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Evaluation of antidiabetic effect of *Murraya koenigii* leaves chloroform extract (MKLCE) in alloxan induced diabetic albino rats

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Abstract

Background: Diabetes is a growing health concern in the world and is now emerging as an epidemic world over. Currently, more attention is being paid to the natural products study as potential antidiabetics. India is considered as the world's diabetic capital. The study of plants having Antidiabetic activity may give a new idea in the approaches of diabetes mellitus treatment.

Objective: The present study was aimed to evaluate the antidiabetic effect of *Murraya koenigii* leaves chloroform extract (MKLCE) in alloxan - induced diabetic albino rats model.

Methods: Albino rats weighing 150- 250 grams were grouped into 5 equal groups taking 6 rats in each. Group A served as control (normal), Group B as diabetic control, received alloxan after overnight fasting. Group C & D was received alloxan + MKLCE suspension at a doses of 250 & 500 mg/kg orally respectively, Group E was given alloxan + standard drug (Glimiperide 2mg/kg) suspension for 21 days & the MKLCE effect on blood sugar was measured at regular intervals. At the end of this research study blood samples were collected from all rats on 0day (initial), on day 7th, 14th, 21st of given test drug MKLCE treatment for biochemical estimation of blood sugar with glucometer & the FBS values were observed.

Results: This present research study revealed that MKLCE has antidiabetic effect against alloxan induced diabetic albino rats on i.p. alloxan injection at 120mg/kg.b.w. & confirms that on i.p. alloxan injection causes a significant raise in fasting blood sugar (FBS) in untreated albino rats when compared to control group. Treatment of diabetic rats with MKLCE for 21 days caused a dose dependent fall in FBS levels. Glimiperide treated diabetic rats also showed a significant ($P<0.00$) fall in FBS levels after 21 days of treatment.

Conclusion: It is concluded that MKLCE has shown significant antidiabetic effect at a doses of 250 & 500 mg/kg. b.w. in alloxan induced diabetic albino rats.

Keywords: MKLCE, glimiperide, antidiabetic, albino rats, blood sugar

Introduction

Medicinal plants are used in many countries to treat and control diabetes mellitus. The hypoglycemic action of these medicinal plants is being studied. [1] Diabetes is a group of metabolic diseases which is characterized by hyperglycemia resulting from defective insulin action or insulin secretion or both. Broad research work on diabetes leads to a number of synthetic oral hypoglycemic agents like thiazolidinedione's, biguanides, and sulphonylureas being used to treat diabetes. But all synthetic oral hypoglycemic drugs have side effects associated with their uses. On the other hand, traditional medicinal plants along with their various biological constituents have been used effectively by the communities since long time to treat diabetes. Several natural products such as polysaccharides, glycosides alkaloids, saponins, terpenoids, flavonoids, and are isolated from these medicinal plants and are being reported to have or possess anti-diabetic activities. In addition, herbal drugs are extensively used for the various diseases treatment due to their effectiveness, minimal side effects and relatively low cost. Therefore, it is very much important to isolate the bioactive molecules from traditional anti-diabetic plants.

As per as the management of this disease concerned which may include lifestyle modifications, exercise, diet, long – term use of insulin therapy or oral hypoglycaemic agents. The search for plants with the hypoglycaemic property is an area that draws attention of research workers globally reviewed 45 medicinal plants and their products that have been used in the Indian traditional system of medicine. Diabetes management without any one of side effect is yet a challenge to the medical community.

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There is continuous search for alternative drugs. Even though herbal medicines have been effectively used from a long time in treating diseases in Asian communities and throughout the world, it is prudent to look for more herbal medicines for diabetes. From ancient times, some of these herbal medicinal plants preparations have been used in the diabetes treatment. So, for the diabetes treatment so many traditional plants were used. The active compounds of these medicinal plants play an important role in the diabetes mellitus management especially in developing countries. Even though, during the past few years some of the new bioactive drugs as biological active medicines isolated from plants showed antidiabetic activity with more efficacy than oral hypoglycemic agents used in clinical therapy.

