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Effects Of Salacia Reticulata Root Bark On Blood Glucose Levels Of Normal And Alloxan-Monohydrate Induced Diabetic Mice

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Abstract:
In the present study, a comparative antidiabetic potential of S. reticulata root bark ethyl acetate and aqueous extracts were studied in vitro. Acute toxicity study of both extracts was carried out in male BALB/c mice, showed no toxicity. Further, in in-vivo studies showed antidiabetic activity in mice with induced type II diabetes mellitus. Type II diabetes mellitus was induced in the experimental rats by intraperitoneal injection of 10% alloxan monohydrate (2,4,5,6 tetraoxypyrimidine; 5-6-dioxyuracil). The mice were orally fed different doses ranging from 50 to 200 mg/kg body weight of S. reticulata root bark extracts for 8 hrs. Blood glucose level, body weight and hematological parameters were analyzed on day 0, 7, 14 and 28 days. Among these extracts, ethyl acetate extract showed decreased the glucose levels in a dose-dependent manner as well as the hematological parameter levels in diabetic mice as compared with aqueous extracts. The HPLC chromatogram showed the abundant presence of phenolic acids and terpenoids.

Keywords: Alloxan diabetic rats, Hypoglycemic activity, Salacia reticulata Weight, Hematological parameter, Blood glucose

INTRODUCTION:

Diabetes mellitus is a principal cause of morbidity and mortality in human. It is a syndrome characterized by hyperglycemia, polydipsia and polyuria and causes complications to the eyes, kidneys, and nerves. It is also associated with an increased incidence of cardiovascular disease. The clinical manifestations and development of diabetes often differ significantly between countries and also between racial groups within a country. This increase can be attributed to many factors, including a stressful lifestyle as well as improper dietary habits. This is of economic concern as the disease requires life-long treatment and is also associated with high morbidity from the resulting complications[1]. According to the IDF statistics, presently every seven seconds someone is estimated to die from diabetes or its complications, with 50% of those deaths (4 million in total per year) occurring under the age of 60 years[2]. This is against the background of a global diabetes prevalence of 8.8% of the world population in 2017, standardized for the age group 20-79 years[3].

The prevalence is expected to further increase to 9.9% by the year 2045. In total numbers, this reflects a population of 424.9million people with diabetes worldwide in 2017 with an estimate of a 48% increase to 628.6million people for the year 2045. Global umbers of diabetes prevalence have continuously risen from 151 million in 2000, when the IDF Diabetes Atlas first was launched, to 285million in 2009 and to 382million in 2013. Disturbingly in this context, some 50% of all individuals with diabetes are undiagnosed, especially in developing countries[4]. Moreover, it was estimated that the number of adults with diabetes in the world had increased from 108 million in 1980 to 422million in 2014 (28.5% due to the rise in prevalence, 39.7% due to population growth and ageing, and 31.8% due to interaction of these two factors). Besides the growth and aging of the world population in general, the global obesity epidemic has turned out to be a key factor for the rise of diabetes incidence together with the immense progress of multifactorial cardiovascular risk management and successful revascularisation therapy of people with diabetes also contributing to the expansion of the worldwide diabetes population[5].

Salacia reticulata (Celastraceae) is a large woody climbing, perennial, woody shrub naturally found in Sri Lanka and Southern region of India. The plant has dichotomous branching pattern. Bark is smooth, greenish grey in colour, thin, and white internally. Leaves are opposite and elliptic-oblong. Leaf-base is

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acute, apex abruptly acuminate, margin are toothed with minute rounded teeth. Flowers are bisexual and arranged as 2-8 clustered in leaf axils. They are greenish-white to greenish-yellow in color. Fruit is a drupe which is globose and tubercular. The drupe assumes pinkish-orange color on ripening. Seeds are 1to 4 in number and resembles with almond[6]. Different species of Salacia have medicinal principles with a high pharmacological significance. In traditional system of medicine, different species of the genus, Salacia are being used as acrid, bitter, termogenic, urinary and as liver tonic. The aerial parts and roots of Salacia are extensively used in Ayurvedic and Unani system of medicine for treating diabetes, gonorrhoea, rheumatism, itching, asthma, ear diseases, leukaemia and inflammations[7].

