EVALUATION OF HYPOGLYCEMIC ACTIVITY OF MURRAYA KOENIGII EXTRACTS IN ALLOXAN INDUCED DIABETIC RATS.

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ABSTRACT

The present study was aimed to evaluate the hypoglycemic activity of ethyl acetate and ethanolic extracts of Murraya koenigii in alloxan induced diabetic rats. Wistar rats of either sex were used for the study. The Wistar rats used for the experimental work were administered with alloxan (120 mg/kg) to induce diabetes. The rats having blood glucose levels above 200 mg/dl were selected and considered as diabetic rats. The diabetic rats were segregated into groups, with a minimum of at least 6 in each group and treated with Glimiperide 2 mg/kg, ethyl acetate and ethanolic extract of low dose 100 mg/kg and high dose 200 mg/kg separately for 21 days. Blood glucose level estimations were recorded on the 1st, 7th, 14th and 21st day of the experiment using glucometer. Blood glucose levels increased in alloxan induced diabetic rats but the extracts of M. koenigii significantly reduced blood glucose levels.

KEYWORDS: Alloxan, Murraya koenigii extracts, hypoglycemic activity, Glimiperide.

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic diseases in which there are high blood sugar levels over a prolonged period is seen. This high blood sugar produces the symptoms of increased urination and increased hunger. Untreated diabetes can cause many complications. Acute complications include diabetic ketoacidosis and nonketotic hyperosmolar coma. Serious long-term complications include heart disease, stroke, kidney failure, foot ulcers and damage to the eyes.

Diabetes is due to either the pancreas not producing enough insulin, or the cells of the body not responding properly to the insulin produced. Three main types of diabetes mellitus; Type
1 DM results from the body's failure to produce enough insulin. This form was previously referred to as "insulin-dependent diabetes mellitus" (IDDM) or "juvenile diabetes"; Type 2 DM begins with insulin resistance, a condition in which cells fail to respond to insulin properly. This form was previously referred to as "non insulin-dependent diabetes mellitus" (NIDDM) or "adult-onset diabetes". The primary cause is excessive body weight and not enough exercise and Gestational diabetes, is the third main form and occurs when pregnant women without a previous history of diabetes develop a high blood glucose level.

Number of plants have been used since ancient times for diabetes control. India is rich in the medicinal herbs and therefore, it can be accurately called the “Botanical Garden of the world”.

Murraya koenigii, belongs to the family Rutaceae, commonly known as curry-leaf tree, is a native of India, Sri Lanka and other south Asian countries. It is found almost everywhere in the Indian subcontinent, it shares aromatic nature, more or less deciduous shrub or tree.

MATERIALS AND METHODS

Plant material: The fresh leaves of plant Murraya koenigii were collected locally from Nanded region and identified botanically, authenticated by referring the standard taxonomic characteristic features and given the voucher number.

Preparation of ethanolic extract of Murraya koenigii: The fresh leaves of plant Murraya koenigii were cleaned, washed well in water, dried in shade and further crushed to powder, and then the powder is passed through the mesh 60 and stored in air tight container for further use.

The extraction method selected for the leaves of Murraya koenigii was continuous hot extraction method using soxhlet apparatus through petroleum ether, chloroform, ethyl acetate and ethanol. Defating of powdered leaves was performed in Soxhlet extractor according to the standard method till colourless solution was observed in siphon tube. 300 gm of the powdered leaves and 1000 ml Petroleum ether was used for extraction, further chloroform then Ethyl acetate was used for extraction. Ethyl acetate extract of powdered leaves was prepared in Soxhlet extractor according to the standard method till colourless solution was observed in siphon tube. Average 260 gm of the powdered leaves and 1000 ml Ethyl acetate was used for extraction. After completion of extraction extract was cooled and dried. The
extract was stored in air tight container till use and finally ethanolic extract of powdered leaves was prepared in Soxhlet extractor according to the standard method till colourless solution was observed in siphon tube, 250 gm of the powdered leaves and 1000 ml Ethanolic was used for extraction. After completion of extraction extract was cooled and dried. The extract was stored in air tight container for further use.\[5\]

**Animals**

Wistar rats of either sex weighing 200 to 300 g were used in the present study. The experimental animals were maintained under standard laboratory conditions in animal house of Nanded Pharmacy College approved by the committee for the purpose of control and supervision on experiments on animals (CPCSEA) (Reg. No. 1613/PO/a/12/CPCSEA) under 12 h light/dark cycle and controlled temperature (24 ± 2°C) and fed with commercial pellet diet and water *ad libitum*. All animals were acclimatized to the laboratory environment for at least one week before the commencement of experiment. The experimental protocol for the study was followed according to the norms of Institutional Animal Ethics Committee.

