EVALUATION OF ANTIDIABETIC EFFECT OF METHANOLIC EXTRACT OF INULA RACEMOSA ROOT IN RATS.

H. B. Ajani[1]*, H. P. Patel[2], G. B. Shah[2], S. R. Acharya[2] and S. K. Shah[2]

[1] National Institute of Pharmaceutical Education and Research, India.

[2] K. B. Institute of Pharmaceutical Education and Research, Gujarat, India.

Summary

To evaluate the antidiabetic effect of methanolic extract of Inula racemosa root in rats. The effect of the chronic treatment of (28day) methanolic extract of Inula racemosa (syn.: Puskarmoola) (300 mg/ kg, p.o.) roots on alloxan-induced hyperglycaemia was investigated in Wistar albino rat. Along with blood sugar level, the effect on MDA, SOD and GSH levels of liver, kidney and heart homogenate were determined. Chronic treatment with the methanolic extract of Inula racemosa resulted into significant reduction in blood sugar level. Also the MDA levels were found to be higher and SOD and GSH levels were found to be lower in drug treated animals than in control animals. The food intake, water intake and urine output was found to rise whereas body weight find to fall in drug treated hyperglycaemic animals. However, in the animals such rise was not observed in all these parameters. On the basis of these findings, it can be assumed that the methanolic extract of roots of Inula racemosa possess significant hypoglycaemic and antioxidant property in alloxan induced hyperglycaemia modal in rats.

Keywords: *Inula racemosa*; Alloxan; Diabetes mellitus; Antioxidant activity.

***HARESH AJANI:** Department of Pharmacoinformatics, National Institute of Pharmaceutical Education and Research, 4 Raja S.C. Mullick Road, Kolkata-700032, India. Mobile No:-91-9925522578

Email Id: - ajani_haresh@yahoo.co.in

Introduction

Diabetes mellitus is a chronic metabolic disease with different aetiologies. It is characterized by derangements in carbohydrate, protein and fat metabolism resulted in high blood glucose levels which may be due to insulin deficiency or insulin resistance.[1,2] It is ranked seventh among the leading causes of death and third when all its fatal complications are taken into account. The disease becomes a real problem of public health in developing countries, where its prevalence is increasing steadily[3] but adequate treatment is often expensive or unavailable. Currently available therapy for diabetes include insulin and various oral anti-diabetic agents such as sulfonylurea, metformin, α- glucosidase inhibitors, troglitazone, etc. Which are used as alone or in combination? But each agent suffers from a number of serious adverse effects.[4] Indian traditional medicine is one of the richest medicinal systems among those available around the world[5,6] In accordance with the recommendations of the WHO[7] expert committee on diabetes mellitus, an investigation of anti – diabetic agents of plant origin used in traditional medicine seems important. Many herbs and plant products have been shown to have antidiabetic action.[8]

Inula racemosa (F: Compositae) (Syn.: Pushkarmoola) is an ayurvedic herb. It grows in the hilly regions in the north -western Himalayas in Kashmir.[9] and collected in the spring and autumn.[10] Its roots have been mentioned as bitter, acrid, alterative, aromatic, stimulant and thermogenic.[11] It has been reported to contain inulin, essential oil, sesquiterpene lactones - Alantolactone, isoalantolactone, isoalloalantolactone, dihydroalantolactone, dihydroisoalantolactone, alantodiene, isoalantodiene, and beta sitosterol, daucosterol, inunal, inunolide.[12]

The present investigation was designed to confirm the effect of the methanolic fraction of *I. racemosa* root on alloxan induced diabetic rats. An attempt was also made to elucidate the mechanism of the action for anti-diabetic activity.

Materials and Methods

Animals

Adult albino rats (250-300 g) of Wistar strains were used. All the animals were housed in clean poly-propylene cages. The laboratory was windowless with automatic temperature (22 ± 10 C) and lighting controls (14 hours light/10 hours' dark). Rats were fed with commercial pelleted rats chow (M/S Pranav Agro Private Limited, Vaghodiya, and Vadodara) and water ad libitum. Animals described as fasted were deprived of food for 16 h but had free access to water. Before starting the experiment permission from Institutional Animal Ethics Committee (IAEC) was obtained under project no KB 0659.

