Evaluation of Antidiabetic activity of *Murraya koenigii* on Alloxan Induced Diabetic rats

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ABSTRACT

The *present* study was carried out to evaluate the antidiabetic effect and histological parameters of *Murraya Koenigii* in Alloxan induced diabetic albino rats. The experimental rats weighed 200-250g were induced for diabetes with single dose of alloxan (120mg/kg body weight). Oral administration of chloroform extracts of Murraya leaf (250 and 500mg/kg body weight) for 30 days resulted in significant decrease of blood glucose from 296.62±20.12 to 80.22 ± 03.63 and decrease in the activities of enzymes of liver. To study the histology of *Murraya Koenigii* in Alloxan induced albino rats, sampling and staining of pancreas, spleen, liver and kidney tissues of diabetic and normal rats showed that strong antigenesity in beta-cells of the islets in control. Degenerative and necrotic changes and shrunken tissues in islets of langerhans were observed in diabetic induced group. Majority of the cells are protected from light degeneration when treated with 25 and 50 ml/kg/bw of Murraya and moderate antigenesity was noted in beta-cells of the islets of langerhans of the pancreatic tissue. Diabetic rats treated with murraya *(*25 ml/kg/bw) showed an improvement in the spleen histology and treated with Murraya *(*50 ml/kg/bw) shows a result similar to that of non- diabetic control. The results showed not only significant anti-hyperglycemic effect of Murraya extracts in experimental model of diabetes mellitus but also indicated a dose dependant activity of the extracts.

Keywords: Diabetes, Alloxan, Hypoglycaemic, *Murraya Koenigii*

INTRODUCTION

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion or insulin action, or both. Broad research on diabetes leads to a number of synthetic oral hypoglycemic agents like biguanides, sulphonylureas and thiazolidinedione's being used to treat diabetes. But all have side effects associated with their uses. On other hand, traditional medicinal plants with their various biological constituents have been used effectively by the communities since long time to treat diabetes. 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. In addition, herbal drugs are extensively 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.

Management of this disease may include lifestyle modifications, diet, exercise, long – term use of oral hypoglycaemic agents or insulin therapy. Since ancient times, plants have been an exemplary source of medicine. The search for plants with hypoglycaemic property is an area that draws attention of research workers globally reviewed 45 of such plants and their products that have been used in the Indian traditional system of medicine (Grover *et al.*, 2002). Management of diabetes without any side effect is still a challenge to the medical community. There is continuous search for alternative drugs. Even though herbal medicines have long been used effectively 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 preparations have been used in the treatment of diabetes. 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 the past few years some of the new bioactive drugs isolated from plants showed antidiabetic activity with more efficacy than oral hypoglycemic agents used in clinical therapy (Mohammed *et al*., 2006)

Murraya koenigii (Rutaceae) commonly known as "Curry Patta" (Hindi) is widely used as a spice and condiment in India and other tropical countries. Various parts of *Murraya koenigii* have been used in traditional or folk medicine for the treatment of rheumatism, traumatic injury and snake bite and it has been reported to have antioxidant, anti-diabetic and anti-dysenteric activities. Curry leaf is used traditionally as a stimulant, antidysenteric and for management of diabetes mellitus.

Alloxan (2,4,5,6-tetraoxypyrimidine; 2,4,5,6- pyrimidinetetrone) is an oxygenated pyrimidine derivative and was originally isolated in 1818 by Brugnatelli and got its name in 1838 by Friedrich Wöhler and Justus von Liebig . Alloxan is a toxic glucose analogue, which selectively destroys insulin-producing cells in the pancreas when administered to rodents and many other animal species. This causes an insulin-dependent diabetes mellitus (called "Alloxan Diabetes") in these animals, with characteristics similar to type 1 diabetes in humans (Lenzen, 2008).

Even though, literature reports elsewhere (Khan *et al*., 1995) indicate that *Murraya koenigii* possess hypoglycaemic property, the plant has not been subjected to scientific investigation. Leaves of this plant is used in the present study to effectiveness of the drug in the treatment of Alloxan induced diabetes, on blood glucose, enzymes of liver and histological effects on pancrease, spleen, liver and kidney tissues in experimental rat model.

