

Effect of murraya koenigii, catharanthus roseus and psidium guajava leaves extract on blood glucose in alloxan induced diabetic rats

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Abstract

The present work was carried out to observe the hypoglycemic activity of Murraya koenigii, Catharanthus and psidium guajava leaves extract in alloxan induced diabetic rats. For this purpose 50 rats were divided into five groups of 10 rats each. The group 1 was control and given normal feed. The group 2 was made diabetic (by alloxan) injection and given normal feed. Group 3 was diabetic given normal feed and treated with Murraya koenigii leaves extract. Group 4 was also diabetic, given normal feed and treated with Catharanthus roseus leaves extract. Group 5 was diabetic, given normal feed and treated with Psidium guajava leaves extract. All the rats were given herbal extracts 200 mg//kg body weight/ day for 42 days. It was observed that Murraya koenigii and Catharanthus roseus decreased blood sugar level more than Psidium guajava. The results were subjected to statistical analysis using SPSS (ANOVA & Mean \pm SE) according to (1), and revealed that Murraya koenigii, Catharanthus roseus and Psidium guajava leaves extract have blood sugar lowering effect significantly higher(P<0.05) in diabetic rats than normal ones who were not given herbal extract.

Keywords

Diabetes, Murraya Koenigii, Catharanthus, Psidium Guajava, Alloxan

1. Introduction

Diabetes mellitus is an endocrine disorder affecting nearly 10% of the population all over the world (2). Diabetes is one of the leading cause of death in humans and animals. In animals it occurs mostly in dogs with an incidence of approximately 0.2%. In the indigenous Indian system of medicine, a number of plants were claimed to be beneficial for treatment of diabetes and some of them have been experimentally approved and active principles were isolated (3).

Catharanthus roseus(Sada Bahar in urdu) belongs to Apocynaceae family & is known with various names in India and all over the world. Hot water decoction of the leaves or the whole plant is used for the treatment of diabetes in several countries i.e. Brazil, Cook Islands, Dominica, England, Jamaica, Mozambique, Pakistan, Taiwan, Thailand and West Indies (4). In a study, researchers found that an aqueous extract could lower blood glucose about 20% in diabetic rats. Dichloromethane and methanol extracts (more similar to ethanol as a solvent) lowered blood glucose 49-58%, significantly better than in control rats, but not used in humans due to unknown reasons and aqueous extract is preferred (5). The active alkaloid ingredients such as Vinblstine and vincristine from its roots are also used in cancer treatment (6).

Psidium guajava is commonly known as guava, guyava and kuawa (amrood in urdu). The place of origin of the guava is uncertain but needs a tropical environment and full sun. This small evergreen tree belongs to the *Myrtaceae* family and has been claimed to be useful in the treatment of diarrhea, dysentery and acute gastrointestinal inflammation (7; 8). Leaves of this plant have been reported to contain several compounds such as terpenoids (9; 10) flavonoids (11) and tannins (12).

*Murraya koenigii (*Curry-leaf Tree in urdu) is a tropical to subtropical tree in the family Rutaceae, which is native to India, commonly known as curry leaf. It is a medicinal plant that grows throughout the South East Asia (13). The aqueous extract of Murraya koenigii leaves has been tried to evaluate the hypoglycemic activity in normal and alloxan induced diabetic subjects. The scientific evaluation of its hypoglycemic activity was therefore explored (14).

The leaves retain their flavour even after drying and hence these are marketed both in fresh and dried forms. There is not much loss of volatile oil during drying either in sun/shade or in cross flow dryer. Oven drying at 50°C is recommended as the best technology for conversion of fresh leaves into dry form (14).

2. Materials and Methods

The whole experimental work was carried out at the Institute of Molecular Biology and Biotechnology, The University of Lahore and some confirmatory analytical work was performed at Fatima Memorial Medical College, Labs, Lahore, Pakistan.. *Murraya koenigii, Psidium guajava* and *Catharanthus roseus* leaves were purchased from the local market which were cleaned, dried and finely powdered.

2.1. Extraction of Plant Material

Dried powder of *Murraya koinigii* leaves (500 g), *Psidium guajava* leaves (500 g) and *Catharanthus roseus* leaves (500 g) was taken and 1000 ml. of distilled water was added in each and boiled and filtered. The final concentration of each extract was 750mg/5ml. The filtrate obtained served as crude extract and administered orally to the experimental rats at the concentration of 200mg/kg body weight/ day for 42 days (15).

2.2. Animals

50 Albino rats (Ratis norvegicus) weighing between 175g to 250 g were included in the study and divided randomly in five groups of 10 rats in each group.

2.3. Induction of Diabetes

Alloxan is known as a useful drug to induce diabetes in experimental animals (16). After overnight fasting diabetes was induced in rats by intraperitoneal injection of Alloxan dissolved in 1ml distilled water at a dose of 65mg/kg body weight. This induces diabetes in 2 to 6 days and after 7 days, the rats which have blood glucose more than 150 mg/dl are considered diabetic and were used for further experiments (17). The treatment with *Murraya koenigii*,(*MK*) *Psidium guajava*(*PG*) and *Catharanthus roseus*(*CR*) leaves extract was started on 8th day after Alloxan injection and considered as the first day of treatment and day zero indicates the day before alloxan injection. The treatment continued for 42 days.