Plant Material

Roots of I. racemosa (Family: compositae) (Syn.: Puskormoola) were collected from Lalubhai Vrjjlal Gandhi in 2005. The roots were authenticated by Department of Pharmacognosy, K. B. Institute of Pharmaceutical Education and Research, Gujarat University and preserved as voucher specimen no KB/PCOG/0501

Preparation of Extract

Roots of I. racemosa were dried in the laboratory at room temperature and powdered. Powder of these roots (300 gm) was macerated in 70 % methanol in a soxhlet apparatus for 24 hours at < 500c. After 24 hours, mixture was filtered. Extract was dried under vacuum at 500C. (Extraction yield was 28 % w/v.)

Preparation of Drug Solution

A suspension of 25 mg/ml of the methanolic extract was prepared and used for administering the dose of 300 mg/kg p.o. after dilution.

Determination of LD50 of Methanolic Extract.

For LD50 determination, the Organization for Economic co operation and Development (OECD) guideline 425 was followed.

The test substance is orally administered daily in graduated doses to a group of rats containing six animals, one dose level per group for a period of 28 days.

During the period of administration the animals are observed closely, each day for signs of toxicity. Animals which die or are killed during the test are necropsied and at the conclusion of the test surviving animals are killed and necropsied. A 28 day study provides information on the effects of repeated oral exposure and can indicate the need for further longer term studies. It can also provide information on the selection of concentrations for longer term studies. The dose of extract at which 50% animal killed is considered as LD50 dose.[13]

Induction of Experimental Diabetes

Diabetes was induced by a single alloxan monohydrate (170 mg/kg, i.p.) in sterile saline.[14] The induction of hyperglycaemia was confirmed after the 14th day of alloxan treatment by estimation of elevated fasting blood glucose level. Only those rats with blood glucose level > 300 mg/dl were included in the study. The animals were allowed free access to water and pellet diet and maintained at room temperature in poly-propylene cages.

Treatment of Animals

The rats used for the study were divided in to groups of six. Group I consisted of normoglycemic animals. Group II, III, IV consisted of hyperglycemic animal and received distilled water, glibenclamide (5 mg/kg/day, p.o.) and the methanolic extract (300 mg/kg/day, p.o.) respectively. The assigned treatment was given once daily for 28 days.

Measurement of Body Weight, Food Intake and Water Intake and Urine Output.

During the study period of 28 days the body weight, food, water intake and urine output were recorded on daily basis.

Collection of Samples

Blood samples were collected by retro-orbital puncture under light anaesthesia using solvent ether on days 0, 7, 14, 21 and 28 of drug administration for blood glucose estimation.

All the four groups of rats were sacrificed on the last day of the treatment and liver; kidneys and heart were collected and

homogenized. The homogenate was used for the malondialdehyde (MDA), super oxide dismutase (SOD) and reduced glutathione (GSH).

Biochemical Estimations

Determination of Blood Glucose Level

Estimation of blood glucose was carried out by glucose oxidase and peroxidase method using commercially available kit (Span Diagnostic Ltd.). Determination of serum glucose was done at 505 nm on UV-Visible spectrophotometer for all the collected samples and expressed as mg/dl.

Determination of SOD, MDA and GSH levels

Preparation of Sample[15]

Liver, kidney and heart were excised and rinsed with ice-cold saline immediately. Homogenate of these organs were prepared in phosphate buffer to attain the strength of 10%w/v. The homogenate was centrifuged at 1000 g for 20 minutes at 40C. The supernatant was separated out and used for estimation of MDA, SOD and GSH levels.

Estimation of SOD[16]

Supernatant(0.1ml) added to mixture was mixed with 0.1 ml EDTA (1*10-4M), 0.5 ml of carbonate buffer (pH 2.7) and 1 ml of an epinephrine (3*10-3M). Optical density due to formed adenochrome was read at 480 nm for 3 min at an interval of 30 seconds. Results were expressed as U/minute/mg of protein.

Estimation of MDA[17]

Estimation of Malondialdehyde (MDA) was carried out by treating the supernatant with thiobarbituric acid. A secondary product of lipid peroxidation reacts with thio-barbituric acid at pH 3.5. The pink color produced was estimated by measuring the absorbance at 532 nm. The amount of MDA was calculated using molar extinction coefficient of 1.56*10 -4 M/cm-1 and reported as moles of MDA/mg of protein

Estimation of Reduced Glutathione[18]

Reduced Glutathione was estimated using Elleman's reagent (5, 51-dithiobis-2-nitrobenzoic acid) [DTNB]. The sulphydryl groups present in glutathione formed a colored complex with DTNB, which was measured colorimetrically at 412 nm. Standard curve of GSH was prepared using standard glutathione concentrations were determined and expressed as moles of GSH/mg of protein.