MATERIALS AND METHODS:

Plant material and study animals

Plant materials- The plant materials of root bark of Salacia reticulata Wight. (Celastraceae) were collected from an area around local forest in Gondia district in Maharashtra and were shade dried.

Animals- The study used male BALB/c mice, 3-5 weeks old that weighed 20-30 g with a mean weight of 25 g. The animals were allowed to acclimatize for a period of two weeks in the animal house at the Department of Pharmacology, Bajiraoji Karanjekar College of Pharmacy prior to the study. The mice were housed in polypropylene cages, maintained under standard laboratory conditions of 12 hour light and dark normal photoperiodicity, at ambient temperature of 25 ± 2ºC and 35-60% humidity. The animals were fed with standard mice pellets and water ad libitum.

Plant Extraction- The air-dried roots of S. reticulata were finely ground using a mechanical blender. 250 grams of each root powder was subjected to hot-reflux extraction separately, using water and ethyl acetate as solvent. Each cycle was repeated 3 times for a period of 3 h and fresh solvent was added to the same after filtering through whatman filter paper No.1. The solvent from 3 cycles was evaporated to 2/3rd of its initial volume using a rotatory evaporator. The powdered extracts obtained after lyophilization were labeled separately as aqueous extract and ethyl acetate extract of S. reticulata.

Phytochemical Analysis- The Phytochemical, such as alkaloids, phenols, flavonoids, saponins, glycosides, steroids, present in the powdered root bark extracts were qualitatively analyzed as mentioned, using standard methods[8].

Induction of hyperglycemia- Hyperglycemia was induced experimentally by intraperitoneal administration of a single dose of 186.9 mg/kg body weight (4.6725 mg in 0.1 ml physiological saline) of a freshly prepared 10% alloxan monohydrate (2,4,5,6 tetraoxypyrimidine; 5-6-dioxyuracil) obtained from Sigma Lab. 48 hours after alloxan administration, blood glucose level was measured using a glucometer. Mice with blood glucose levels above 2000 mg/L were considered diabetic and used in this study. Prior to initiation of this experiment, the animals were fasted for 8-12 hours but allowed free access to water until the end of this experiment.

Experimental design- The experimental animals average weight 25 g were randomly divided into six groups of five animals each. Group I consisted of normal mice orally administered with 0.1 ml physiological saline; Group II consisted of alloxan induced diabetic mice orally administered with 0.1 ml physiological saline; Group III consisted of alloxan induced diabetic mice orally administered with 0.075 mg of glibenclamide (3 mg/kg body weight) in 0.1 ml physiological saline; Group IV consisted of alloxan induced diabetic mice orally administered with 1.25 mg extract (50 mg/kg body weight) in 0.1 ml physiological saline; Group V consisted of alloxan induced diabetic mice orally administered with 2.50 mg extract (100 mg/kg body weight) in 0.1 ml physiological saline; and Group VI consisted of alloxan induced diabetic rats either intraperitoneally or orally administered with 5 mg extract (200 mg/kg body weight) in 0.1 ml physiological saline.

In vivo single dose toxicity test- The mice were randomly divided into three different groups of five mice each. Group I consisted of untreated control mice orally administered daily for 28 days with 0.1 ml physiological saline. Group II and Group III consisted of normal control mice orally administered with

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aqueous and ethyl acetate extracts of the plant at 25 mg (1 g/kg body weight) in 0.1 ml physiological saline daily for 28 days. During this period, the mice were allowed free access to mice pellet and water and observed for any signs of general illness, change in behavior and mortality. At the end of 28 days, the mice were sacrificed.

Determination of body weight- The body weight of each mouse was assessed after every seven days during the dosing period up to and including the 28th day and the day of sacrifice. On the day of sacrifice, all the animals were euthanized and blood samples taken by cardiac puncture of each sacrificed animal divided into two parts: one part for estimation of hematological parameters and the other part for estimation of biochemical parameters.

