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Research Article

ANTI-HYPERGLYCEMIC ACTIVITY OF MURRAYA KOENIGII IN COMPARISON WITH PIOGLITAZONE IN EXPERIMENTAL SMALL ANIMAL MODELS

Bhopal Chandra.V[1], *Madhavulu Buchineni[2], Kudagi B.L[3], Rama Mohan Pathapati[4], Umar Sufyan[5], Sreeharsha[6] Haritha.M[7]

Associate Professor[2, 4], Tutor[1, 7], Professor & Head[3], PG Students [5-6] ,Department of Pharmacology, Narayana Medical College, Nellore

Corresponding Author: Dr B Madhavulu Buchineni*, Associate Professor, Department of Pharmacology & Therapeutics, Narayana Medical College & Hospital, Nellore – 524 002 (A.P),Ph: +919440713718

Abstract

Background: Murraya koenigii is commonly known as curry leaf plant and is habitually used as an imperative spice in India and other tropical countries. There were inadequate studies available to establish anti-hyperglycemic activity of Murraya in animal studies. In this study we have evaluated anti hyperglycemic activity of Murraya koenigii leaf (aqueous extract) in comparison with Pioglitazone in alloxan induced diabetic rats. Methodology: The study were conducted on 18 Albino rats (Wister) for three groups consists of Diabetic control, diabetic and Pioglitazone and diabetic and Murraya koenigii. Statistical values were being expressed as mean ± SEM, Statistical difference in mean were analyzed by one way ANOVA. Results: Administration of the Murraya koenigii, produced statistically significant reduction in the blood glucose level ($p<0.0001$) which was 240.1 before treatment and reduced to 224 after treatment. Pioglitazone has produced peak reduction in blood glucose level 199.8 and was statistically significant ($p<0.001$) compared to the value of 232 before drug administration. Conclusion: from our study it was evident that Murraya koenigii have beneficial effects in the management of alloxan induced diabetes in male wister rats as an anti-hyperglycemic agent in comparison with known insulin sensitizer Pioglitazone.

Keywords: Murraya koenigii linn, Pioglitazone, Insulin sensitizers, alloxan, Type-2 Diabetes

INTRODUCTION

Diabetes mellitus (DM) is the most common endocrine disorder which currently affects more hundreds of million people worldwide and the number of people with diabetes is increasing due to population growth, aging and increasing prevalence of obesity and physical inactivity. It is also a leading cause of death and disability worldwide. (1)

Diabetes mellitus (DM) is a complex multi systemic disorder characterized by a relative or absolute insufficiency of insulin secretion and/or concomitant resistance to the metabolic action of insulin on target tissues. (2) In India, it is estimated that presently 19.4 million individuals are affected with this deadly disease, which is likely to go up to 57.2 million by the year 2025.(3) DM may lead to potential complications like the diabetic retinopathy, nephropathy, peripheral neuropathy, macro-angiopathies. (4) Type 2 diabetes mellitus (DM2) is characterized by excessive hepatic gluconeogenesis, increased insulin resistance and a progressive inability of pancreatic beta cells to produce sufficient insulin. The onset of insulin

resistance itself seems to be an early event in the progression of DM2, precedes the development of overt hyperglycemia, and may contribute substantially to the development of cardiovascular disease independent of the hyperglycemia associated with DM2. [5] We have so far very few anti-hyperglycemic agents like Metformin and Pioglitazone drugs available for the management for insulin resistance. Pioglitazone is a thiazolidinedione (TZD), a class of drugs used to treat T2D. Briefly, pioglitazone activates peroxisome-proliferator activated receptor gamma (PPARγ) in a variety of cells, improving insulin sensitivity in the liver (decreasing gluconeogenesis) and in the adipose tissue as measured by the ability of insulin to inhibit lipolysis [6]

Pioglitazone has been consistently associated with reduced cardiovascular risk including that of myocardial infarction and mortality.[7] However, Pioglitazone is associated with fluid retention that may worsen heart failure.[8] This emphasizes the urgent need for more effective anti-hyperglycemic agents. Herbal drugs constitute an important part of traditional medicine and literature shows that there are more than 400 plant species showing antidiabetic activity.[9] In this study we have evaluated anti hyperglycemic activity of Murraya koenigii leaf (aqueous extract) in comparison with Pioglitazone which was known insulin sensitizer in alloxan induced diabetic rats.

