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A Study On The Hypoglycemic Activity Of Aqueous Extract Of Zanthozylum Armatum Dc Leaves In Normal And Diabetic Albino Rats

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Abstract

Background: Medicinal plants are used for the treatment of many ailments. *Zanthoxylum armatum* DC which has various medicinal properties is investigated for its effects on blood glucose level of the experiment animals.

Methods: The cleaned and dried leaves of *Zanthoxylum armatum* was Soxhleted with water for *Zanthoxylum armatum* aqueous extract (ZAE). 59 albino rats were recruited for the present study. The acute toxicity test of the extract was done by administering up to the dose of 2000mg/kg to 5 albino rats but no toxicity was observed. The ZAE at doses of 200mg/kg, 400mg/kg were administered orally at 0hr, ½ hr, 1hr and 2hr to 24 normal rats & 30 STZ diabetic induced rats. Similarly, the standard drug Glibenclamide (0.5mg/kg) was also administered orally. Then the blood glucose levels (BGL) of the rats were estimated.

Results: The mean blood glucose levels (MBGL) of the rats after the administration of the extract and standard were compared to the MBGLs of the normal control. The result was not statistically significant. ZAE does not have hypoglycemic properties The MBGLs of the diabetic rats after administration of the extract and standard were compared to the MBGLs of the diabetic control. The effect of the extract on BGLs of the diabetic rats were found to be statistically significant. But the observation on the comparison of the MBGLs of the standard and diabetic control was insignificant.

Conclusion: Since the ZAE contains many phytochemicals like flavonoids, the probable mechanism of action of the ZAE is the increasing of sensitivity of insulin or act as an insulin secretagogue. Therefore, ZAE may have antihyperglycemic property. It is suggested to study the effect of the extract in some other experimental animals for definitive detail data.

Keywords: Zanthoxylum armatum extract, blood glucose level, glibenclamide, streptozotocin, antihyperglycaemic, albino rats

Introduction

Medicinal plant products are in great demand for primary health care management because of their wide biological and medicinal activities, higher safety margin as well as low cost. Since the products are developed mostly on the basis of traditional knowledge, there is a few and limited clinical studies. The induction of AYUSH in the health care system and the emergence of Reverse pharmacology in the allopathic teaching globally, the study of Traditional system of medicine is widely accepted. It was reported that certain resistant cases of diabetes have responded well to medicinal plant products alone or in combination with oral hypoglycemic agents.[1] As a result of decrease in systemic insulin sensitivity

people suffers from T2DM.[2] A large number of medicinal plant products have been reported to possess antidiabetic activity.[3] Among the medicinal plants *Zanthozylum armatum* DC (Mukthrubi in Manipuri, tejphal/darmar in hindi) is reported to have many medicinal properties. The leaves of *Zanthozylum armatum* are used to treat diabetes.[4] The present study is undertaken to investigate the effect of *Zanthozylum armatum* aqueous extract on blood glucose level in normal and diabetic albino rats.

Aims and objective:

The effect of aqueous extract of *Zanthoxylum armatum* DC on –

1. Blood glucose level in normal rats
2. Blood glucose level in Streptozotocin induced diabetic rats

Materials and methods:

The Protocol of the study was approved by Institutional Animal Ethics Committee (IAEC) of RIMS (Regd. No: 1596/GO/a/12/CPCSEA). The identification of the plant was authenticated by the Life Science Dept, Manipur University (Acc No 004320 of MUH). Experimental study was conducted in the PG Research Lab, Dept of Pharmacology, Jawaharlal Nehru Institute of Medical Sciences, Imphal

Fresh leaves of the *Zanthozylum armatum* were collected and cleaned with water and then the leaves air dried in shade for several days. Aqueous extraction of *Z. armatum* was done with some modification (Verma SCL & Agarwal SL).[5] 50gm of powdered leaves was Soxhleted and a yield of 25.50 gm was obtained. The test doses of 200mg/kg and 400mg/kg body weight of aqueous extract of *Z. armatum* were selected and given orally (Bajaj and Srinivasam BP)[6]

The Diabetic agent- streptozotocin (STZ) 50mg/kg intraperitoneally[7] and Standard drug – Glibenclamide 0.5mg/kg orally (Ignacimuthu S & Amalraj T)[8] selected.