MATERIALS AND METHODS

Preparation of Murraya Koenigii Extracts

The leaves of *Murraya Koenigii* was collected from kristu jayanti college campus, Bangalore, at an altitude of 920m (3021ft) from the sea level. The collected plant leaves were shade dried, powdered and stored in air tight containers. Leaf powder of *Murraya Koenigii* was extracted with chloroform following the method of Bakus *et al.,* (1981) with certain modifications. A crude residue (5.98g) was obtained giving a yield of 1.19%. The antidiabetic effects were evaluated by intra peritoneal injection of single dose (120mg/kg/b.w) of alloxan to induce diabetes in rats (Ragavan *et al*.,)

Experimental animals

Wistar albino strain of either sex weighing about 150 – 200 g obtained from the Easma institute of Technology, Karur, Tamilnadu were used for the study. They were fed with a standard rat pellet diet (Lakshmi feeds, karur) and Animals were housed in open air cages (60x45x45 cm) at 23 ± 20C temperature with 12h light/ dark photoperiod, and water was provided *adlibitum* and maintained under standard laboratory conditions. (Temperature 24-28°C, relative humidity 60 - 70%) Animals described as fasted were deprived of food for 16 hours but had free access to water. Sigma Chemical Co, (Mumbai) dissolved in sterile saline. Ethical clearance for performing the experiments on animals was obtained from Institutional Animal Ethics Committee (IAEC) Easma institute of technology, Aravakurichy Karur, Tamilnadu

Alloxan Induced diabetes

Alloxan monohydrate (S.D. Fine, Mumbai)was used to induce diabetes by a single ip injection (120mg/kg) Sigma in sterile saline (Ravivijayavargia e tal., 2003) After 72 hours of alloxan injection, the diabetic rats (glucose level > 250 mg/dl) were separated and used for the study(Perfumi *et al*., 1996). Fasting blood glucose [FBS] level was monitored in blood samples using a glucometer before administration of the drugs.

Experimental design

The animals were divided into 4 groups of 6 animals each. Group I served as normal healthy control. Group II untreated diabetic control. Group III diabetic rats were given *Murraya Koenigii* extract (250 mg/kg body weight). Group IV diabetic rats given *Murraya Koenigii* extract (500 mg/kg body weight). The crude extract was administered for a period of 30 days (Ragavan *et al*., 2006).

Specimen Collection

After the experimental regimen, the animals were sacrificed by cervical dislocation under mild chloroform anaesthesia. Blood was collected on decapitation and serum was separated by centrifugation (for 20 min at 2000 rpm). The pancreas, spleen, liver, and kidney tissues were quickly removed, washed in ice cold, isotonic saline and blotted individually on ash-free filter paper and organ weights were measured**.** Organ slices fixed for 48hr in 10% formalin were processed for paraffin embedding following the standard micro technique sections (5mm). All the respective organs were stained with haemotoxylin and eosin are subjected to evaluation for histopathological changes under a light microscope. Histopathological findings were graded for degree of tissue cell damage. The essential features of micro techniques are collection and preparation of material, fixation, dehydration and clearing of material, embedding of material in wax and block making, microtomy, staining and mounting the sections mounted on a slide. Tissues fixed in Bovin's are embedded in paraffin, sectioned, spread over clean slides, deparaffinized and dehydrated were used for histological staining and observation.

The serum and tissues were collected and used for biochemical experiments.

Biochemical parameters

Serum glucose was estimated by GOD/POD method (Brandstrup *et al*. 1957 and Baginsky *et al*. 1992). Glucokinase and Glucose -6-phosphatase were assayed in liver and kidney.

Body weight analysis

Animals were weighed on 0, 7th, 14th and 21st days after diabetes induction to detect any changes in their body weights (Vijayalakshmi e.tal.,2008).

RESULTS AND DISCUSSION

As shown in (Table 1) the levels of glucose in serum of alloxan induced diabetic rats were found to be significantly elevated as compared with control rats. Oral administration of *Murraya Koenigii* leaf extract 250 mg and 500 mg /kg body weight for 30 days showed significant reduction in glucose.

Table 1: Effect of Murraya Koenigii leaf extract on serum glucose, of control and experimental rats.

Parameters	**Group-I Normal control**	**Group-II Diabetic control**	**Group-III Diabetic treated with 250mg /kg/bw**	**Group-IV Diabetic treated with 500mg /kg/bw**
Serum Glucose(mg/dl)	94.23 ± 03.46	296.62±20.12	118.50 ± 20.50	80.22 ± 03.63

Values are expressed as Mean ± SD (n=6)

Administration of Leaf extracts of *Murraya* to diabetic animals increased the activity of glucokinase and Glucose 6 phosphatase in liver were depicted in (Table2). The extract induced decrease in the concentration of blood glucose in alloxan-treated rats may be the result of increased glycolysis. The activity of glucose-6- phosphatase was inhibited after administration of the extracts suggests that glucose -6-phosphate is not utilized for the synthesis of glucose in the glycogenic pathway, but may be used as a substrate for glycogenesis.