2.4. Experimental Design

The rats were divided into five groups and with 10 rats in each group (Table: 1). The data thus obtained was subjected to statistical analysis using SPSS (ANOVA and interpretation with Mean \pm SE.

Groups	Animals Conditions	Treatment
1	Non-diabetic	Normal Feed.
2	alloxan Diabetic	Normal Feed.
3	alloxan Diabetic	Normal Feed + Leaves Extract of <i>Psidium guajava</i> (200mg/kg B.W).
4	alloxan Diabetic	Normal Feed + Leaves Extract of <i>Catharanthus roseus</i> (200mg/kg B.W).
5	alloxan Diabetic	Normal Feed + Leaves Extract of <i>Murraya koenigii</i> (200mg/kg B.W).

2.5. Collection of Blood Samples

1ml of blood was collected from coccygial vein of each rat and was transferred into sample tubes for analysis. The serum was obtained by centrifuging each blood sample at 3000rpm for 10 minutes and stored at 1-4 C^0 till analyzed.

3. Results

The present research work was conducted to observe the hypoglycemic effect of *Murraya koenigii, Catharanthus roseus* and *Psidium guajava* leaves extract on serum glucose of diabetic rats. The change in glucose level of rats serum due to the herbal treatmentss for 42 days is presented in table 2. The collected data was statistically analyzed using SPSS(ANOVA & Mean±SE).

The 1st. group was control and given normal feed. In this group the mean glucose level was 111.37±4.30 mg/dl at 0 day, 109.25±3.01 mg/dl at 1st day, 109.12±3.31 mg/dl at 21st day, and 110.25±4.59 mg/dl at 42nd day and the differences were non significant(P>0.05). (table 2). The 2nd group was diabetic and was fed normal feed and no treatment. The glucose level of this group was 109.62±4.47 mg/dl at 0 day (before induction of diabetes with alloxan), 178.50±4.56 mg/dl at 1st day, 179.12±6.24 mg/dl at 21st day and 172.12±3.97 mg/dl at 42nd day. The rats were made diabetic but not given any treatment. The serum glucose levels (table 2) were significantly higher (P<0.05) when compared to control as the values were elevated from 109.62±4.47 to 178.50±4.56 on day 1, 179.12±6.24 on day 21 and 172.12±3.97 on day 42. The differences between

diabetic rats, serum glucose did not show any significant difference(P>0.05). The 3rd group was diabetic, fed with normal diet and treated with Murrava koenigii leaves extract at the dose of 200 mg//kg body weight. The serum glucose levels of this group were 109.87±3.58 mg/dl at 0 day, 178.87±4.58 mg/dl at 1st day, 146.12±8.18 mg/dl at 21st day and 111.37±2.87 mg/dl at 42nd day. This showed that Murraya koenigii leaves decreased the serum glucose level in diabetic rats from 178.87±4.58 on day 1 to 111.37±2.87 on day 42. This revealed significant decrease(P<0.05) in serum glucose in diabetic rats due to effect of Murraya koenigii leaves treatment and these levels were significantly higher (P<0.01) than control (table 2). The 4th group was diabetic, fed with normal diet and Catharanthus roseus leaves extract at the dose of 200 mg//kg body weight. The glucose levels of this group were $109.50\pm4.20 \text{ mg/dl}$ at 0 day, $182.25\pm5.52 \text{ mg/dl}$ at 1st day, 151.00±4.84 mg/dl at 21st day and 110.87±3.35 mg/dl at 42nd day. This showed that *Catharanthus roseus* leaves decreased the serum glucose level in diabetic rats from 182.25±5.52 on day 1 to 110.87±3.35 on day 42 which revealed significant decrease(P<0.05) in serum glucose in diabetic rats due to effect of Catharanthus roseus leaves treatment. Elisa et al(6) also reported that C.Roseus aqueous extract at 250mg//Kg body weight showed significant (P< 0.05) reduction in blood glucose levels in normal and diabetic mice. These findings are in agreement with the present results (table 2). Similar results were reported by Scopus et al.(20) who studied effect of C. Roseus methanol extract on alloxan induced diabetic rate and reported significantly(P<0.05) reduction in serum glucose levels as compared to control rats .The 5th group was also diabetic, fed with normal feed and treated with Psidium guajava leaves extract at the dose of 200 mg/kg body weight. The glucose levels of this group were $111.12\pm4.48 \text{ mg/dl}$ at 0 day, $178.62\pm6.11 \text{ mg/dl}$ at 1st day, 158.37±3.24 mg/dl at 21st day and 114.87±3.31 mg/dl at 42nd day. This showed that *Psidium guajava* leaves decreased the serum glucose level in diabetic rats from 178.62±6.11 on day 1 to 110.87±3.35 on day 42. This showed significant decrease in serum glucose in diabetic rats due to effect of Psidium guajava leaves treatment. These levels were significantly higher than control (table 2).