Selection and grouping of animals:

Albino Rats have been recruited for its small size, low cost, omnivorous, resemble human being nutritionally. A total of 59 healthy albino rats (24 rats for normal and 30 rats for diabetic induced study, 5 rats for acute toxicity testing) of either sex weighing 125 gm were recruited. The animals were kept in the lab on balance diet and water ad libitum for acclimatization. The animals were fasted for 18 hrs prior to the experiment and care was taken to avoid coprophagy.

Procedure:

24 normal healthy rats were divided into 4 groups (normal control of 6 rats, test dose 1 group of 6 rats, test dose 2 group of 6 rats, standard group of 6 rats). Blood was collected from tails at 0hr, ½ hr, 1hr and 2hr (Babu v et al)[9] and blood glucose levels (BGL) were estimated by Glucose oxidase method (Barham D and Trinder P)[10]

Streptozotocin powder was dissolved in citrate buffer and administered a single dose of 50mg/kg body wt intraperitoneally to the healthy 30 rats (Udhaya Lavinya B et al).[7] Those rats were fed 5% glucose solution for 24 hrs to prevent hypoglycaemia. The rats were divided into 5 groups (normal control group of 6 rats, diabetic control group of 6 rats, test dose 1 group of 6rats, test dose 2 group of 6rats, standard group of 6 rats). Blood was collected from the tails and the glucose levels were estimated at 0hr, ½ hr, 1hr and 2hr. The BGL above 300mg/dl were selected for this study (Babu V et al).[9]

Toxicity testing:

Acute toxicity test of the extract was conducted to 5 rats by giving the extract orally up to the dose of 2000mg/kg as per OECD/ OCED Guidelines 425.[11]

Statical Analysis:

1. Descriptive statistics, mean, SD, SE
2. ANOVA
3. Post- hoc Dunnett's ''t'' test
4. Probability level of $P< 0.05$

Results and observations:

Acute toxicity test: No visible signs like salivation, changes in skin, eyes, furs was observed.

Table 1:

The mean BGLs of the test doses (I and 2) were observed higher when compared with the mean BGLs of the control (normal) and standard (glibenclamide) but the mean BGLs at 2hr were observed lower when compared with the mean BGLs of the control but not with the mean BGL of the standard. Hence it is not significant statistically.

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Table 1: Effect of *Zanthoxylum armatum DC* leaves aqueous extract of blood glucose level in normal rats. (Mean±SEM, n=6)

Group	Drugs &dosage	Blood sugar level (mg%) at			
		Fasting	30min	60min	120min
Control	Gum acacia 2% in D/W	89.0±1.52	90.33±1.64	93.50±0.99	96.33±1.58
Test $Dose_1$	ZAE(200mg/kg) in 2% gum acacia suspension	94.33±6.74	95.50±1.08$^{\alpha}$	93.67±1.11	87.50±3.24
Test $Dose_2$	ZAE(400mg/kg) in 2% gum acacia suspension	95.57±1.05*	94.83±0.94$^{\beta}$	93.67±0.66	89.33±2.52
Standard	Glibenclamide (0.5mg/kg)	91.67±1.74	87.67±1.62	85.67±2.21	83.83±3.03*
One Way ANOVA	F	2.39	7.50	8.33	4.18
	DF	3,20	3,20	3,20	3,20
	P	0.09>0.05	0.001<0.05	0.001<0.05	.019<0.05

*P< 0.05 compared to control group;

$^{\alpha}$P<0.01, $^{\beta}$P< 0.05 as compared to standard group (by applying ANOVA, Dunnett t-test)

Table 2: The mean BGLs of the test doses (I and 2) at 0hr, ½ hr, 1hr and 2hr were observed lower when compared with the mean BGLs of the diabetic control. The reduction of the BGLs was statistically significant (P < 0.001). But the mean BGLs of the test doses (I and 2) at 0hr, ½ hr, 1hr, and2hr were observed higher when compared with the mean BGLs of the standard (glibenclamide). Hence the ZAE has antihyperglycemic effect.