Determination of hematological parameters- The blood sample collected in K3-EDTA tubes was used to estimate hematological parameters. Blood was examined using standard protocols Red blood cells (RBC), white blood cells (WBC), hemoglobin (Hb), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), mean cell volume (MCV) and platelets (PLT) were determined using the Coulter Counter System. Differential white blood cell count for neutrophils (N), lymphocytes (L), and monocytes (M) were determined from stained blood films using a hemocytometer. Air-dried thin blood films stained with giemsa stain were examined microscopically using magnification 400 and 1000 for differential WBC counts and cell morphologies, respectively[9,10].

Data management and statistical analysis- Results were expressed as Mean ± Standard Deviation (SD) of the blood glucose levels per the number of mice used in every study point. One-way ANOVA and post-ANOVA (Bonferroni-Holm) test, $P< 0.05$ was considered statistically significant.

RESULT:

Yields of various extracts from the root bark of S. reticulata

The ethyl acetate and aqueous extracts yielded a dark brown paste and a light brown paste, respectively, of concentrations, 215.7 and 168.0 mg/g dry weight, respectively.

Phytochemical analysis of S. reticulata root bark extracts

The phytochemical analysis of S. reticulata root bark extracts revealed the presence of alkaloids, flavonoids, phenols, saponins, steroids, and terpenoids in varying concentrations.

Table 1: Preliminary phytochemical analysis

Phytochemicals	Salacia reticulate extracts
Tannins	+++
Phenols	++
Saponins	+
Terpenoids	++
Flavonoids	++
Steroids	++
Glycosides/ reducing sugar	++
Alkaloids	++

Effects of oral administration of extracts of Salacia reticulata

Oral administration of ethyl acetate and aqueous dried root bark extracts of Salacia reticulata at 50, 100 and 200 mg/kg body weight to mice is depicted in Table 2; Figure 1and Table 3; Figure 2 respectively.

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Table 2: Hypoglycemic effects of oral administration of **ethyl acetate** dried roots extracts of Salacia reticulata at 50, 100 and 200 mg/kg body weight in alloxan-induced diabetic male BALB/c mice.

Group	Treatment	Blood glucose levels at varying times (mM)				
		0 hr	2 hr	4 hr	6hr	8 hr
Normal control	Saline	5.1±0.1	5.1±0.1	5.1±0.2	5.2±0.2	5.3±0.1
Diabetic control	Saline	24.0±0.8^{A}	25.0±0.8^{B}	27.7±0.9^{Ab}	28.7±0.9^{Ab}	29.9±0.9^{Bb}
Diabetic	Glibenclamide (3 mg/kg body weight)	23.2±0.7^{A}	14.5±0.9^{Aa}	9.4±0.6^{Bb}	5.8±0.6^{c}	5.2±0.1^{Ad}
Diabetic	Extracts					
	50 mg/kg body weight	17.4±1.0^{B}	10.0±0.7^{Ca}	4.8±0.5^{b}	3.3±1.0^{Bc}	3.1±0.3^{C}
	100 mg/kg body weight	19.7±0.8^{C}	15.3±0.8^{Aa}	10.2±0.3^{Cb}	6.7±0.7^{B}	5.9±0.6
	200 mg/kg body weight	24.4±3.7^{C}	22.7±0.8^{A}	20.0±0.9^{C}	15.3±0.5^{B}	13.3±0.7^{A}

Results were expressed as Mean ± Standard deviation (SD) of five mice per group. Means followed by similar upper case letters in the same column are not significantly different at $p \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test.

Table 3: Hypoglycemic effects of oral administration of **aqueous** dried roots extracts of Salacia reticulata at 50, 100 and 200 mg/kg body weight in alloxan-induced diabetic male BALB/c mice.

