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STUDY OF ANTIDIABETIC AND HYPOLIPIDEMIC ACTIVITY OF *ROSCOEA PURPUREA* (ZINGIBERACEAE)

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ABSTRACT

The concerned study reveals the experimental investigation of the biological activity of *rhizome of Roscoea purpurea* used as a traditional antidiabetic and hypolipidemic agent in past and present culture. To study the effect of *R.purpurea* in both normal and alloxan induced diabetic rats. The methanolic rhizome extract of *R.purpurea* at the dose of 400, 600 and 800 mg kg^{-1} body weight was administered orally once a day to the groups for 30 days. The fasting blood glucose, cholesterol, HDL cholesterol and serum triglyceride content were estimated in both normal and alloxan induced diabetic rats. The fasting blood glucose, cholesterol and serum triglyceride content were found to be significantly reduced($p<0.05$) in treated rats whereas the extract also showed the potent elevation in the level of serum HDL cholesterol. The study reveals that *R.purpurea* has significant antidiabetic activity and a hypolipidemic activity in alloxan induced and normal fasting rats. The extract seems promising for the development of a phytomedicine for diabetes mellitus.

INTRODUCTION

Diabetes mellitus is a chronic disease characterized by insufficient production of insulin by pancreatic glands and decrease in absorption of glucose by the cells in the human systems and result in increase in the concentration of glucose in blood and usually occurs in obese individuals and is associated with hypertension and dyslipidemia[1].It is also produced due to the hereditary Characters. Due to increase glucose level in blood causes various deficiencies and hampers the normal physiological effects of the human system like blood vessels and nerves system etc. It is projected that the Diabetic is the main disease which can increase the deaths retain next coming 25 years in Asian countries and Africans[2]. Now days there are number allopathic drugs are available to treat this disease. But all these agents causing serious side effects after prolong use.

Hence to overcome the adverse effects like Hematological effects, coma, disturbance of liver and kidney etc . Many traditional plants medicines are used throughout the world to treat the Diabetic diseases. When compared with synthetic drugs, the plant drugs have less toxic effects with fever side effects. Despite considerable progress achieved in the treatment ofdiabetic mellitus by oral hypoglycemic agents, search for newer drugscontinues because the existing synthetic drugs have several limitation.In recent times, herbal medicines are being used for the alternativetherapy of diabetes. More than 1200 plants have been describedas hypoglycemic agents. They provide a usefulsource of new oral hypoglycemic agents for development as pharmaceuticalentities or as simple dietary adjuvants to existing therapies[3]**.** The aim of this study is to evaluate the antidiabetic and hypolipidemic action of rhizome of *R. purpurea* commonly known askhirakakoli. In ethnobotanical practice, the various plants parts like leaves, roots, bark, and flower etc are used for the treatment of diabetic,hypertension, diarrhea, fever, inflammation etc[3-4]. Hence the present investigation was under taken to evaluate the Antidiabetic and hypolipidemic activity of methanolic extract of *R.purpurea* rhizome in alloxan induced diabetic rates to confirm the Pharmacological evidence in support of Folklore claim.

MATERIALS AND METHODS

Plant Material

Rhizomes of *R.purpurea* were collected Simali ban near Butwal and was authentified by TM Shakya, Assistant Professor, Department of Botany Kathmandu University, Kathmandu, Nepal. Voucher Specimen No. 12B34H is kept for further future reference at Kathmandu University, Kathmandu, Nepal.

Extraction

The collected rhizomes were washed with water and dried and Powdered in a grinder mixture to get coarse powder and then passed through 40 mesh sieve. The Powdered rhizomes (100g) were defatted with hexane and later extracted with methanol. The extract was evaporated to dryness in a rotary evaporator, gave a residue 12% W/W.The extract was preserved in an air tight container[5].

Animals

Wister albino rats (200-250g) of both sexes were purchased from BanaspatiBhibhag, Kathmandu. Before and during the experiment rats were fed with standard diet (Gold Mohr, Lipton India Ltd).These animals were used for the acute toxicity and antidiabetic activity. After randomization in to various groups and before initiation of experiment, the rats were acclimatized for a period of 7 days under standard environmental conditions of temperature, relative humidity, and dark/light cycle. Animals described as fasting were deprived off food and water for 16 hours *ad libitum*. The animals were handled gently to avoid giving them too much stress, which could result in an increased adrenal out put. Ethical clearance for animal study was obtained from institutional animal ethics committee (IAEC/ACP/1220/ a/23).

Acute Toxicity Studies

The animals were fasted overnight and next day extract (suspended in 5% v/v Tween 80 solution) was administered orally at a dose level of 2000 mg/kg to 5 female animals. Then the animals were observed continuously for three hour for general behavioral, neurological, autonomic profiles and then every 30 min for next three hour and finally for mortality after 24 hour till 14 days. An acute toxicity study was carried out to determination of LD50 values by using different doses of the extract. From the toxicity study, it was indicated that the extract is safe up to dose 1000mg/kg body weight. It is very safe for further studies at different doses[6].