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Table 2: Effect of *Zanthoxylum armatum DC* leaves aqueous extract on streptozotocin induced diabetic rats. (Mean±SEM, n=6)

Group	Drugs & dosage	Blood sugar level (mg%) at			
		Fasting	30min	60min	120min
Control	Gum acacia 2% in D/w	86.83±2.22	90.33±2.40	89.50±2.46	90.00±2.63
Diabetic Control	Gum acacia 2% in D/w	221.17±3.26*	225.17±2.48*	227.17±2.56*	230.83±2.27*
$Test_1$	ZAE(200 mg/kg) in 2% gum acacia suspension	211.50±2.60*α	208.33±3.25*#α	204.00±3.64*#α	201.17±3.67*#α
$Test_2$	ZAE (400mg/kg) in 2% gum acacia suspension	207.83±3.08*α	204.83±3.65*#α	201.17±4.07*#α	193.33±4.61*#α
Standard	Glibenclamide (0.5mg/kg)	186.00±2.81*#	184.33±2.60*#	178.83±3.33*#	171.17±3.95*#
One Way ANOVA	F	381.35	336.55	266.79	226.93
	DF	4,25	4,25	4,25	4,25
	P	0.0001<.001	0.0001<.001	0.0001<.001	0.0001<.001

*P< 0.001 as compared to control group

# p< 0.01 compared to diabetic control group

αP<0.01 compared to standard group (by applying ANOVA and Dunnett t- test)

Discussion:

The treatment of T2 DM is mainly done with oral hypoglycemic agents (OHA). But these agents are of synthetic nature having certain side effects. Now a days many medicinal plants and their products are used as they are of cost effective with less side effects. Therefore, *Zanthoxylum armatum* , a food item, is selected for investigation of its hypoglycemic properties in albino rats. The aqueous extraction of *Zanthoxylum armatum* was undertaken with some modification (Verma SCL and Agrawal SL).[5] The test doses of 200mg/kg and 400mg/kg were selected (Bajaj S and Srinivasan BP).[6]

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The hypoglycemic effect of ZAE was assessed in normal and STZ induced diabetic albino rats. The rats suffered from diabetes due to pancreatic beta cell DNA fragmentation and necrosis.[12] Glibenclamide was used as standard drug and administered orally at the dose of 0.5mg/kg after extrapolation from human dose (Ignacimuthu S & Amalraj T).[8]

The hypoglycemic effect was studied vide Babu et al.[9] Estimation of BGL was done by Glucose oxidase Method which needs very small amount of serum (0.01ml) for analysis of blood glucose.[10] The method is specific for D glucose with relatively little effect on other sugar present in blood and true glucose was also obtained (Mc Lauchlan DM).[13] The BGLs of the rats (normal and diabetic) were assessed after administration of the ZAE test doses (200mg/kg, 400mg/kg,) and standard drug Glibenclamide (0.5mg /kg) orally at 0hr, ½ hr, 1hr and 2hr respectively.

The mean BGL of the normal control group (89.0 ±1.52) which was also consistent with the report of Farswan et al (90.0±-0.2).[14] The mean BGLs of the test doses (1 and 2) after 2hr of treatment showed decreased BGL though there was no statistical significance. The reduction in BGL after two hrs of treatment with the standard (83.83±3.03 was consistent with findings of Das et al.[15]

Similarly, mean BGLs of diabetic control group (221.17± 3.26) was conformed with those mentioned in the report of Himani Karki et al.[16] The mean BGLs of the diabetic rats at 0hr ½hr, 1hr, 2 hr after treatment with test dose 1 & 2 of ZAE (200 mg/kg, 400mg/kg) were found to be lower when compared to those of diabetic control group. This reduced effect in BGL was statistically significant ($P < 0.01$). However, the reduction effect of mean BGL of the test doses was not statistically significant when compared with those of the standard drug. Persistent increased BGL of diabetic control group i.e. from 221.17± 3.26 *to* 230.83 ± 2.27 *was seen.* Standard drug lowered BGL in diabetic rats significantly ($P < 0.05$) which conformed with the findings of Ignacinmuthu S & Amalraj T.[8]

The possible cause of lowering of the BGL with the ZAE may be due to presence of flavonoids, steroids, terpenoids, phenolic acid in the extract.[17] Flavonoids regenerates the damage beta cells and act as insulin secretagogue.[18] Aqueous extract of Z armatum show notable effectiveness in reducing blood glucose level and addressing several diabetes- related issues.[19]

Conclusion:

The aqueous extract of *Z armatum* does not produce lowering effect on the normal blood glucose level. But the extract produces the lowering effect on the elevated blood glucose level of STZ induced diabetic rats. Therefore, ZAE has antihyperglycemic (not hypoglycemic activity). The antihyperglycemic activity of ZAE may be due to the presence of many phytochemicals like flavonoid. Therefore, there is need for further elaborate studies on other experimental animals and human beings so that more definitive data regarding its therapeutic potential and therapeutic utilization.

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