Screening of Anti Diabetic Potential of Zizyphus Xylopyrus Fruits in Type-2 Diabetic Animal Model

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ABSTRACT
India has a century old tradition of using medicinal plants and herbal medicines for the alleviation of various diseases and ailments. Zizyphus, locally known as sider or ber is a multipurpose tree species used for food, fodder, medicine and desertification control in arid lands. Zizyphus xylopyrus is known for its medicinal properties for sore throats, dysentery, inflammation of the uterus and anti depressant etc. in all over world. This research was undertaken to test the effect of zizyphus xylopyrus fruits extract on the diabetes of rat. Zizyphus xylopyrus fruits extract (100 mg/kg, 200 mg/kg) was administered orally to rat with the help of oral feeding needle. A total of 84 rats divided in 14 groups. Alloxan induced diabetes model was used in whole study. In this research fruit extract was significantly ($P<0.001$) reduce blood glucose level in alloxan induced diabetic rats. It also decrease total cholesterol level and triglyceride level. This drug was also increase ($P<0.001$) total protein level.

KEYWORD
Rhamnaceae, Diabetes, blood glucose, zizyphus xylopyrus.

1. INTRODUCTION
Zizyphus xylopyrus is spiny shrubs and small tress in the buckthorn family,Rhamnaceae.Rhamnaceae is a commonly called "Buck thorn family",with 51 genera and about 850 species of tress,shrubs and some climbers with simple leaves and often bearing thorns[(1)] The fruit is an edible drupe ,yellow brown,red,or black,1-5 cm long often very sweet and sugary. Zizyphus xylopyrus fruits is also called a kat-ber in hindi. The major chemical composition of zizyphus xylopyrus are rich in flavonoids in quercetin, quercitrin, tannin(7.2%),d-7,3,4-trihydroxyflavan-3,4-diol and oleanolic acid.[(2)] Diabetes mellitus is a chronic metabolic disorder,mainly disruption in carbohydrate ,fat and protein metabolism caused by the complete or relative insufficiency of insulin action.Diabetes mellitus is one of the most common chronic diseases in the whole world. it is a complex ,multifactorial disease which affects the quality,quantity and style of an individual's life. Diabetes mellitus is characterized by abnormal insulin secretion,derangement in carbohydrate and lipid metabolism and is diagnosed by presence of hyperglycemia.[(3-4-5)] Oxidative stress which defined as an imbalance between the generation of oxidants and antioxidants defence capacity of the body is suggested as a

mechanism underlying diabetes and diabetic complications like many other diseases[6-7] Zizyphus xylopyrus fruits for antidiabetic activity using alloxan induced diabetes model.

2. MATERIALS AND METHODS

Plant material

Fruit of zizyphus xylopyrus were collected from the month of march and got authenticated from department of botany. Extract Of these fruits was administered in different concentration daily for duration of 15 days to rats with the help of a oral feeding needle.

Animal

Wistar rat (150-200g) were group housed (n=6) under a standard 12 hour light/dark cycle and controlled condition of temperature and humidity[8] Rats Received standard rodent chow and water ad libitum. rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All The experiments were carried in a noise free room between 08.00 to 15.00 h.separate group (n=6) of rats was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee(IAEC),constituted for the purpose of control and supervision of experimental animal by Ministry of Environment and Forests, Government of India, New Delhi.

Drug

Alloxan monohydrate, Normal saline, Glibenclamide and Zizyphus Xylopyrus . Normal saline (0.5 ml distilled water/day/rat), Glibenclamide(600 µg/kg) and zizyphus xylopyrus extract (100 mg and 200 mg per kg body.wt) were administered for 15 successive days to rats. Biochemical studies were carried on after 15 day of treatment.

Hypoglycemic Activity in Rats

Animals were divided into three groups of 6 rats each.

Group I: Rats served as normal-control and received the vehicle (0.5 ml distilled water/day/rat).
Group II: Rats (normal) were administered ZX (100 mg/kg.body wt/day) in distilled water as a fine aqueous suspension orally for 7 days.
Group III: Rats (normal) were administered ZX (200 mg/kg body wt/day) in distilled water as a fine aqueous suspension orally for 7 days.

Induction of Experimental Diabetes in Rats

After fasting diabetes was induced by a single intraperitoneal injection of 120 mg/kg.body weight of 'Alloxan monohydrate' in distilled water. The animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycemia. These animals were

tested for diabetes after 15 days and animals with blood glucose (fasting) were selected for experimentation.