METHODOLOGY:

Chemicals & drugs

Alloxan (150mg/kg) (Sigma Pvt. Ltd.,)
Pioglitazone (1 mg/kg) (Cipla Pvt. Ltd,)

Animals

The study were conducted on Albino rats (Wister) of 180-250 g .They were housed in standard polypropylene cages & maintained under standard conditions (room temperature 24-27^{o}C and humidity 60-65%) with 12 h light and dark cycle. The food in the form of dry pellets and water were available *adlibitum*. The animal experiments were conducted after obtaining approval by the ethics committee of the narayana medical college with the proposal number – 17/2011/NMC.

Each model consists of 3 groups (six animals in each group).Groups are divided as follows.

I. Diabetic control
II. Diabetic+ Pioglitazone
III. Diabetic + Murraya koenigii

Plants Materials/Preparation of the Extract [10]

Fresh leaves of the plant *(Murraya koenigii)* were obtained locally. The fresh leaves were ovendried at 45^{0}C, ground into powder and 20g will be soaked in 300ml of distilled water overnight at room temperature. The filtrate obtained was evaporated in a hot-air oven at 45^{0}C. The extract was weighed (2g) and reconstituted in appropriate volume of distilled water before administration to the rats.

Alloxan induced Diabetes

Alloxan monohydrate dissolved in 0.9% sodium chloride solution (normal saline) is used in this study

PROCEDURE:

Alloxan induces diabetes in almost all species of animals. Diabetogenic dose varies with species and the optimal dose given in rats is 150 g/kg i.p. The blood glucose shows the triphasic response, with hyperglycemia at 1 hour, followed by hypoglycemia, which lasts for 6 hours, and stable hyperglycemia by 24-48hours after Alloxan administration. These hyperglycemic rats were used for study. The rats were divided in 3 groups and each group consists of 6 rats.

a. Group I received Alloxan (150mg/kg/i.p) serve as diabetic control
b. Group II received Pioglitazone (1 mg/kg/p.o)
c. Group III (Diabetic) was administered orally - aqueous leaf extract of *M. koenigii* at dose of 200 mg/kg/p.o.

Blood Collection and Biochemical Analysis [10]

At the end of seven days of extract administration, 1ml of blood samples were collected from the tail directly into anticoagulant bottles contains sodium fluoride. The plasma was separated after centrifugation. Diagnostic reagent kit were used to estimate plasma glucose concentration.

Estimation of serum glucose

Glucose reagent (Transasia bio-medicals Ltd.,) diagnostics kit was used for the estimation of serum glucose. Estimation was carried out by GOD/POD (Glucose oxidase and peroxidase) method.

STATISTICAL ANALYSIS:

Values were be expressed as mean ± SEM, Statistical difference in mean were analyzed by one way ANOVA

RESULTS:

In the control group, the blood glucose values were 245.8 and 257 respectively before and after administration of control (normal saline). There was an increase in the blood glucose level indicating that vehicle (normal saline) used to dissolve test and standard drugs was not having any blood glucose lowering action. Pioglitazone produced highest reduction in blood glucose level 199.8 and was statistically highly significant ($p<0.001$) compared to the value of 232 before drug administration. Administration of the test drug Murraya koenigii, produced statistically significant reduction in the blood glucose level ($p<0.0001$) which was 240.1 before treatment and reduced to 224 after treatment. Thus, overall the blood glucose lowering action was more with pioglitazone and Murraya koenigii then control.

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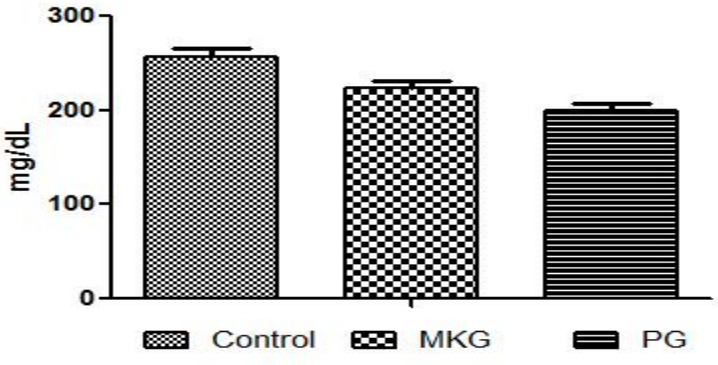
Table.1: Shows Levels Of glucose before and after Test and Standard Drug Administration

	Murraya koenigii+ Alloxan	
	Before	**After 7days**
Glucose (mg/dl)	240.1 ± 6.6	**224 ± 7.9*** **

Table.2: Inter Group Comparison between Test (Murraya) and Standard Drug (Pioglitazone)

	Glucose (mg/dl)
Control	257 ± 8.9
Pioglitazone	**199.8 ± 7.8*** **
Murraya koenigii	**224 ± 7.9*** **

Diagram 1: Blood sugar control in comparison with Murraya and Pioglitazone.