Table 2: Effect of Murraya Koenigii on the enzymes in liver of control and experimental rats

Parameters	**Group-I Normal control**	**Group-II Diabetic control**	**Group-III Diabetic treated with 25Micro mol/kg/bw**	**Group-IV Diabetic treated with 50Micro mol/kg/bw**
Glucokinase (Micromol of glucose-6-Po4 formed /min/mg protein)	202.5±5.3	112.3±7.9	160.0±1.2	162.7±4.2
Glucose-6-phosphatase (Micro mol of Pi liberated/min/mg protein)	0.120±0.011	0.242±0.028	0.192±0.006	0.201±0.036

Values are expressed as Mean ± SD (n=6)

Body weight analysis

Reduction in body weight was observed in all the diabetic animals. Table 3 shows the average weekly body weights of both control and treated groups are depicted in (Table-3)Moreover, animals treated with Murraya extract (250 &500 mg/kg) registered a less gradual decrease in body weights on 14th and 21st days of the study. It was noted that there was a significant decrease in body weight, comparison of 250 & 500 mg/Kg/bw of murraya extract treated group on 14th and 21st day, compared to diabetic control group.

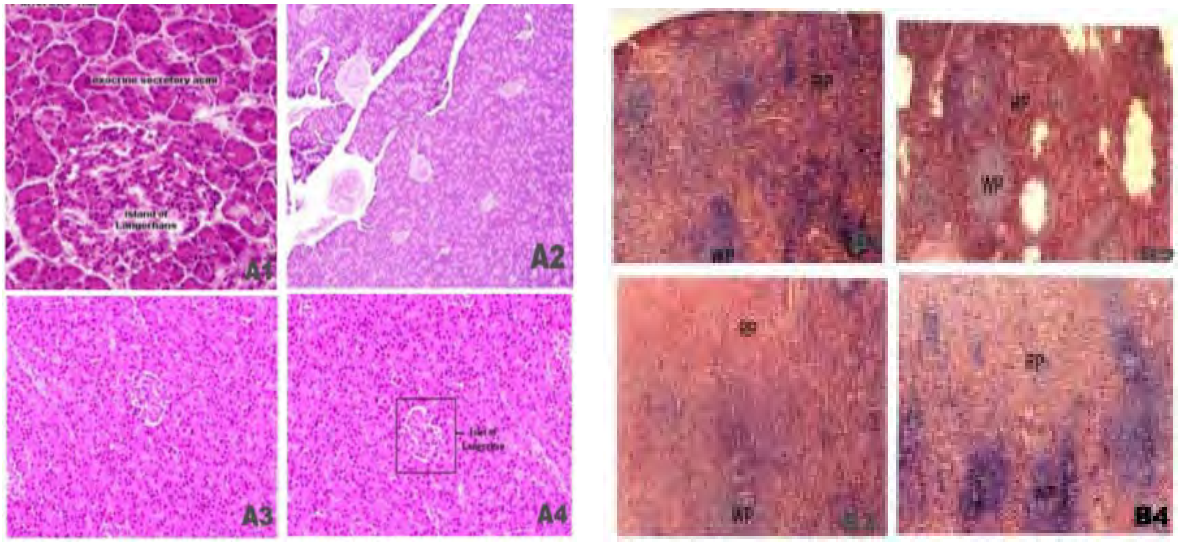
Table 3: Effect of Murraya extract at the dose of 250 and 500 mg/kg on body weight in Alloxan -induced diabetic rats

Groups	0day	7^{th} day	14thday	21^{st} day
Normal control	210.50 ± 7.12	213.00 ± 6.34	214.00 ± 3.85	215.00 ± 5.09
Diabetic control	194.60 ± 6.53	168.50 ± 9.12	143.50 ± 8.85	101.30 ± 8.69
Diabetic treated with 250mg/kg/bw of murraya extract	196.62 ± 5.53	173.50 ± 6.16	160.50 ± 3.85	130.30 ± 6.89
Diabetic treated with 500mg/kg/bw of murraya extract	200.60 ± 31.10	181.0 ± 13.80	168.0 ± 15.47b	141.33 ± 13.60

All values expressed are means ± SD, n=6; a $P < 0.05$ vs normal group; b $P < 0.05$ vs diabetic control group