Murraya koenigii is belongs to Family Rutaceae (Rutaceae) and is commonly known as “Curry Patta” in Hindi & is widely used as a condiment and spice in India & other tropical countries and is a native plant of India, Sri Lanka & other south Asian countries as well. It is found usually almost everywhere in the Indian subcontinent. Various parts of this plant have been used in folk medicine or traditional medicine for the treatment of traumatic injury, rheumatism, and snake bite and it has been reported to have antioxidant, anti-diabetic, & anti-dysenteric activities. Curry leaf is traditionally used as, antidiabetic and a stimulant for the diabetes mellitus management.

Chemically, alloxan (2,4,5,6 tetraoxypyrimidine) is an oxygenated derivative of pyrimidine & a cyclic urea analog which was reported to produce permanent diabetes in experimental animals such as rabbits, rats. It is a well known diabetogenic agent that is used to induce type- 2 diabetes in experimental animals^[2, 3]. One of the most potent methods to induce experimental diabetes is chemical induction by Alloxan^[4].

Even though, literature reports indicate that the *Murraya koenigii* plant possess hypoglycaemic property, the plant has not been subjected to scientific investigation. This plant leaves are used in the present research study to check the effectiveness of the drug in the treatment of Alloxan induced diabetes, on blood sugar levels in experimental albino rat model.

Materials and Methods

Chemicals

Alloxan monohydrate ((Hi- Media Lab Pvt. Ltd., Mumbai), Tab Glimiperide 2mg (ABEPRIDE), glucometer, chloroform.

Animals

Both sexes of the animals (Albino rats) with a weight between 150gm – 250 gm were used in this experiment. Albino rats were housed in a group of 6 in polypropylene cages at a controlled room temperature of 25 ± 2 °C, and a relative humidity of 55% & 12 hrs. light: dark cycle. Albino rats were fed with the supplied standard food pellet diet & water ad libitum during this experiment. Prior to this experiment the wistar rats were fasted for a exact time period of 12 hrs with water ad libitum given & weighed. All the study protocols of this research study were cleared and approved by CPCSEA(Committee for the Purpose of Control & Supervision of Experiments on Animals) & were cleared by Institutional Animal Ethics Committee (IAEC) clearance at Mamata Medical College, Khammam, Telangana state.

Plant Material and Preparation of Test Extract

The *Murraya koenigii* leaves was collected from local regions near Khammam city at rotary nagar in the month of October – November and authenticated by Assistant Professor and Head, Department of Botany, Govt. SRBJNR PG College, Khammam. The collected plant leaves were thoroughly washed with distilled water & shade dried, powdered and stored in air tight containers. Extract was prepared by using chloroform following the method of Bakus *et al.*, (1981) with certain minor modifications. A crude residue (5.98g) was obtained giving a yield of 1.19%.

Experimental Induction of Diabetes

Rats were fasted overnight and blood is withdrawn from tail vein of the rats of each group before the treatment i.e (initial) day (0 day) and on 7th, 14th, 21st day of the given test drug MKLCE from rats of each group using glucometer and FBS levels were analyzed and were observed. Serum of collected blood was used for estimation of biochemical parameter like blood sugar. Animals were fasted upto 18 hrs. before Diabetes induction. A single dose (120 mg/kg, b.w., i.p.) of alloxan monohydrate was dissolved in normal saline was used for induction of type -2 diabetes in rats after overnight fasting. After 72 hrs of alloxan administration, the animals were fed standard pellets and water ad libitum. The animals were stabilized for a week and animals showing blood sugar level >250 mg/dL were selected for the study. Fasting blood sugar [FBS] level was monitored in blood samples with a glucometer before administration of the drugs.