Statistical Analysis

The mean and SEM for six observations were calculated for each parameter ANOVAs statistical test was used to determine whether the observations from control and drug treated animals are statically significant at $P< 0.05$.

Results

General Characteristics of Diabetes Effect on Body Weight, Food Intake, Water Intake and Urine Output.

The body weight did not change significantly in diabetic rat treated with either glibencalamide (5 mg/kg/day, p.o.) or methanolic extract (300 mg/kg p.o.) the reduction in body weight was significantly lesser then that observed in the diabetic rats and same as significantly in diabetic rat treated with either glibencalamide (5 mg/kg/day, p.o.) or methanolic extract (300 mg/kg p.o.) Treatment prevented increase food water intake and urine output gradually.

TABLE – I: - Effect of chronic treatment of methanolic extract of roots of I. racemosa on general characteristic of diabetes. (n=6 rats per group)

Group	Normal control	Diabetic control	Diabetic + Glibenclamide	Diabetic + methanolic extract
Food intake (gm)	17.32±1.85	24.18±2.17[1]	20.82±2.06[2]	17.83±0.89[2]
Water intake (ml)	35.77±0.11	43.43±0.41[1]	39.78±1.47[2]	31.37±0.47[2]
Body weight (gm)	292.11±0.57	268.19±1.07[1]	283.61±2.3[2]2	279.82±2.41[2]
Urine output(ml/day/rat)	3.057±0.05	23.00±2.93[1]	4.41±0.13[2]	5.19±0.57[2]

Values are mean ± S.E.M. (n=6); [1]P <0.05 when compared with normal control group. [2]P<0.05 when compared with diabetic group by ANOVA statistical test.

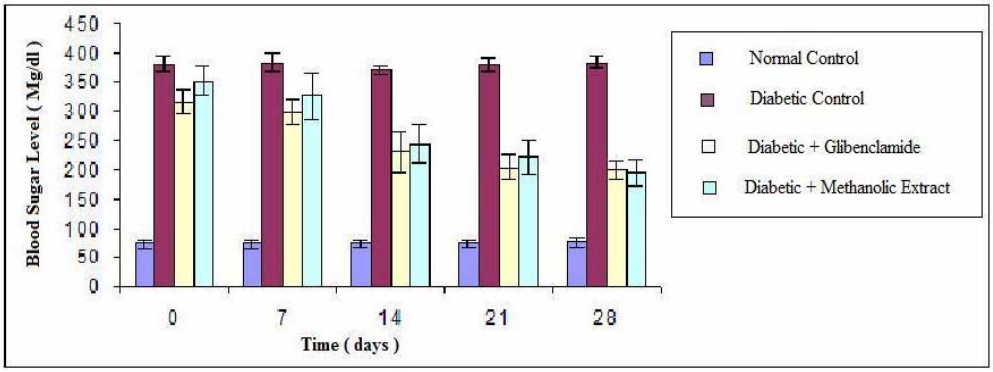
Determination of LD_{50} of Methanolic Extract in Rats.

LD_{50} of methanolic extract was found to be greater than 3 g/kg, when given orally.

Measurement of Blood Glucose Level

Fasting blood glucose levels of diabetic rats were significantly higher than those in normal rats. A significant decrease in blood glucose levels was observed in the diabetic group treated with glibenclamide group from an initial level of 341.25 ± 17.70 to 249.58 ± 13.68 mg/dl and with methanolic extract from 341.10 ± 22.50 to 256.16 ± 21.69mg/dl. (Figure- I)

FIGURE-I: - Effect of chronic treatment of methanolic extract on blood sugar level.



Values are mean ± S.E.M. (n = 6)
P < 0.05 when compared with normal control group.
P < 0.05 when compared with diabetic group. Student's'

Effect on Oxidant-Antioxidant Stress Parameters

MDA level were found to be significantly higher in diabetic rats as compared to that in normal rats. Also GSH and SOD were significantly lesser in diabetic rats. (Table- II).

