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Original Research Article

Effect of aqueous extract of *Ardisia colorata* Roxb. leaves on blood sugar in Albino rats

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ABSTRACT

Background: Diabetes is a group of common metabolic disorders that share the characteristic features of hyperglycemia. *Ardisia colorata* Roxb. leaf extract (AEAC) is reported to be used for the treatment of diabetes. So, the present study is undertaken to evaluate the effect of *Ardisia colorata* Roxb. leaves on blood sugar by using different hyperglycemic models in Albino rats.
Methods: In both the glucose induced hyperglycemia model and streptozotocin induced hyperglycemia model, animals were divided into 4 groups of 6 animals each. 2% gum acacia were taken as control (group 1) and glimepiride 0.2 mg/kg were taken as standard (group 2). Hyperglycemic activity was checked at two different doses i.e., 200 mg/kg (test 1) and 400 mg/kg (test 2) of AEAC (given at group 3 and group 4 respectively) by assessing the decreased in blood sugar level using glucometer by following methods of these two models. The results were analyzed using one-way analysis of variance (ANOVA) followed by Bonferroni test. P value <0.05 was considered significant.
Results: Test 2 showed significant reduction in blood sugar level when compared to control after 1 hour and 2 hours of drug administration in both the models whereas test 1 showed significant reduction only in glucose induced hyperglycemia model. There was significant difference when test 2 was compared to test 1 after 1 hour and 2 hours in both the models.
Conclusions: The present studies showed that AEAC leaves produced significant reduction in blood sugar level. It might be suggested that flavonoids, alkaloids, tannins and terpenoids were responsible for the hypoglycemic activity of AEAC leaves.

Keywords: Hyperglycemia, *Ardisia colorata* Roxb., Glimepiride

INTRODUCTION

Diabetes is a group of common metabolic disorders that share the characteristic features of hyperglycemia. Various vascular or nonvascular complications can develop in diabetes like nephropathy, neuropathy, retinopathy, coronary heart disease, cerebrovascular disease, peripheral arterial disease, skin changes, infections, and hearing loss.[1]

Globally, the prevalence of diabetes mellitus has increased from an estimated 30 million cases in 1985 to 415 million cases in 2017.[1] In India, the number of individuals with diabetes are expected to be increased to 101 million by 2030 and 134.2 million by 2045.[2] This rise in prevalence may be due to multiple factors like sedentary lifestyle, lack of healthy diet, and tobacco use.[3] So, being a multifactorial disorder, different approaches should be made for the management of the disease. Drugs like sulfonylureas which increase insulin secretion have drawbacks like

chances of developing hypoglycemia, hypersensitivity reaction and weight gain. Others drugs which overcome insulin resistance like thiazolidinediones may also cause liver dysfunction, fluid retention and increased risk of fracture. Even metformin which is considered to be one of the safest oral hypoglycemic drugs have limitations like in case of renal insufficiency, heart failure, hypotensive state.[4]

Ardisia colorata Roxb. is a large shrub or small tree which belongs to the Myrsinaceae family and is locally known as uthum. Decoctions prepared from the leaves are believed to cure diabetes and urinary problems.[5,6] The leaf extract of the plant is also reported to be used for the treatment of diabetes.[7]

However, limited data are available to support the above reports. So based on the properties of which have been reported in the traditional medicine and in view of current trend towards research based on natural product, the present study is undertaken.

Streptozotocin (STZ) is a broad-spectrum antibiotic which is derived from *Streptomyces achromogens.* The mechanism of STZ is by causing β cell damage by methylation, generation of free radicals and production of nitric oxide. In rats, STZ induced diabetes by giving 3 phases of blood sugar level with hyperglycemia at 1h of administration followed by hypoglycemia for 6 hours and stable hyperglycemia at 24-48 hours.[8]

METHODS

Study design

It is a non-randomized controlled experimental study conducted in the Department of Pharmacology, Regional Institute of Medical Sciences, Imphal after getting the clearance from the Institutional Animal Ethics Committee, RIMS, Imphal (registration no: 1596/GO/a/12/CPCSEA) for a duration of two years. 24 healthy adult Wistar albino rats of either sex weighing 100-200 gm obtained from the Central Animal House, RIMS, Imphal were included and pregnant rats were excluded.

Authentication and collection of plant material

The plant *Ardisia colorata* Roxb. was identified and authenticated by Botany Department D.M College, Imphal, having the Acc. No. 007.10 DMU. The leaves of *Ardisia colorata* Roxb. were collected from the valley area of Imphal East District, Manipur.