Group	Treatment	Blood glucose levels at varying times (mM)				
		0 hr	2 hr	4 hr	6hr	8 hr
Normal control	Saline	4.8±0.5	5.2±0.2	5.2±0.2	5.5±0.4	5.4±0.3
Diabetic control	Saline	23.2±3.5^{A}	24.6±2.7^{Aa}	25.8±2.5^{Ab}	27.9±1.6^{Ac}	29.0 ±1.0^{Ad}
Diabetic	Glibenclamide (3 mg/kg body weight)	23.1±4.0^{A}	15.0±0.6^{Ba}	16.8±1.5^{Bbe}	7.5±0.9^{Bcfh}	5.9±0.6^{dgij}
Diabetic	Extracts					
	50 mg/kg body weight	20.9±1.8^{A}	20.3±0.7^{Ca}	16.6±1.2^{Cbe}	12.8±1.0^{Ccfh}	10.6±0.6^{Bdgij}
	100 mg/kg body weight	25.6±0.7^{A}	20.6±0.9^{Ca}	18.2±0.7^{Dbe}	13.5±0.9^{Ccfh}	9.5±0.9^{Bdgij}
	200 mg/kg body weight	23.4±1.3^{A}	17.0±0.8^{Da}	14.9±0.8^{Cbe}	11.9±0.6^{Ccfh}	7.7±0.5^{Cdgij}

Results were expressed as Mean ± Standard deviation (SD) of five mice per group. Means followed by similar upper case letters in the same column are not significantly different at $P \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test. Means followed by similar lower case letters in the same row are not significantly different at $P \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test.

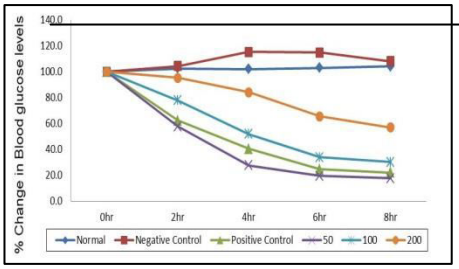

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Figure 1: Percentage reduction in blood glucose levels at varying times after oral administration of ethyl acetate dried roots extracts of Salacia reticulata at 50, 100 and 200 mg/kg body weight.

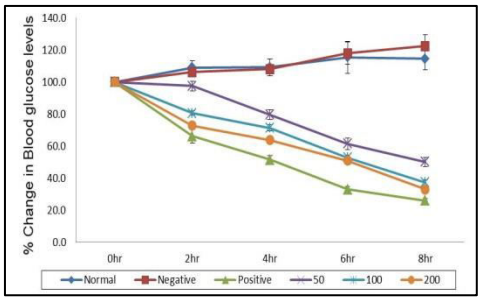

Figure 2: Percentage reduction in blood glucose levels at varying times after oral administration of aqueous dried roots extracts of Salacia reticulata at 50, 100 and 200 mg/kg body weight.

Effect of oral administration of aqueous plants extracts on body weight and mean weekly body weight change

As depicted in Table 4, oral administration of ethyl acetate and aqueous extracts of Salacia reticulata root bark at 1 g/kg body weight daily in mice for 28 days significantly reduced the body weight and mean weekly body weight gain compared to that of the normal control mice.

Table 4: Effects of oral administration of ethyl acetate and aqueous plants extracts

Treatment	Weekly Weight of Mice (g)					Weight/Week
	0	1	2	3	4	
Control	23.06±1.82	23.31±1.80	23.90±1.44	24.82±0.88	26.96±1.62	0.98±0.34
Ethyl Acetate extract	21.86±4.44	22.16±4.43	22.42±4.52	22.94±4.43	23.18±4.72	0.23±0.22*
Aqueous extract	18.24±1.68 *	18.54±1.70 *	18.86±1.76 *	19.36±1.70 *	20.43±1.30 *	0.39±0.08*

Determination of hematological parameters

Table 5: Effects of oral administration of aqueous plants extracts at 1 g/kg body weight daily in mice for 28 days on some end point hematological parameters in mice.