Pancreas and Spleen histology

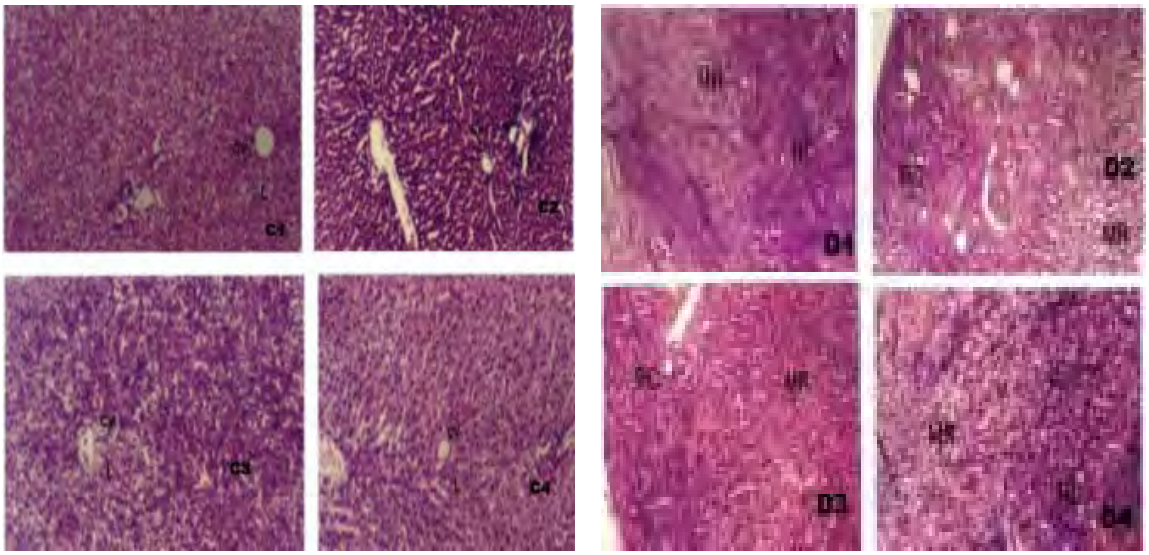
The histology of pancreatic islet cells was normal in control group. In histological sections of pancreatic tissues stained with haematoxylin and eosin were the degenerative and necrotic changes, and shrunken islets of Langerhans was observed in induced group. *Murraya Koenigii* treatment protected the majority of cells of Langerhans islet. In histochemical staining of the pancreatic tissues in diabetic Murraya *treated* rats (25 & 50 ml/kg/bw) there was moderate insulin antigen positivity in the majority of Beta-cells of the islets of Langerhans. Under low magnification view of a spleen section, red pulp and white pulp were observed. Diabetic rat treated with Murraya (25 ml/kg/bw) shows an improvement in the spleen histology. Although the red pulp and the white pulp are not prominent, spleen of diabetic rat treated with Murraya (50 ml/kg/bw) shows a picture similar to that of non-diabetic control (**Plate I -A1, A2, A3& A4 & Plate II-B1, B2, B3, B4**).



Liver and Kidney Histology

Diabetic rats show several alterations when compared to control rats. Mostly destruction of the cells leads to large gaps in between the lobules. The cells appear swollen

and it clearly reveals that cell necrosis and inflammatory infiltration of lymphocytes and kupffer cells have taken place around the central vein. Liver of diabetic rats treated with Murraya *(*25 ml/ kg/bw) shows destruction of cells leading to gap and inflammatory infiltration of lymphocytes and kupffer cells is minimized**.** Liver of diabetic rats treated with Murraya *(*50 ml/kg/bw) extract showed a recovery from the diabetic condition. Diabetic control group shows necrosis in the cortex. Diabetic rat treated with Murraya (25 ml/kg/bw) shows a healing effect on the necrosis. There is a decrease in the number of vacuoles in comparison to diabetic control rats. The cortex of diabetic rat treated with Murraya (50 ml/kg) shows architecture similar to that of the control rat (**Plate III-C1, C2, C3, C4 & Plate IV-D1, D2, D3, and D4**).



CONCLUSION

The results of this investigation indicate that the leaf extracts of *Murraya Koenigii* have a hypoglycemic effect on alloxan-induced diabetes in rats. One possible mechanism of action is increased insulin secretion and enhancement of the glycogenesis process. The extracts were effective in regulating the biochemical indices associated with diabetes mellitus such as activities of glucokinase and glucose-6-phosphatase. Further studies are in progress to isolate the active principle(s) of the extracts as well as to elucidate their exact mechanism of action. Histological studies showed damages caused by alloxan to pancreas, spleen, liver and kidney. *Murraya Koenigii* shows protective effects in experimental diabetes, possibly by decreasing Oxidative stress and preservation pancreatic âcell integrity. But to elucidate the exact mechanism of this modulatory effect, bioactive compounds and to examine its potential therapeutic effects further studies are essential.

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