Experimental study Design

30 albino rats were divided into 5 groups (A, B, C, D, E) of 6 animals (n=6) each. Group A served as normal control or non-diabetic received vehicle (1ml/kg in 0.5% sodium CMC) orally for 21 days on routine diet. Group B was served as diabetic control received single dose of alloxan monohydrate (120 mg/kg, b.w., i.p.) dissolved in sterile 0.9 % normal saline was used for type 2 diabetes induction in rats after overnight fasting. Where as the Group C, D(Experimental Groups) were treated as Diabetic and received alloxan (120 mg/kg, b.w., i.p.) with MKLCE suspension at a doses of (250, 500 mg/kg b.w. in 0.5% sodium CMC)/day respectively) for 21 consecutive days. Group E was received alloxan (120 mg/kg, b.w., i.p.) plus suspension of Glimiperide (2mg/kg b.w. in 0.5% sodium CMC) orally for 21 consecutive days. After 30 minutes of treatment, rats of each group were given glucose (5gm/kg) in distilled water orally. Blood samples were collected on day before the experiment i.e. (0 day (initial day) of the experiment, and experimental days like on day 7th,14th, 21st days of the given test drug MKLCE from rats of each group with a glucometer and blood sugar levels were analyzed and blood sugar(FBS) values were observed.

Statistical analysis

Results of biochemical estimation like blood sugar are reported as mean \pm SD of six animals in each group (n=6). The data were subjected to one-way analysis of variance (ANOVA) for multiple comparisons followed by Dunnett's test was applied for determining statistical significance of difference in blood serum glucose. P values of less than 0.05 ($p < 0.05$) were considered statistical significant.

Results

The present investigation revealed that the MKLCE has antidiabetic activity against alloxan induced diabetic albino rats on intra peritoneal injection of alloxan at a dose of 120mg/kg body weight. This study also confirms that on intra peritoneal injection of alloxan at a 120mg/kg b.w. dose causes significant raise in blood sugar level in untreated albino rats (diabetic control) groups when compared to control group was shown in (Table 1). Treatment of diabetic rats with MKLCE for 21 days caused dose dependent fall in blood sugar levels (FBS) in diabetic albino rats. Glimiperide treated diabetic rats also showed significant ($P<0.00$) decrease in blood sugar levels after 21 days of treatment as shown in (Table 1)



Fig 1: *Murraya koenigii* leaves

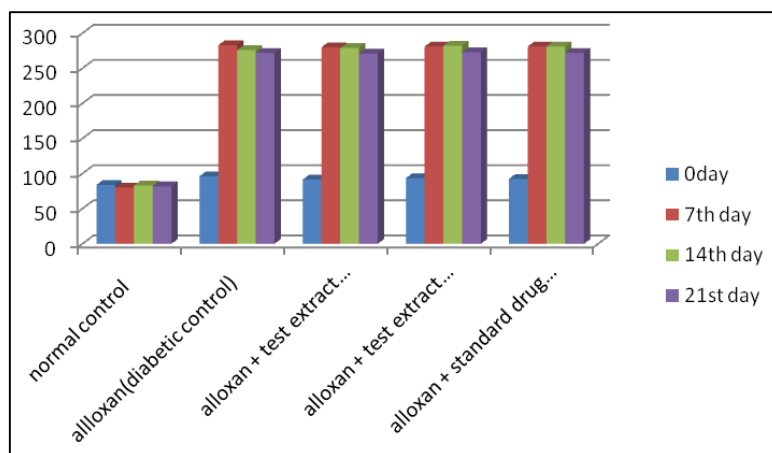


Fig 2: Effect of *Murraya koenigii* leaves chloroform extract (MKLCE) on fasting blood sugar levels in alloxan induced diabetic albino rats.

Group treatment Fasting blood serum glucose (mg/dl)

Table 1: Effect of *Murraya koenigii* leaves chloroform extract (MKLCE) on fasting blood sugar levels in alloxan induced diabetic albino rats.

	0 th day	7 st day	14 th day	21 st day
Normal control group (given only (1ml/kg in 0.5% sodium CMC))orally	84.6±1.8	80.8±1.3	83.83±2.4	82.83.7±2.1
Diabetic control (given saline 10 ml/kg + Alloxan (120 mg/kg) in a single i.p. dose	96.3±7.4	283.8±5.1	276.3±4.3	272.7±3.9
Diabetic+ <i>Murraya koenigii</i> leaves chloroform extract (MKLCE) (250mg/kg/ b.w. /day) orally	91.5±5.6	280.8±6.3*	279.8±6.4**	271.5±2.6**
Diabetic+ <i>Murraya koenigii</i> leaves chloroform extract (MKLCE) (500mg/kg/ b.w. /day) orally	93.5±5.6	281.2±5.3*	282.3±5.6**	273.3±3.2**
Diabetic+standard drug glimiperide(2mg/kg b.w.day) orally	92.5±5.6	281.8±6.3*	281.3±7.0**	272.5±2.6**