Treatment	Control	Aqueous extracts	Ethyl acetate extracts
RBC ($\times 10^6/\mu L$)	9.92±1.13	10.74±1.05*	5.02±1.46*
PCV (%)	56.24±0.47	25.70±1.48*	22.80±2.16*
Hb (g/dL)	13.66±1.32	7.52±1.41*	6.68±2.11*
MCHC (g/dL)	23.32±1.15	30.32±2.62*	34.76±1.20*
MCV (fL)	59.28±2.82	42.78±1.39*	42.40±2.29*
MCH (pg)	13.80±0.63	12.82±1.43*	12.78±0.91*
PLT ($\times 10^3/\mu L$)	732.00±202.27	526.80±44.21	475.80±80.16*
WBC ($x10^9/L$)	3.70±0.44	10.78±1.00*	11.37±0.51*
DLC ($x10^9/L$)			
Granulocytes	0.95±0.12	1.13±0.18	1.64±0.17*
Lymphocytes	2.21±0.24	8.30±0.88*	8.17±0.32*
Monocytes	0.54±0.12	1.35±0.28*	1.56±0.21*

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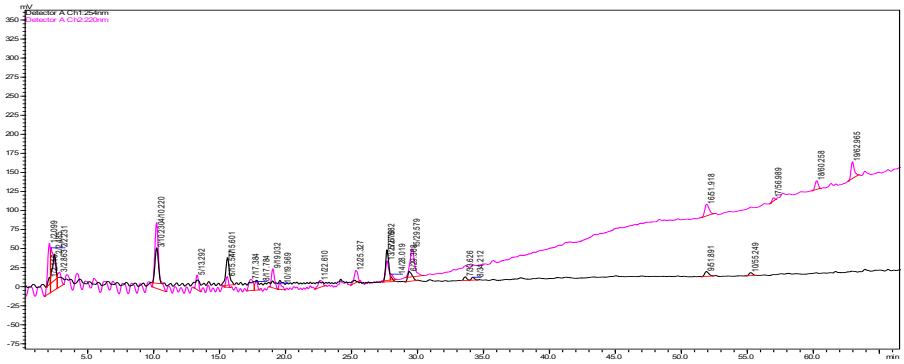
ROOTSPALE- *Salacia reticulata*



Amines/Phenolic acids

Flavonoids Tocopherol/ Terpenoids

Figure 3. A representative high-performance liquid chromatography with a diode-array detector chromatogram of Salacia reticulata extract using reversed-phase C18 column.

DISCUSSION:

Alloxan induces diabetes by damaging the insulin secreting cells of the pancreas leading to hyperglycaemia[11]. An observation in this study correlates with the previous research finding, in that the blood glucose levels significantly increased in alloxan untreated diabetic rats. In the present study, the continuous treatment with ethyl acetate and aqueous extracts of Salacia reticulata for a period of 4 weeks caused a significant decrease in the blood glucose levels of treated diabetic rats but no effect was observed in normal treated rats. The possible mechanism by which ethyl acetate and aqueous extracts of Salacia reticulata brings about its hypoglycaemic action may be, by potentiating the insulin effect, either by increasing the pancreatic secretion of insulin from the cells of islets of Langerhan's or its release from bound insulin[12]. We have noticed a significant reduction in food and water intake and increased in the body weight in alloxan diabetic rats. This could be the result of improved glycaemic control produced by the plant. Phenolic acids, flavonoids, and terpenoids are the main antidiabetic components in medicinal plants. Hence, we can conclude that the anti-diabetic effects of the Salacia reticulata might be due to these phytochemicals. Abundant evidence generated in human studies collectively suggests that the intake of polyphenols and their major food sources may exert beneficial effects on improving insulin resistance and related diabetes risk factors, such as inflammation and oxidative stress[13].

CONCLUSIONS:

The obtained data by the present experiment approves the antihyperglycemic features of ethyl acetate and aqueous plants extracts in alloxan-induced diabetic rats and introduced the Salacia reticulata root bark extract as a safe and efficient applicant in fighting against diabetes. In summary, intake of polyphenols, especially flavan-3-ols and their food sources, have demonstrated overall beneficial effects on decreasing insulin resistance, chronic systematic inflammation, oxidative stress, and improving other cardiometabolic risk factors.

CONFLICT OF INTERESTS:

The authors have declared that there is no conflict of interests.

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