TABLE – II: Effect of chronic treatment of methanolic extract on oxidant-antioxidant parameters. (n=6 rats per group)

Group	*SOD (units/mg of protein)*			*MDA (moles /mg of protein)*			*Reduced glutathione (moles/mg of protein)*		
	Liver	*Kidney*	*Heart*	*Liver*	*Kidney*	*Heart*	*Liver*	*Kidney*	*Heart*
Normal control	8.16±0.83	4.15±0.56	9.23±0.65	1.54±0.24	0.75±0.02	0.51±0.06	0.71±0.01	0.09±0.02	0.14±0.03
Diabetic control	3.97±0.74[1]	1.76±0.27[1]	5.87±0.32[1]	1.95±0.53[1]	1.01±0.24[1]	1.02±0.21[1]	0.07±0.01[1]	0.06±0.04[1]	0.11±0.07[1]
Diabetic + Glibenclamide	4.68±0.67[2]	2.95±1.51[2]	8.58±0.78[2]	1.31±0.18[2]	0.91±0.15[2]	0.74±0.01[2]	0.07±0.01[2]	0.07±0.01[2]	0.12±0.04[2]
Diabetic + methanolic extract	5.54±0.71[2]	3.88±1.41[2]	8.22±0.47[2]	1.19±0.17[2]	0.94±0.04[2]	0.77±0.03[2]	0.07±0.02[2]	0.07±0.01[2]	0.01±0.01[2]

Values are mean ± S.E.M. (n=6) [1]P <0.05 when compared with normal control group. [2]P<0.05 when compared with diabetic group by ANOVA statistical test

Discussion

Diabetes mellitus is a chronic metabolic disorder posing a major challenge to health status throughout the world. Large number of drugs from herbal origin are claimed to produce beneficial effects in diabetes mellitus. *I. racemosa* has been reported to produce significant hypoglycemic effect.[19] In our study we have studied the antidiabetic activity of *I. racemosa* in alloxan induced hyperglycemia in rats. Chronic treatment of methanolic extract of *I. racemosa* produced significant reduction in blood sugar level in alloxan induced hyperglycemia status in rats. The other parameter such as body weight, Food intake, water intake and urine output affected due to hyperglycemia were significantly reversed to normal by drug treatment. The hyperglycemic effect of *I. racemosa* could be because of increased utilization of glucose by peripheral tissues without affecting insulin secretion.[19] Additionally because of B-blocking activity of *I. racemosa*, it might be sensitizing insulin receptor in the periphery.[20] Recently cortisol lowering activity along with antiperoxidative and hypoglycemic activity has been reported by[21] Many of the complications of diabetes mellitus are associated with free radicals. We therefore assessed the status of antioxidant parameters in various organs due to alloxan induced hyperglycemia and the effect of *I. racemosa* on this parameter. In all the three organs liver, kidney and heart observed the decrease in SOD and GSH levels and increase in MDA due to alloxan treatment. In animals treated for 28 days with the methanolic extract of *I. racemosa* roots we observed significantly higher levels of SOD and GSH and lower levels of MDA than in alone alloxan treated animals. These results are in agreement with the antiperoxidative effect reported by[21] glibenclamide treatment also produced similar result [22] has reported that glibenclamide increase SOD activity in liver and kidney. We further strengthen antioxidant property of this drug. *I. racemosa* possesses large number of sesquiterpene lactones and essential oils. Recently sesquiterpens have shown the great scope for the treatment of diabetes. As *I. racemosa* has sesquiterpene lactones, which are responsible for the antidiabetic and antioxidant property of *I. racemosa.* Many other activities for this plant have been reported which includes antianginal, [23] antiallergic[24] and antimicrobial activity.

Phyotochemical tests showed that the methanol extract of plant *I. racemosa* contains tannins and flavonoids, herbal extracts containing flavonoids and tannins were reported to demonstrate antidiabetic activity.[25] The plant extracts is safe below 300 mg/kg. p.o. doses to the rodents as it not produced any behavioural changes in them.

Previous study reported that the other species such as Inula viscose, Inula japonica, and Inula britannica posses antidiabetic activity. [26, 27, 28]

It can thus be expected that the methanolic extract of roots of *I. racemosa* possess significant potential in treatment of variety of chronic disorders.

In conclusion methanolic extract *I. racemosa* roots possess significant hypoglycemic effect in alloxan treated rats. The antioxidant property could be beneficial in reducing various complications of diabetes mellitus.

Conclusions

The plant extract of roots of Inula racemosa is capable of ameliorating hyperglycemia in alloxan induced diabetic rats as it lowers blood sugar level. Probable mechanism of action for anti - hyperglycemic activity can be its anti-oxidant effect which may be due to presence of rich sesquiterpenes. However, further studies are required to isolate and characterize active sesquiterpenes from roots and their precise mechanism of action as anti-hyperglycemic agent. A potential source for isolation of new orally active agent for diabetes mellitus. Plant could be an antioxidant activity. The result of this study shows that the roots of methanolic extract of I. racemosa prevent alloxan induced diabetes that may be due to antioxidant activity of its compounds.

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