Preparation of plant extract

The leaves of *Ardisia colorata* Roxb. were collected and dried under shade and then grind into a moderately coarse powder. Preparation of aqueous extract was done by the method described by Verma et al using Soxhlet apparatus.[9] Yield was 10.66%.

Acute toxicity testing

It was done in healthy albino rats according to OECD guidelines 423.[10] Limit test was done with the dose 2000 mg/kg using six animals (three animals per step). Animals were observed once during the first 30 minutes and then for 4 hours daily for 14 days. No mortality was observed till 14 days at this dose. $1/10^{th}$ of maximum test dose is the maximum safe dose so, 200 mg/kg and 400 mg/kg of aqueous extract of *Ardisia colorata* Roxb. leaves (AEAC) were selected for the study as test 1 and test 2 respectively.

Phytochemical screening

The preliminary phytochemical analysis of the AEAC was done by using standard procedure to identify various constituents.[11-14]

Collection of blood

Blood was collected by tail snipping method by cutting the tip of rat tail under general anaesthesia with proper aseptic and antiseptic condition.[15]

Preparation

Test drugs

200 mg/kg and 400 mg/kg orally of AEAC. The drug was prepared as suspension of 2% (w/v) gum acacia and fed in a volume of 1 ml/100 g.

Standard drug

Glimepiride [Dr Reddy's Laboratories Ltd, Nalagarh road, Baddi, Distt. Solan (HP)] 0.2 mg/kg orally. Aqueous suspension of glimepiride was prepared by 2% gum acacia in a volume of 1 ml/100 g.

Vehicle

Aqueous 2% gum acacia suspension was prepared and used in the control group at the dose of 1 ml/100 g per orally in albino rats.

Inducing agent

Streptozotocin (Sisco Research Laboratories Pvt Ltd, Maharashtra India) 50 mg/kg was prepared in 0.1 M sodium citrate buffer, pH 4.5 intraperitoneally.

D-Glucose [Dabur India Ltd, Narendrapur, Kolkata (W.B)] at the dose of 3 g/kg orally.

Experimental design

In the following experiments, healthy albino rats of either sex weighing 100-200 g were selected and fasted overnight. Proper care was taken to prevent coprophagy

and to provide free access to water. They were used for the following experiments:

Effect of Ardisia colorata Roxb. on oral glucose induced hyperglycemia

Healthy albino rats were selected and the method of Puri et al is followed with slight modification.[16] Fasting blood glucose samples were collected using glucometer and animals were divided into 4 groups of 6 animals each and treated as in Table 1.

Table 1: Treatment for oral glucose induced hyperglycemia model.

Groups	Drugs
1 (control)	2% Gum acacia (10 ml/kg p.o.) followed by oral glucose load 3 g/kg
2 (standard)	Glimepiride (0.2 mg/kg in 2% gum acacia p.o.) followed by oral glucose load 3 g/kg
3 (test 1)	AEAC (200 mg/kg in 2% gum acacia p.o.) followed by oral glucose load 3 g/kg
4 (test 2)	AEAC (400 mg/kg in 2% gum acacia p.o.) followed by oral glucose load 3 g/kg

Blood sample were collected at 1 hour and 2 hours after the glucose load and blood sugar level were estimated using glucometer. In this experiment, glucose was given immediately at the dose of 3 g/kg p.o. after the treatment. Drug wash out period of 10 days was maintained in between the two tests.

Effect of Ardisia colorata Roxb. on streptozotocin induced hyperglycemia

In this study, same set of animals were used for the experiment after the drug wash out period, to avoid the interference of the action of one particular drug with the other. Hyperglycemia were induced by the streptozotocin injection by following the method of Gupta.[8] Single intraperitoneal injection of streptozotocin was given. After three days, blood glucose samples were collected after overnight fasting, and animals were treated as in Table 2.

Table 2: Treatment for streptozotocin induced hyperglycemia model.

Groups	Drugs
1 (control)	2% Gum acacia (1 ml/100 gm p.o.)
2 (standard)	Glimepiride (0.2 mg/kg in 2% gum acacia p.o.)
3 (test 1)	AEAC (200 mg/kg in 2% gum acacia p.o.)
4 (test 2)	AEAC (400 mg/kg in 2% gum acacia p.o.)

Blood samples were collected at 1 hour and 2 hours after the drug administration and the blood sugar levels were estimated using glucometer.

Analysis of results

The results were analyzed for statistical significance using one-way analysis of variance (ANOVA) followed by Bonferroni test using IBM statistical package for the social sciences (SPSS) software version 21 (IBM Corp., Armonk, NY, USA). P value <0.05 was considered significant.

