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Original Research Article

EFFECT OF ETHYL ACETATE EXTRACT OF CINNAMOMUM ZEYLANICUM BARK ON ADRENALINE INDUCED HYPERGLYCAEMIA IN ALBINO RATS

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ABSTRACT

Background: The aim is to evaluate the effect of ethyl acetate extract of Cinnamomum zeylanicum (EAECZ) bark on adrenaline induced hyperglycaemia in albino rats.

Materials and Methods: Twenty four healthy albino rats of either sex with weight within the range of 100-200 gm were divided into four groups of six animals each and kept overnight fasting. Hyperglycaemia was induced in all the animals by adrenaline (0.5mg/kg) intraperitoneal injection. Different groups were treated as follows- control group with 2% gum acacia (1ml/100g), standard group glibenclamide 0.5mg/kg per orally (p.o) and test groups with EAECZ (500 and 750) mg/kg respectively. Fasting blood glucose samples were collected using glucometer after stipulated time of treatment. Data were analysed using One way ANOVA and Bonferroni test. P value less than 0.05 was considered significant.

Results: EAECZ at the dose of 750mg/kg showed significant reduction in blood sugar compared to control after 1/2 hour and 1 hour of drug administration. There was significant difference in blood sugar level when EAECZ 500mg/kg was compared to EAECZ 750mg/kg after 1 hour of drug administration.

Conclusion: Cinnamomum zeylanicum lowers blood sugar level due to inhibitory effect on gluconeogenesis and promoting peripheral uptake of glucose or insulin released by beta cells.

Keywords: Diabetes mellitus, Cinnamomum zeylanicum, glibenclamide.

INTRODUCTION

Diabetes mellitus is a spectrum of metabolic disorders that arises from myriad of pathogenic mechanisms, all resulting in hyperglycaemia. Major causes of morbidity seen with diabetes are the chronic complications of which that arise from prolonged hyperglycaemia, including retinopathy, neuropathy, nephropathy and cardio vascular diseases.[1]

The International Diabetes Federation projects that 642 million individuals will have diabetes by the year 2040. The countries with the greatest number of individuals with diabetes in 2019 were China(116.4 million), India (77 million), United States (31 million), Brazil (16.8 million) and the Mexico (12.8 million).[2]

The atlas estimates that as of 2020, there are 33 million children in India who are living with overweight and obesity. This works out to an overall prevalence of 9% of overweight and obesity among children below the age of 20 years.[3]

The aetiology of diabetes in India is multifactorial and includes genetic factors coupled with environmental influences such as obesity associated with rising living standards, steady urban migration and lifestyle changes.[4] Emphasis has been given on the underlying molecular mechanisms, the new technologies that have been introduced to facilitate early diagnosis, and the new potential therapies for these complications includes diabetic neuropathy, nephropathy, retinopathy, macrovascular and miscellaneous complications.[5] The drugs used to overcome the insulin resistance like biguanides, thiazolidinediones and alpha glucosidase inhibitors, the major limitation is inability to achieve normoglycaemia by themselves in many patients, especially moderate to severe cases.[6]

Many potential anti diabetic drugs are currently being studied. There is a great interest in inhibitors of protein kinase C because of the implicating activation of this pathway in the development of vascular diabetic complications.[7]

Cinnamomum zeylanicum locally known as 'Ushingsha' in Manipuri and 'Dalchini' in Hindi is a small tree or shurbs.[8] Every part of the plant including bark, leaves, flower, fruits and roots have some medicinal properties. The bark is flavoured and used in bronchitis, diarrhoea, itching, disease of heart and urinary diseases. The oil is also used as emmenagogue, in inflammation, abdominal pains and cold.[9]

Therefore, considering the various claims made so far and in view of present orientation toward the complementary and alternative systems of medicine, it will be an interesting effort to evaluate the effect of Cinnamomum zeylanicum on blood sugar in albino rats and hence, we take up the present study.

Adrenaline mediates the release of glucocorticoids which in turn affect glucose metabolism in liver, reduction in utilisation and are also known to inhibit insulin release by pancreas thereby inducing hyperglycaemia.[10]

MATERIALS AND METHODS

Place and duration of study: The study was conducted in the chemical laboratory of Department of Pharmacology, Regional Institute of Medical Sciences (RIMS), Imphal, Manipur, after getting a clearance from Institutional Animal Ethics Committee, RIMS, Imphal (registration no: 156/GO/a/12/CPCSEA) at the duration of October 2018 to November 2020. The study was non-randomised experimental study conducted on healthy albino rats of either sex obtained from Central animal house, RIMS, Imphal.