Table2. Serum Glucose level of rats (mg/dl) with different treatments

Crowne	Glucose Level (mg/dl) Means±S.D				
Groups	Zero Day before Alloxan	Day1 (after Alloxan)	Day21	Day42	
1. Non-diabetic (control) normal feed	111.37±4.30	109.25±3.01	109.12±3.31	110.25±4.59	
2. Diabetic+ Normal feed	109.62±4.47	178.50±4.56	179.12±6.24	172.12±3.97	
3. Diabetic, Normal feed +200ml//Kg BW, MK	109.87±3.58	178.87±4.58	146.12±8.18	111.37±2.87	
4. Diabetic, Normal feed +200ml//Kg/ BW, CR	109.50±4.20	182.25±5.52	151.00±4.84	110.87±3.35	
5. Diabetic, Normal feed +200ml//Kg BW, PG	111.12±4.48	178.62±6.11	158.37±3.24	114.87±3.31	

MK (Murraya koenigii), CR (Catharanthus roseus), PG (Psidium guajava)

4. Discussion

Diabetes mellitus is one of the major metabolic disorders, afflicting a large proportion of the population all over the world (18). *)*. Present number of diabetics worldwide is 150 million and this is likely to increase to 300 million or more by the year 2025 (19). Reasons for this rise include increase in sedentary lifestyle, consumption of energy rich diet, obesity, higher life span etc (, 20).

The primary objectives of search for different types of drugs for the treatment of all types of diabetes include alleviation of symptoms of hyperglycemia, prevention and treatment of associated complications and disorders, improvement of the quality of life and hence reduction in mortality caused by the disease (21). Diabetes mellitus is a major cause of disability and hospitalization and it results in significant financial burden (22.).The increasing incidence of diabetes represents an enormous socioeconomic burden in the developing countries. The World Heath Organization estimates that over 300 million people worldwide will have diabetes mellitus by the year 2025 with alarming proportions from developing countries

(23.). The present work was focused on hypoglycemic effect of Murraya koenigii, Catharanthus roseus and Psidium guajava leaves extract in alloxan induced diabetic rats. For this purpose 50 rats were divided into 5 groups with 10 rats in each group. The group 1 was control and given normal feed. The group 2 was diabetic and was given normal feed. The group 3 was diabetic and treated with Murraya koenigii leaves extract. Group 4 was diabetic and treated with Catharanthus roseus leaves extract. Group 5 was also diabetic and treated with *Psidium guajava* leaves extract. According to the results presented in table 2, it was observed that the diabetic rats who were fed Murraya koenigii at the dose of 200 mg/kg body weight for the period of 42 days showed significant decrease(P<0.05) as compared to day 1 (table 2). Although the serum glucose levels were significantly higher on day 1 and day 21, but on day 42 it was reduced almost similar to 0 day level when they were non diabetic. This showed that Murraya koenigii has hypoglycemic effect on diabetic rats. These findings are correlated with the findings of Foster (24) who reported the hypoglycemic effect of aqueous leaf extract of Murraya koenigii in normal and alloxan- induced diabetic rats. The extract was administered orally at 100 mg/Kg, 150 mg/Kg and 200 mg/Kg bodyweight to respective groups of animals (Groups I, II and III) for seven days. Group IV received normal saline and served as control for the normal rats. For the alloxan-induced diabetic rats, the same dosage pattern was administered to three groups of rats for seven days (Groups V, VI and VII) while group VIII which received normal saline served as control. According to the results of this trial presented in table 2, it was observed that the diabetic rats who were fed Catharanthus roseus at the dose of 200 mg/kg body weight for the period of 42 days showed that the serum glucose level in diabetic rats significantly decreased from day 1 to day 42 (table 2). Although the serum glucose levels were significantly higher on day 1 and day 21, but on day 42 it was reduced almost similar to 0 day level when they were non diabetic. This showed that *Catharanthus roseus* has hypoglycemic effect on diabetic rats. These findings are in agreement with the findings of Prasad et al (25). According to the results presented in table 2, it was observed that the diabetic rats who were fed Psidium guajava at the dose of 200 mg/kg body weight for the period of 42 days, the serum glucose level decreased from day 1 to day 42 (table 2). Although the serum glucose levels were significantly higher on day 1 and day 21, but on day 42 it was reduced almost similar to 0 day level when they were non diabetic. This showed that Psidium guajava has hypoglycemic effect on diabetic rats. These findings are correlated with the findings of Prasad et al (25).

The rat is genomically very close to human beings and these herbal treatments may be effective in reducing serum glucose levels in diabetic humans and for this purpose further such trials should be conducted before the extract is recommended for treatment of diabetes in human beings.

The initial research is always done on laboratory animals to determine the direction of medicinal effect because no new medicine can be tried on human beings without knowing its significance and side effects. The rats being genomically more than 90% close to human beings, thus were selected for this study because the research was focused on human diabetes treatment. After a few more studies the results will be discussed in scientific conference for trials on human volunteers.

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