**Procedure**

Rats were injected with a single dose of alloxan monohydrate [120 mg/kg body weight] dissolved in normal saline by i.p. route. The diabetic state was confirmed 48 hours after alloxan injection by hyperglycemia. The blood glucose was measured by one touch glucometer. The alloxan induced rats were allowed to drink 5% glucose solution during 48 hrs of alloxan treatment. Blood glucose levels show triphasic response with hyperglycemia for one hour followed by hypoglycemia that lasts for six hours & stable hyperglycemia after 48 hours. Animals showing fasting blood glucose level above 200 mg/dl after 48 hour of alloxan administration were considered diabetic & selected for the further study. For a period of three weeks, drug samples were administered orally.\[7\]

**Evaluation**

Evaluation was carried out by estimating blood glucose level and at 7th, 14th and 21st day of treatment. Blood samples were collected from 8 hour fasting animals through a retro orbital vein, serum is separated by centrifuge (3000 rpm) under cooling (2-4 °C) for ten minutes and the serum glucose level is estimated by glucose oxidase-peroxidase method [GOD-POD kit] using auto analyser.
Experimental design for alloxan induced diabetes model

Wistar albino rats of either sex weighing 200-300g, obtained from animal house of college. The Animals were randomly divided as following groups of six animals each namely-
1. Control group receive Alloxan 120 mg/kg
2. Control + Standard drug glimiperide 2 mg/kg
3. Test group (MKEA extract, dose 100 mg/kg)
4. Test group (MKEA extract, dose 200 mg/kg)
5. Test group (MKE, dose 100 mg/kg)
6. Test group (MKE, dose 200 mg/kg).

The control group administered with only alloxan. The other diabetic groups were administered with glimiperide the standard drug, MKEA and MKE of two different doses, separately. Oral administration of standard drug, MKEA and MKE was carried out for 21 days. On the 21st day, the blood samples were collected for the estimation of blood glucose levels.

Statistical analysis

The data were expressed as mean ± Standard Error of Mean (SEM). Statistical analyses were performed by one way analysis of variance (ANOVA) followed by T – test.

RESULTS AND DISCUSSION

![Chart 1. Blood glucose levels in all groups of animals during experimental period](image)

MKEA= *Murraya koenigii* ethyl acetate extract;  MKE= *Murraya koenigii* ethanolic extract
Table – 1 Blood Glucose Levels

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Group</th>
<th>Day 1</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>540.0±3.8</td>
<td>436.33±8.9</td>
<td>554±8.6</td>
<td>473.4±0.1</td>
</tr>
<tr>
<td>2</td>
<td>Standard</td>
<td>557.3±8.4</td>
<td>375.8±13.7</td>
<td>173.3±8.8</td>
<td>98.3±4.8</td>
</tr>
<tr>
<td>3</td>
<td>MKEA 100 mg/kg</td>
<td>512.5±2.4</td>
<td>431.6±5.3</td>
<td>280.5±5.1</td>
<td>170.8±6.1</td>
</tr>
<tr>
<td>4</td>
<td>MKEA 200 mg/kg</td>
<td>558.7±6.2</td>
<td>465.81±4.1</td>
<td>296.9±7.2</td>
<td>138.5±5.3</td>
</tr>
<tr>
<td>5</td>
<td>MKE 100 mg/kg</td>
<td>507.4±9.3</td>
<td>405.8±5.8</td>
<td>269.2±3.9</td>
<td>139.6±7.2</td>
</tr>
<tr>
<td>6</td>
<td>MKE 200 mg/kg</td>
<td>473.7±4.8</td>
<td>283.81±2.8</td>
<td>138.6±1.7</td>
<td>99.5±4.7</td>
</tr>
</tbody>
</table>

The results in Table-1 and Chart -1 confirms the antihyperglycemic activity of ethanolic and ethyl acetate extract of *Murraya koenigii* leaves in alloxan induced diabetic rats. The plant extracts might have initiated the release of insulin from the existing β-cells of pancreas, thus reducing the Blood glucose level. Glimipride is a sulfonylurea drug that is effective in moderate diabetic state. It reduces the blood glucose level.

Present study investigated the antidiabetic potential of extracts of *Murraya koenigii* leaves. The results showed that the highest dose (200mg/kg) of different extracts of leaves possesses significant antidiabetic activity when given orally in a daily single dose. The findings suggest effect of two different doses of different extracts (100 mg/kg and 200 mg/kg) of *Murraya koenigii* leaves is probably mediated through its ability to cause a significant decrease in blood glucose level of diabetic rats.

The antidiabetic activity of *Murraya koenigii* leaves was evaluated in alloxan-induced diabetic rats by testing its effect on fasting blood glucose level using auto analyser glucose kit. Alloxan, at a dose of 120 mg/kg body weight, caused sufficient damage to pancreatic β cells so that secreted insulin was not enough to regulate blood glucose and resulted to a significant increase in blood glucose levels.

The result of study indicates that the ethyl acetate and ethanolic leaf extract of *Murraya koenigii* leaves at the test dose used and the reference drug Glimipride (2 mg/kg) exhibited a time dependent reduction of the blood glucose levels of the alloxan-induced diabetic rats. It is well documented that antidiabetic drugs treat diabetes mellitus by lowering glucose levels in the blood. The result obtained from this preliminary study clearly shows that *Murraya koenigii* leaves caused marked antihyperglycemic activity in alloxan-induced diabetic rat model which indicates antidiabetic potentials of the extract. The dose of the extract (200 mg/kg) produced the highest antidiabetic effect and this may suggest that this dose may be the effective antidiabetic dose of the crude extract. However Glimipride was superior in
activity when compared to the test doses of the extract which may be attributed to the crude nature of the plant extract.

CONCLUSION
It is concluded that the extracts of *Murraya koenigii* leaves significantly reduced the blood glucose level in alloxan treated diabetic rats thus proving its antidiabetic activity. The blood glucose level was reduced either due to the bioactive phytochemical component of the plant that stimulated the β cells of pancreas to secrete insulin or due to any biochemical mechanism. Though this plant is recommended for the treatment of diabetes, further investigations need to be carried out to know the actual mechanism involved in its action.

REFERENCES