Drug Administration

Alloxan was purchased from Sigmachemicals (St. Louis, Mo, USA).After seven days of alloxan induction, the extract was administered orally through intragastric tube at the following doses of 400, 600 and 800 mg kg^{-1} body weight.

Experimental Induction of Diabetes

The rats were injected intraperitoneally with alloxan monohydrate dissolved in sterile normal saline at a dose of 150 mg kg^{-1} body weight. Blood samples were collected before the

administration of alloxan and after 5 days of alloxan administration. Diabetic state was confirmed when the blood sugar level was above 200 mg/dl. The rats with moderate diabetes and hypolipidemia were used for the experiment[7].

Animal Allotment

After the induction of diabetes the rats were divided in to a five different groups of six rats each.

1. Group I: Control rats received normal saline and fed on normal diet.
2. Group II: Diabetic control
3. Group III: Diabetic rats received extract (400 mg kg^{-1} body weight) daily using an intragastric tube for 30 days.
4. Group IV: Diabetic rats given extract (600 mg kg^{-1} body weight) daily using an intragastric tube for 30 days.
5. Group V: Diabetic rats received extract (800 mg kg^{-1} body weight) daily using an intragastric tube for 30 days

At the end of 0 , 10^{th}, $20^{th}$and 30^{th} day blood was collected in tubes containing potassium oxalate and sodium fluoride solution for the estimation of glucose and lipid profile.

BIOCHEMICAL ANALYSIS[8]

Estimation of Blood Glucose: Blood glucose was determined by the O-toluidine method .

a. **Estimation of Total Cholesterol (TC):** Total cholesterol level was determined by the commercially available reagent kit (Erba Mannheim, Transansia biomed, Daman, India). It is based on (CHD-PAP) enzymatic methods.

b. **Estimation of HDL-cholesterol:** HDL-cholesterol level was determined by commercially available reagent kit (Erba Mannheim, Transansia biomed, Daman, India) based on phosphotungustate method.

c. **Estimation of Triglyceride (TG):** Triglyceride level was estimated by commercially available kit (Erba Mannheim, Transansia biomed, Daman, India). Its working is based on enzymatic colorimetric method. This reagent kit was made for *in vitro* quantitative determination of triglycerides in serum or plasma. Our study was carried out by serum.

Histopathological studies[9-10]

Animals were sacrificed on $5^{th}$and 10^{th}day during prolonged treatment. Pancreas, liver andkidney were removed, washed with cold saline and preserved in 10% formalin in buffered form.

Blocks from tissues were routinely processed and embedded in paraffin. Thin sections were cut using rotary microtome and stained with hematoxilin and eosin for histomorphology evaluation.

Statistical Analysis

All values were expressed as mean ± SEM. Thedata were statistically analyzed by ANOVA Followed byfollowed by Tukey's multiple comparison. Values were considered statistically significant when at $p<0.05$.

RESULTS AND DISCUSSION

Table:1 Toxicity Study

Treatment	Dose (mg/kg body wt)	No. of animals	No. of Survival	No. of death	Percentage of morality
Control	Tween80	5	5	0	0
	10	5	5	0	0
	100	5	5	0	0
	1000	5	5	0	0
	3000	5	5	0	0
	5000	5	5	0	0
	1000	5	5	0	0

Table 2: Changes in fasting blood glucose levels of control and experimental animals

		Fasting blood glucose (mg/dl)			
SN	**Group**	**0 Day**	**10^{th} Day**	**20^{th} Day**	**30^{th} Day**
1	Normal	85±1.6	87.6±2.4	87.3±2.6	89.3±2.4
2	Diabetic (control)	299±3.4	311±2.3	310.2±2.4	301±2.6
3	Extract (400 mg kg-1	284±2.3	123.6±2.8	119.2±3.1	110.5±2.3
4	Extract (600 mg kg^{-1}	291.6±2.1	112.6±2.0	106.2±2.7	100±2.
5	Extract (800 mg kg^{-1}	289.6±2.6	98±2.9	95.7±2.8	90±2.5

Results are the means of triplicate determinations on ± standard deviation

Table 3: Changes in total cholesterol (TC) levels of control and experimental animals

		Total cholesterol (mg/dl)			
SN	**Group**	**0 Day**	**10^{th} Day**	**20^{th} Day**	**30^{th} Day**
1	Normal	138.6±1.6	138.6±3.2	136±2.5	134±2.9
2	Diabetic (control)	265.3±2.1	265.7±2.8	279.5±2.9	275±3.2
3	Extract (400 mg kg-1	261.6±3.3	213.5±2.6	166±3.2	151±2.5
4	Extract (600 mg kg^{-1}	256.3±3.3	210±2.5	157.7±2.8	145.2±3.0
5	Extract (800 mg kg^{-1}	259±2.9	210±3.5	145.5±4.7	139±2.7