RESULTS

Phytochemical screening

The AEAC leaves were tested for the phytochemicals present by their respective tests and found the presence of tannins, flavonoids, protein, amino acids, carbohydrates, steroids, triterpenoids, terpenoids and alkaloids.

Oral glucose induced hyperglycemia

At fasting, there was no significant difference between the groups in blood sugar level (Table 3).

At 1 hour, test 2 (400 mg/kg) and standard (glimepiride 0.2 mg/kg) showed highly significant ($p<0.001$) and test 1(200 mg/kg) showed significant ($p<0.05$) when compared to control in reduction of blood sugar. When test 1 and test 2 were compared to standard, test1was significant ($p<0.01$). And test 2 when compared to test 1 was significant ($p<0.05$).

At 2 hours, when all the groups (standard, test 1 and test 2) were compared to control in reduction of blood sugar, standard and test 2 were highly significant ($p<0.001$) and test 1 was significant ($p<0.05$). When compared to standard, test 1 showed significant ($p<0.05$). Test 2 also showed significant ($p<0.05$) when compared to test 1.

Streptozotocin induced hyperglycemia

At fasting, there was no significant difference between the groups in blood sugar level (Table 4).

At 1 hour when all the groups (standard, test 1 and test 2) were compared to control in reduction of blood sugar, standard (glimepiride 0.2 mg/kg) was highly significant ($p<0.001$) and test 2 (400 mg/kg) was significant ($p<0.05$). When test 1 and test 2 were compared to standard, test 1 (200 mg/kg) was highly significant ($p<0.001$) and test 2 was significant ($p<0.05$). And test 2 when compared to test 1 was significant ($p<0.05$).

At 2 hours when all the groups (standard, test 1 and test 2) were compared to control in reduction of blood sugar, test 2 and standard were highly significant ($p<0.001$). When test 1 and test 2 were compared to standard, test 1 was highly significant ($p<0.01$) and test 2 ($p<0.05$) was significant and test 2 when compared to test 1 was significant ($p<0.01$).

Table 3: Effect of *Ardisia colorata* Roxb. leaves on oral glucose induced hyperglycemia.

Groups (n=6)	Treatment	Blood glucose		
		Fasting	1 hour	2 hours
Control	2% Gum acacia (10 ml/kg per oral p.o.) followed by oral glucose load 3 g/kg	67.50±9.094	106.5±8.361	97.5±9.418
Standard	Glimepiride 0.2 mg/kg in 2% gum acacia followed by oral glucose load	66±8.922	72.5±8.503**	65.33±7.941**
Test 1	AEAC 200 mg/kg in 2% gum acacia p.o. followed by oral glucose load	68.33±7.554	91.5±10.766*††	83±10*†
Test 2	AEAC 400 mg/kg in 2% gum acacia p.o. followed by oral glucose load	65±5.797	75.83±7.195**‡	68.50±5.992**‡
One way ANOVA				
Df		3	3	3
F		0.211	18.969	18.204
P		0.887	0.000	0.000

The results were expressed as mean±SD, p<0.05 was considered significant. **p<0.001, *p<0.05 when compared with control group, ††p<0.01, †p<0.05 when compared to standard, ‡ p<0.05 when test 1 group was compared with test 2 group (one-way ANOVA followed by Bonferroni test)

Table 4: Effect of *Ardisia colorata* Roxb. leaves on streptozotocin induced hyperglycemia.

Groups (n=6)	Treatment	Blood glucose		
		Fasting	1 hour	2 hours
Control	Gum acacia (10 ml/kg p.o.)	357±22.882	360.50±22.528	364.83±22.185
Standard	Glimepiride (0.2 mg/kg p.o.)	363±9.011	274.17±8.256**	241.33±5.610**
Test 1	AEAC 200 mg/kg in 2% gum acacia p.o.	366.50±24.509	359.83±26.731††	337.17±25.506††
Test 2	AEAC 400 mg/kg in 2% gum acacia p.o.	365±34.223	316.50±34.564*†‡	285.50±34.355**†‡‡
One way ANOVA				
Df		3	3	3
F		0.176	16.418	30.678
P		0.912	0.000	0.000

The results were expressed as mean±SD, p<0.05 was considered significant. **p<0.001, *p<0.05 when compared with control group, ††p<0.01, †p<0.05 when compared to standard, ‡‡p<0.01, ‡p<0.05 when test 1 group was compared with test 2 group (one-way ANOVA followed by Bonferroni test)

DISCUSSION

Diabetes is a complicated health issue that has reached alarming level. Globally, around half a billion people are living with diabetes.[2] Even though there are wide variety of possibilities for the drug management of diabetes, they are associated with problems like hypoglycemia, weight gain, liver dysfunction, renal insufficiency, heart failure etc.[4] So, the current trend in the management of diabetes have highlighted an immediate requirement of extensive research for identification of plant products in drug discovery as they are cost effective, lesser side effects and easily available.[17]

In the present study, the blood sugar activity of the AEAC were evaluated by using oral glucose and streptozotocin induced hyperglycemia in albino rats. Glimepiride was used as standard drug.