Plant extract preparation: The Plant Cinnamomum zeylanicum was identified and authenticated by Botany Department, D.M College, Imphal, having the Acc. No. DM 11:40. The barks were collected during the month of September to October 2018 from the foothills of Langol Range, Imphal, Manipur and cleansed, dried under shade and powdered by mixer grinder. The ethanolic extract of Cinnamomum zeylanicum barks were obtained by using Soxhlet apparatus. The percentage yield was 7%.

Phytochemical analysis: The preliminary phytochemicals analysis of the extract was carried out using standard procedure to identify various constituents.[11-14] It revealed the presence of tannins, flavonoids, saponins, triterpenoids and proteins.

Acute Toxicity Testing: Acute toxicity testing was done in healthy albino rats according to OECD guidelines 423.15 Limit test with 5000 mg/kg was carried out with six animals (three animals per step). The extract was dissolved in 2% gum acacia and freshly prepared extract was given at uniform volume of 1mL/100 g of body weight in a single dose by gavage. After administration of the extract, food was withheld for a further 3-4 hours. Animals were observed once during the first 30 minutes and then for 4 hours daily for 14 days. There was no mortality observed at the dose of 5000 mg/kg till 14 days. So maximum safe dose 500 mg/kg (1/10th of maximum test dose) and 750 mg/kg of EETC were selected for the study.

Experimental animals: 24 healthy albino rats (Wistar strain) weighing between 100-200gm of either sex were used for each experiment after acclimatized to laboratory condition. Pregnant albino rats were excluded. They were divided into 4 groups of 6 animal each and fasted overnight before the experiment. They were maintained at a temperature of 22 ± 10 C in a 12 hr light/ dark cycle with free access to water and standard diet. Glibenclamide 0.5 mg/kg and test drugs were suspended in 2% gum acacia and administered orally to standard and test groups respectively. The control group were given 2% gum acacia in distilled water (DW).

Table 1: Allotment of animals to different groups and their treatment

Groups	Drugs
1(Control)	2% Gum Acacia followed by adrenaline 0.5mg/kg intraperitoneal injection.
2(Standard)	Glibenclamide 0.5mg/kg in 2% Gum Acacia followed by adrenaline 0.5mg/kg intraperitoneal injection
3(Test 1)	EAECZ(500mg/kg in 2% Gum Acacia p.o.) followed by adrenaline 0.5mg/kg intraperitoneal injection.
4(Test 2)	EAECZ(750mg/kg in 2 % Gum acacia p.o.) followed by adrenaline 0.5 mg/kg intraperitoneal injection.

Glucose concentrations were estimated at 1/2hr. and 1hr. after the adrenaline hydrochloride administration.

Blood was collected by cutting the tip of rat tail with proper aseptic, antiseptic condition under general anesthesia (cutting tip).[16]

Estimation of blood glucose level was measured using glucometer (ACCU-CHEK® Active strips in Accu-Chek® Active test meter, Roche Diagnostics, Germany).

Statistical analysis: The results were analysed for statistical significance using One-way ANOVA followed by Bonferroni test using IBM SPSS software version 21(IBM Corp., Armonk, NY, USA). P value less than 0.05 was considered significant.

RESULTS

Table 2: Effect of Cinnamomum zeylanicum on adrenaline induced hyperglycemia

Groups (N=24)	Treatment	Blood glucose(mg/dl)		
		Fasting	1/2hr	1hr
Control	2% Gum Acacia followed by adrenaline 0.5mg/kg intraperitoneal injection	73.17 ± 1.797	160.67 ± 4.18	136.50 ± 4.19
Standard	Glibenclamide 0.5mg/kg in 2% Gum Acacia followed by adrenaline 0.5mg/kg intraperitoneal injection	74.50 ± 1.821	112.67 ± 5.58*	96.33 ± 4.18*
Test 1	EAECZ(500mg/kg in 2% Gum Acacia p.o.) followed by adrenaline 0.5mg/kg intraperitoneal injection.	72.67 ± 2.704	155.83 ± 3.76ϕ Ω	133.00 ± 4.72ϕΩ
Test 2	EAECZ(750mg/kg in 2% Gum Acacia p.o.) followed by adrenaline 0.5mg/kg intraperitoneal injection.	75.33 ± 3.739	128.33 ± 5.377#	104.83 ± 1.25#
One way ANOVA				
Df		3	3	3
F		0.214	18.955	14.807
P		0.885	0.000	0.000

