 min
	Effect in normoglycemic			
Normal	78.7 ± 3.14	77.79 ± 3.96 (-1.17)	78.34 ± 4.69 (+0.46)	77.96 ± 3.47 (-0.94)
Vehicle control	77.73 ± 3.77	76.13 ± 5.80 (-2.10)	76.75 ± 4.68 (-1.27)	76.54 ± 2.53 (-1.55)
MKL-300	75.58 ± 3.74	72.06 ± 3.20 (-4.88)	71.45 ± 2.68 (-5.78)	67.36 ± 2.95 (-12.20)
MKL-500	77.24 ± 4.42	72.3 ± 3.1 (-6.83)	68.19 ± 2.82 (-13.27)	62.9 ± 2.07 (-22.79)
	Effect in OGTT			
Vehicle control	76.31 ± 4.15	137.36 ± 5.14 (+80.0)	130.14 ± 4.65 (+70.54)	116.36 ± 6.77(+52.48)
Glibenclamide	75.23 ± 3.28	110.87 ± 4.97(+47.37)	94.35 ± 4.95 (+25.41)	85.86 ± 5.36(+14.13)
MKL-300	74.09 ± 5.02	124.69 ± 4.94(+68.29)	114.54 ± 5.13 (+54.59)	104.5 ± 4.75(+41.04)
MKL-500	74.75 ± 6.48	115.36 ± 5.56(+54.32)	105.36 ± 5.53 (+40.94)	91.37 ± 5.58(+22.23)

Table 2: Antidiabetic activity of M.koenigii in streptozotocin induced diabetic rat.

Time Interval	Vehicle control	Diabetic	Glibenclamide	MKL-300 mg/kg	MKL-500 mg/kg
0 h	80.93 ± 3.84	308.56 ± 18.83*	302.60 ± 14.21*	304.66 ± 14.56*	307.98 ± 10.22*
1 h	80.61 ± 6.20	309.62 ± 19.08	291.038 ± 15.48	294.87 ± 16.63	294.45 ± 11.52
3 h	79.48 ± 4.35	309.99 ± 19.11	276.58 ± 17.10[a]	283.51 ± 15.43	280.73 ± 12.29[a]
5 h	80.89 ± 5.51	309.06 ± 20.20	260.42 ± 16.21[a]	273.72 ± 14.82[a]	267.65 ± 10.92[a]
5th Day	79.84 ± 4.55	310.55 ± 21.4	223.15 ± 15.43	239.65 ± 16.00	236.95 ± 9.36
10th Day	78.56 ± 4.41	312.85 ± 21.67	194.49 ± 16.08	215.77 ± 16.65	204.92 ± 9.80
15th Day	79.54 ± 3.84	316.12 ± 18.52	169.57 ± 11.98[ab]	195.94 ± 14.93[ab]	174.39 ± 12.56[ab]

In the present study, the diabetic rats had shown higher levels of glycosylated hemoglobin compared to those in normal rats indicating their poor glycemic control; while after 15 days of MKL treatment, the glycosylated hemoglobin significantly decreased as comparable to standard glibenclamide, indicating a decrease in the status of glycation. Lipid peroxidation of unsaturated fatty acids is commonly used as an index of increased oxidative stress and subsequentcytotoxicity.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 because of higher level of glycosylated haemoglobin. In present study, lipid peroxidation was measured in terms of TBARS and results indicated to significant increase in liver TBARS content in diabetic rats compared with control rats. However oral administration of MKL extract reduces the level of TBARS indicating a decrease rate of lipid peroxidation.

Table 3: Glycosylate haemoglobin in STZ induced diabetic rats.

Groups	Glycosylated haemoglobin (%Hb)
Vehicle control	8.34 ± 0.731
Diabetic control	16.43 ± 0.141[a]
MKL-300	11.098 ± 0.921[ab]
MKL-500	9.32 ± 1.043[ab]
Glibenclamide	11.98 ± 0987[ab]

These finding suggest that the MKL extract prevents the formation of glycosylated haemoglobin which ultimately protect from the formation of reactive oxygen species and induction of lipid peroxidation by STZ in diabetic rats. Thus based on our present finding as well as reported phytochemical literatures demonstrated that the presence of antioxidant carbazole alkaloids of M. koenigii might be involved in stabilization glycemic level. Literature also suggestsdirect or indirect antioxidant nature of the MKL extract, which could be due to the free radical scavenging of carbazole alkaloids present in the M. koenigii leaf acting as a strong free radical scavenger, thereby improving the antioxidant nature in STZ diabetic rats. 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 finding would be helpful in diabetic patient for prevention of diabetic complications related to level of oxidative stress.

4. CONCLUSION

The collected leaves of M. koenigii (L) were dried under shade and undergone crushing in electric blender to form powdered and subjected to extraction by maceration in air tight container by using ethanol as a solvent (125 g/500 ml ratio). The extract was concentrated by evaporation at room temperature and used for pharmacological studies.

Suspension of ethanolic extract was prepared in 0.5% carboxymethyl cellulose using Tween 20 (0.2% v/v) as a suspending agent. The extract was administered in a dose of 300 and 500 mg/kg respectively to streptozotocin induced diabetic Wister rats. Control groups were given only 0.5% carboxymethyl cellulose with Tween 20 (0.2% v/v).

After fasting, diabetes was induced by single intravenous (i.v.) injection of STZ (55 mg/kg) dissolved in 0.1 M cold sodium citrate buffer, pH 4.5. The control rats received the vehicle alone. The animals were allowed to drink 5% glucose solution overnight for prevention of mortality due to hypoglycemic convulsing reaction. After 13 days of STZ administration the remaining survival animals which showed blood glucose concentration more than 250 mg/dl considered as diabetics and were used for experimentation.

13 days after streptozotocin, control and survival diabetic rats were randomly divided in seven groups, each consisting of three 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 diabetic animals received glibenclamide (10 mg/kg, p.o.) Group 4 and 5 diabetic animals received 300 and 500 mg/kg of MKL extract respectively. After 15 days of above treatments schedule animal's blood was withdrawal by retro-orbital plexus (fasted animals) for determination of glucose and glycosylated haemoglobin, triglyceride, and cholesterol. Liver was isolated from respective group of animals for determination of thiobarbituric acid reactive substance.

Fasting blood glucose was estimated by glucose estimation kit by GOD-POD method while glycosylated hemoglobin was assayed according to reported method by using enzymatic kit.

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. The diabetic group showed considerable loss of body weight and increase in blood glucose levels and degeneration of the glomeruli and renal convoluted tubules and atrophied islets with disintegration of β-cells. Treatment of diabetic rats with MK extract showed significant ($p < 0.001$) improvement in blood glucose levels and body weight gain. The MK extract also caused an improvement in tissue injury induced by STZ injection in the kidney and islets of Langerhans.

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.

To conclude, present study shows a better result in management of diabetes when drugpowder used in therapy was well tolerated by animal models.

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