All values are expressed in Mean±SD. Analyzed by one way ANOVA followed by Dunnet’s test for multiple comparison tests. * = $p<0.05$ when compared to normal control group (significant p value). ** = $p<0.00$ when compared to diabetic control group (Highly significant p value).

Discussion

Chemically, alloxan (2, 4, 5, 6 tetraoxyypyrimidine) is an oxygenated pyrimidine derivative & a cyclic urea analog which was reported to produce permanent diabetes in experimental animals such as rabbits, rats. It is a well Known diabetogenic agent that is used to induce type- 2 diabetes in experimental animals [2, 3]. One of the most potent methods to induce experimental diabetes is chemical induction by Alloxan [4]. In the present study, alloxan caused significant reduction in body weight and marked increase in fasting serum glucose levels in diabetic rats. As the alloxan is a highly reactive molecule which is readily reduced to diuleric acid, which is then gets auto-oxidized back to alloxan which ultimately results in the production of free radicals which actually damaged the DNA of β – cells of pancreatic islets & cause cell death [3].

In addition, it has been widely used to produce experimental diabetes in animals such as rabbits, rats, mice and dogs with different grades of disease severity by varying the dose of alloxan used [4, 5].

As the alloxan has been widely accepted that alloxan

selectively destroys the insulin-producing β -cells found in the pancreas, hence it is used to induce diabetes in laboratory animals. The toxic action of alloxan on pancreatic β –cells involve oxidation of essential sulphhydryl (-SH groups), inhibition of glucokinase enzyme, generation of free radicals and disturbances in intracellular calcium homeostasis [6, 7, 8]. Fresh leaves, dried leaf powder, and essential oil are widely used for flavouring soups, curries, fish and meat dishes, eggs dishes, traditional curry powder blends, seasoning and ready to use other food preparations. The essential oil is also utilized by soap and cosmetic aromatherapy industry [9]. Curry leaves are boiled with coconut oil till they are reduced to blanked residue which is then used as an excellent hair tonic for retaining natural hair tone and stimulating hair growth. It is traditionally used as a whole or in parts as antiemetics, anti-diarrheal, febrifuge, blood purifier, antifungal, depressant, anti-inflammatory, body aches, for kidney pain and vomiting [10-22].

Treatment of diabetic rats with chloroform extract of *Murraya koenigii* leaves extract at a dose of 250 mg/kg and 500 mg/kg for 21 days caused significant reduction in fasting blood sugar

level in a dose dependent manner when compared to diabetic control group. Most of the studies of *Murraya koenigii* on diabetes revealed that there is remarkable improvement in the condition of damaged β -cells in histological study of the pancreas of leaves *Murraya koenigii* chloroform extract treated diabetic albino rats. However, the *Murraya koenigii* leaves chloroform extract did not restore the disturbed biochemical parameter like blood glucose levels to normal value in diabetic rats. Hence, the extract can be used in combination with other established anti-diabetic drugs or herbal formulations for more effective outcomes.

Conclusion

In the present research study, our experimental data finding clearly confirmed and also revealed that the test extract of our study, i.e. chloroform extract of the leaves of *Murraya koenigii* extract at a dose of 250mg/kg and 500 mg/kg b.w. possess potent antidiabetic activity. Thus, *Murraya koenigii* leaves can be used as potential antidiabetic drug. It could be a novel antidiabetic agent and also a dietary adjunct for the management of type 2 diabetes and its complications. However, the exact mechanism of the antidiabetic effect of *Murraya koenigii* chloroform extract is unknown. However, further studies are required to identify the probable mechanism of action to establish its antidiabetic effect. Further human studies are necessary and found the active component of *Murraya koenigii* and role of these herbal drugs in controlling diabetes and its complications.

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Declarations

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee.

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