Glimepiride is a potent second-generation sulfonylureas group of antidiabetic drugs. It stimulates release of insulin by binding to the specific site on β cell K_{ATP} channel complex and inhibiting its activity. This drug has longer duration of action and can be prescribed in a single daily dose.[4,18]

The blood glucose level was measured using glucometer (Sugarchek advance strip in Sugarchek advance test meter, Taidoc Technology Corporation). It used glucose oxidase technology (GOD). This glucometer measured the amount of glucose present in the whole blood. It is based on the measurement of electrical current generated by reaction of the reagent in the test strip with the glucose. The amount of glucose in the blood sample is directly proportional to the strength of the current produced by the reaction. The meter calculates the blood glucose level by measuring the current and displays the result.[19]

Flavonoids like catechin, epicatechin and quercetin has free radical scavenging and insulinomimetic activity. Flavanol, flavones and flavanones which are also flavanoids and alkaloids like harmine, pinoline and

ginkgolide increased insulin secretion. Tannins caused regeneration of β cells of the pancreas and also increased insulin secretion.[20] Terpenoid showed improvement in diabetic condition by regenerating β cells of pancreas or by increasing secretion of insulin.[21]

Phytochemical constituents of *Ardisia colorata* Roxb. leaves give alkaloids, tannins, flavonoids, saponins, reducing sugar, steroid and terpinoids.[22] Leaves of *Ardisia colorata* is rich in bergenin and it can be used in diabetic complications as therapeutic medication.[23]

Oral glucose induced hyperglycemia was evaluated by following the method of Puri et al with slight modification.[16] Fasting, 1 hour and 2 hours blood glucose samples were evaluated using glucometer after the glucose load of 3 g/kg in an overnight fasted rat. 200 mg/kg and 400 mg/kg of the test drug and 0.2 mg/kg of the standard drug glimepiride were given before oral glucose load and significant decreased in blood sugar after 1 hour and 2 hours of drug administration was evaluated. The result in the present study [i.e., 83±10 at 2 hours observation by test 1 (200 mg/kg)] was similar to that Barik et al [i.e., 83.54±1.36 at 2 hours observation by aqueous root extract of *Ichnocarpus frutescens* (250 mg/kg)].[24] The blood glucose lowering effect of AEAC after glucose load of 3 g/kg may be due to a strong stimulus to stimulate pancreatic beta cells to secrete insulin.

Streptozotocin is an analogue of nitrosourea in which carbon 2 of hexose is linked to N-methyl N nitrosourea moiety. It is selectively gathered in the β cells of the pancreas by GLUT 2 glucose transporter in the plasma membrane. It causes β cell toxicity and necrosis by methylation of DNA resulting in DNA fragmentation. It also causes β cells functional defect by protein methylation.[25]

Streptozotocin induced hyperglycemia was evaluated by the method of Gupta.[8] Using albino rats the blood sugar was evaluated by recording at fasting, 1 hour and 2 hours after drug administration. The test drug at the dose of 400 mg/kg and standard drug glimepiride 0.2 mg/kg showed significant decreased in blood sugar after 1 hour and 2 hours of drug administration. The result in the present study was 359.83±26.731 and 337.17±25.506 for test 1 and 316.50±34.564 and 285.50±34.355 for test 2 at 1 hour and 2 hours respectively. And the result for that of Sunder et al was 263.6±4.1 and 252.5±2.1 for methanolic extract of *T. portulacastrum* (i.e., METP 100 mg/kg) and 255.9±3.3 and 239.3±4.1 for METP 200 mg/kg at 1 hour and 2 hours respectively.[26]

CONCLUSION

To conclude, the finding of the present study showed that AEAC leaves significantly reduced blood sugar levels in albino rats. It might be suggested that flavonoids, alkaloids, tannins and terpenoids were responsible for the hypoglycemic activity. However, further studies are required to understand the exact mechanism of hypoglycemic activity caused by its phytochemical constituents.

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