According to table 2, the results were expressed as mean + SEM, n=6, P<0.05 was considered significant
At ½ hour interval
*Indicates P<0.001 and # indicates P <0.01 when compared with control group, ϕ indicates P<0.05 when test 1 group is compared with test 2 group, Ω indicates P<0.001 when compared to standard group.
At 1 hour interval
*Indicates P<0.001 and # indicates p<0.05 when compared to control group, ϕ indicates P<0.001 when test 1 group is compared to test 2, Ω indicates P<0.001 when test 1group compared to standard group.(One way ANOVA followed by Bonferroni test).
Blood glucose levels (mg%) after overnight fasting of the albino rats were 73.17 ± 1.797, 74.50 ± 1.82, 72.67 ± 2.704, 75.33 ± 3.739 for control, standard drug (glibenclamide 0.5mg/kg), test 1(EAECZ 500mg/kg) and test 2 (EAECZ 750mg/kg) treated group respectively.
Blood glucose level after ½ hr of treatment of control (gum acasia followed by adrenaline 0.5mg/kg), standard drug (glibenclamide 0.5mg/kg followed by adrenaline 0.5mg/kg), test 1(EAECZ 500mg/kg followed by adrenaline 0.5mg/kg) and test 2 (EAECZ 750mg/kg followed by adrenaline 0.5mg/kg) were 160.67 ± 4.177, 112.67 ± 5.578, 155.83 ± 3.763 and 128.33 ± 5.377#respectively.
Blood glucose level after 1 hr of treatment of control (gum acasia followed by adrenaline 0.5mg/kg), standard drug (glibenclamide 0.5mg/kg followed by adrenaline 0.5mg/kg), test 1(EAECZ 500mg/kg followed by adrenaline 0.5mg/kg) and test 2 (EAECZ 750mg/kg followed by adrenaline 0.5mg/kg) were 136.50 ± 4.193, 96.33 ± 4.177, 133.00 ± 4.71 and 104.83 ± 1.25 respectively.
At ½ hr: test1(EAECZ 500mg/kg) showed very highly significant(P<0.001) comparing with the standard and significant(P<0.05) comparing with the test2 (EAECZ 750mg/kg) in reduction of blood sugar. Standard (glibenclamide 0.5mg/kg) showed very highly significant(P<0.001) comparing with control and test 2(EECZ 750mg/kg) showed highly significant (P<0.01) compared to control.
At 1hr: test1(EAECZ 500mg/kg) showed very highly significant(P<0.001) comparing with the standard and highly significant(P<0.01) comparing with the test2 (EAECZ 750mg/kg) in reduction of blood sugar. Standard (glibenclamide 0.5mg/kg) showed very highly significant(P<0.001) comparing with control and test 2(EAECZ 750mg/kg) showed significant (P<0.05) compared to control.

DISCUSSION

In the above study, the hypoglycaemic activity of Cinnamomum zeylanicum was evaluated in adrenaline induced hyperglycaemia in albino rats. Glibenclamide is an sulfonylureas group of antidiabetic drugs that enhance insulin secretion by blocking K+ ATP channel. Glibenclamide at the dose of 0.5mg/kg a was used as standard drugs.[6]
Phytochemical constituents of Cinnamomum zeylanicum from the bark gives alkaloids, glycosides, terpenoids, saponins, flavonoids, anthraquinones, phlobatanins, steroids, phenolic, amino acids, proteins, quinones, tanins, reducing sugars.[17] Tannins may be employed medicinally in antidiarrheal, haemostatic, and antihemorrhoidal compounds. They have been also reported to have anti-viral, antibacterial, and antiparasitic effects. Flavonoids are also known to regenerate the damaged β-cells and act as insulin secretagogues. Steroids, terpenoids and phenolic acids are known to be bioactive anti-diabetic components.[18]
Hyperglycaemia was induced by the method adopted by SS Gupta.10 Following an overnight fast, blood sugar of fasting, 1hr and 2hrs blood glucose samples were collected and measured using glucometer after adrenaline 0.5mg/kg intraperitoneally. The test drug at doses of 500mg/kg and 750mg/kg and standard drug glibenclamide 0.5mg/kg was given before adrenaline and significant decreased in blood sugar after 1/2hr of drug administration was evaluated. The result in this present study were comparable to that of Mandlik RV et al.[19] The blood glucose lowering effect of EAECZ after adrenaline may be due to promoting peripheral uptake of glucose or insulin released by beta cells.

CONCLUSION

In the study entitled "Effect of ethyl acetate extract of Cinnamomum zeylanicum on adrenaline induced hyperglycaemia in albino rats", the ethyl acetate extract of Cinnamomum zeylanicum(EAECZ) at the dose of 750mg/kg showed the significant reduction in blood sugar level in adrenaline induced hyperglycaemia. However, more studies are needed to elucidate the exact mechanism of reduction of blood sugar effect offered by its phytoconstituents and its clinical application in lowering of blood sugar.

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