MKG – Murraya Koenigii

PG - Pioglitazone

DISCUSSION

Insulin resistance is one of pathogenic factors for non-insulin-dependent diabetes mellitus (NIDDM). Pioglitazone can improve glucose and lipid metabolism by reducing insulinresistance on the post-binding system.(11) There were many studies available to prove hypoglycemic activity of Murraya leaves in comparision with glibenclamide. The study by, H.A. Lawal et al (2008) Murraya koenigii produced dose dependant decreased in the blood glucose level in Streptozotocin induced diabetic rats and the effect was comparable to standard antidiabetic glibenclamide.(12)It is also suggested that Murraya keonigii aqueous leaf extract possesses hypoglycaemic activity in normal and alloxan – induced diabetic rats and murraya koenigii acts by decreasing glycogenesis and gluconeogenesis. Dineshkumar et.al (2010) also suggested that possible mechanism by which the murraya koenigii decreases blood sugar level may be by potentiating the effect of insulin either by increasing the pancreatic secretion of insulin from beta cells of islets of langerhans or by increasing the peripheral glucose uptake. (13) William Felicia et al (1993) observed a significant reduction in fasting blood sugar and postprandial blood sugar after feeding Murraya keonigii to Non Insulin Dependent Diabetes Mellitus patients (NIDDM).(14)

Insulin Resistance pattern is rising in India urban and rural areas and as we have limited to drugs to treat Insulin resistance so, Murraya can have add on benefit as adjuvant agent.(15) In continuation of our research work with Murraya koenigii leaves, we have further extended our study for in small animal diabetic models.Murraya koenigii Spreng (Rutaceae), a medicinally important herb of Indian origin, has been used for centuries in the Ayurvedic system of medicine. Leaves, fruits, roots and bark of this plant are a rich source of carbazole alkaloids. These alkaloids have been reported for their various pharmacological activities such as antitumor, antiviral, anti-inflammatory, antidiarrhoeal, diuretic and antioxidant activities. Apart from these activities, the plant is reported to possess a wide spectrum of biological activities like anti-diabetic and hypolipidemic action. Mahanimbine is one of the Phytochemical compound which was responsible for the antidiabetic activity, antioxidant and free radical-scavenging activities. (16)

In this study the hypoglycemic and hypolipidemic action of murraya koenigii aqueous leaf extract has been evaluated and its efficacy is compared with Pioglitazone. Administration of alloxan (150 mg/kg, i.p.) produced significant increase in blood glucose level after 3 days. Alloxan chemically 2, 4, 5, 6-tetraoxo pyrimidine is a potent destructor of pancreatic β-cells through generation of reactive oxygen species. It has been postulated that glucose transporter and glucokinase are the target molecule for alloxan, leading to decreased insulin levels and uncontrolled blood glucose level. (17)

Alloxan produces uncontrolled diabetes with blood glucose level greater than 250 mg/dl until the death of the animals. The progressive increase in blood glucose level produced by alloxan was significantly reduced by murraya koenigii and pioglitazone after treating the diabetic rats for seven days. Murraya koenigii showed definitive anti hyperglycemic action when compared to the control but the reduction of blood glucose level was less when compared to that of pioglitazone. The present study was in accordance with the previous studies done by Tembhurne et al (2010). (18)

This study of anti diabetic action of Murraya keonigii was in agreement with in the study of Yadav et al (2002) showed reduction in blood glucose level for mild and moderate diabetes rats on feeding with extract of Murraya keonigii as diet, proving its potential as antihyperglycaemic activity. (19)

CONCLUSION

Murraya koenigii as an anti-diabetic agent was not investigated thoroughly, so that the present study was under taken. The present study evaluated the anti diabetic effect of the aqueous leaf extract murraya koenigii by using alloxan to induce diabetes in rats. At the end of the study it was concluded that aqueous leaf extract of Murraya koenigii has anti hyperglycemic effect in diabetic rats. Thus our study suggests that Murraya koenigii may have beneficial effects in the management of type II DM. However further extensive studies need to be done to isolate the active ingredient and to study the pharmacodynamics and pharmacokinetic, adverse effects of them and if found suitable it can be tried in human studies.

CONFLICT Of INTEREST - NONE

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