Results are the means of triplicate determinations on ± standard deviation

Table 4: Changes in HDL-cholesterol content of control and experimental animals

		Serum HDL (mg/dl)			
SN	**Group**	**0 Day**	**10th Day**	**20th Day**	**30th Day**
1	Normal	44.3±1.2	42±2.5	40±2.4	40±2.3
2	Diabetic (control)	49.6±1.8	50.2±3.1	51.7±2.7	54.5±2.2
3	Extract (400 mg kg-1)	51±2.9	44.5±2.8	59.2±2.6	61.5±1.6
4	Extract (600 mg kg^{-1})	48±2.9	55.2±2.9	61.7±2.7	61.7±2.2
5	Extract (800 mg kg^{-1})	49.3±2.3	59±2.2	63±2.9	4.2±1.9

Results are the means of triplicate determinations on ± standard deviation

Table 5: Changes in serum triglyceride (TG) content of control and experimental animals

		Serum triglyceride (mg/dl)			
SN	**Group**	**0 Day**	**10th Day**	**20th Day**	**30th Day**
1	Normal	80.6±1.6	81.5±2.6	78±2.7	79.7±2.5
2	Diabetic (control)	187±2.6	192.5±2.5	186.7±1.4	179.5±2.5
3	Extract (400 mg kg-1)	191.3±2.3	111.7±2.6	107±3.0	106.5±2.2
4	Extract (600 mg kg^{-1})	189.6±2.6	115±2.2	101±2.5	101.5±2.3
5	Extract (800 mg kg^{-1})	186±2.9	110.5±2.6	97.2±2.9	92.5±2.3

Acute Toxicity studies (Table 1) on *R.purpurea* rhizome extract showed no mortality at a dose of 1000 mg/kg, during a time period of 14 days. The behavioral, neurological, autonomic responses were studied and during the study no noticeable responses were seen in the rats. This helps to predict that it does not contain any type of toxicity and is safe.

The various doses of extract of *R.purpurea rhizomes* were given to the diabetic rats once a day and changes in fasting blood glucose, total cholesterol, HDL cholesterol and triglyceride were measured on day 10, 20 and 30 from the day of first dose of experiment. An effective reduction in fasting blood glucose level was observed on above mentioned time. Reduction was examined at all doses of given plant extracts but highest concentration (800 mg kg^{-1}body weight) was resulted maximum on all observed days. Although, a drastic reduction of fasting blood glucose was found to be at 400 mg kg^{-1} body weight compared with other doses. (Table 3). Diabetic control showed negligible change whereas maximum percentage reduction of 61, 66 and 69% were recorded on 30th day in group fed aqueous leaf extract at 400, 600 and 800 mg kg^{-1} body weight respectively.

The change in the cholesterol, HDL-cholesterol and triglyceride level was measured and observed on potent reduction in serum cholesterol, triglycerides and effective elevation in HDL-cholesterol level over diabetic control when the rats fed rhizome extract. The level of serum cholesterol was lower in normal rats that were not treated with alloxan and elevation were found in diabetic control whereas maximum percentage reduction 19, 44 and 46 % were seen on 10 , 20 and 30 day during the course of 800 mg kg^{-1} body weight treatment. In respect to HDL-cholesterol, it showed decrement in normal rats but maximum elevation of 20, 29 and 30% were recorded on 10 , 20 and 30 day due to 800 mg kg^{-1} body weight concentration of rhizome extract. However, similar trends of HDL-cholesterol elevation were observed at all doses of treatments with given time periods. Rats fed rhizome extract were showed inhibition in serum triglyceride content (Table 5) which was recorded in percentage 44, 47 and 50% on 10^{th}, 20^{th} and 30^{th} day but remarkable reduction of serum triglyceride was noted on feeding at 400 mg kg^{-1} body weight extract at all days of observations.

Histopathological examination of pancreas, liver and kidney showed the recovery of damaged tissues when section of treated groups compared with diabetic control. In this study, the feeding of *R.purpurea* extract resulted in significantly decreased total cholesterol and serum triglycerides and significantly increased HDL-cholesterol level; these findings are correlated with the experiment. Ingestion of *R.purpurea* produced a significant lowering of cholesterol in a hypertension model. It is possible that the drug may be acting by potentiating the pancreatic secretion or increasing the glucose uptake. Insulin is potent inhibitor of lipolysis since it inhibits the activity of the hormones sensitive lipases in adipose tissue and suppresses the release of triglycerides. The increase in HDL-cholesterol levels may be beneficial awing to the negative correlation between HDL-cholesterol levels and cardiovascular diseases.

CONCLUSION

Diabetes mellitus is a well known clinical entity with various late complications like retinopathy, neuropathy, nephropathy etc. The rhizome of *R.purpurea* has significant antidiabetic as well as hypolipidemic activity so that it can be used as an adjuvant along with allopathic treatment of medicine to treat diabetes as well as to delay the late complications of diabetes. Thus the claim made by the traditional Indian systems of medicine regarding the use of rhizome juice of this plant in the treatment of diabetes stands confirms. Further study is underway in our laboratory to isolate the active principle and to study the mechanism of its action.

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