Experimental Design

Hypoglycemic Activity in Rats- Animals was divided into five groups of 6 rats each.

Group I: Rats served as normal-control and received the vehicle (0.5 ml distilled water/day/rat).

Group II: Rats served as diabetic-control and received the vehicle (0.5 ml distilled water/day/rat).

Group III: Rats (Diabetic) were administered ZX (100 mg/kg body wt./day) in distilled water as a fine aqueous suspension orally for 15 days.

Group IV: Rats (Diabetic) were administered ZX (200 mg/kg body wt./day) in distilled water as a fine aqueous suspension orally for 15 days.

Group V: Rats (diabetic) were administered glibenclamide (600 µg/kg) in distilled water as a fine aqueous suspension orally for 15 days.

Biochemical Determinations

After 15th day of treatment, blood was collected from the retro orbital sinus of overnight fasted rats. The serum was separated and triglycerides and cholesterol level were determined by using, triglycerides test kit and cholesterol test kit (Span diagnostic Ltd., Surat) respectively. And total protein is determined by folin- ciocaltue reaction, In this reaction, a deep blue colored compound is formed when a phenol reagent is added to alkalinized protein solution.[9]

Statistical Analysis

The data were expressed as mean ± SEM. The data of hypoglycemic activity, oral glucose tolerance test and anti diabetic activity were analyzed by one way analysis of variance (ANOVA) followed by "Tukey's post hoc test." *p* value less than 0.05 was considered as statistically significant.

Result of Anti Diabetic study

Table 1: Effect of Zizyphus xylopyrus on hypoglycemic activity in normal rats

Group	Treatment	Hypoglycemic (mg/dl)	
		Onset of study	End of study
I	Normal	91.11±1.88	93.13± 2.16
II	Zizyphus xylopyrus (100 mg/kg)	93.14±2.98	95.10±1.76
III	Zizyphus xylopyrus (200 mg/kg)	90.32±1.65	92.11±0.97

Value are expressed as mean +- S.E.M.(n=6). Values are statistically significant at *P<0.001vs normal group;(one way ANOVA followed by Tukeys post hoc test).

Table 2: Effect of Zizyphus xylopyrus treatment on blood glucose (mg/dl) in normal and Diabetic rats

Group	Treatment	Blood Glucose(mg/dl)	
		Onset of study	End of study
I	Normal	101.16±3.36	105.0±5.61
II	Diabetic control	249.81 ± 2.0	260.11±2.41***
III	Zizyphus xylopyrus (100 mg/dl)	243.03±2.5	184.80±2.59
IV	Zizyphus xylopyrus (200 mg/dl)	251.07±2.7	163.76±2.78
V	Glibenclamide (600µg/kg)	249.10±1.8	121.80±2.59

Value are expressed as mean ± S.E.M (n=6). Values are statistically significant at *P<0.001vs normal group; ***p<0.001vs diabetic control group (one way ANOVA followed by Tukeys post hoc test).

Table 3: Effect of Zizyphus xylopyrus treatment on biochemical parameters in normal and Diabetic rats

Group	Treatment	TC(mg/dl)	TG(mg/dl)	Total protein(g/dl)
I	Normal	91.12±3.12	78.16±6.12	8.60±0.10
II	Diabetic Control	189.11±9.00	121.01±10.5	6.02±0.10
III	Zizyphus xylopyrus (100mg/kg)	116.14±5.12***	91.23±5.19***	7.00±0.19
IV	Zizyphus xylopyrus (200mg/kg)	106.5±6.10***	88.53±8.15***	7.70±1.10
V	Glibenclamide (600µg/kg)	101.23±7.43***	82.04±7.20***	8.04±1.00***

TC- Total Cholesterol, TG- Total Triglycerides

Value are expressed as mean ± S.E.M (n=6). Values are statistically significant at ***P<0.001vs normal group; ***p<0.001vs diabetic control group (one way ANOVA followed by Tukeys post hoc test).

3. CONCLUSION

At the end of the study it can be concluded that aqueous root extract of zizyphus xylopyrus has hypoglycemic effect in diabetic rats and it does not have hypoglycemic action in normal rats. The hypoglycemic activity is comparable to that of Glibenclamide in diabetic rats, we can say that intake of this plant product may help not only in glycaemic control but also in minimizing the complications associated with diabetes; our study provides a way to study the anti diabetic study of the extract for the development of ant diabetic formulation. In our study the biochemical parameters are significantly reduced which may be helpful in diabetic complications.

4. REFERENCES

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