Journal of Applied Pharmaceutical Science Vol. 2 (11), pp. 075-078, November, 2012
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2012.21113
ISSN 2231-3354

Effect of *Cynoglossum zeylanicum* (Vehl ex Hornem) Thunb. Ex Lehm on Oral Glucose Tolerance in rats

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ARTICLE INFO

Article history:
Received on: 15/10/2012
Revised on: 29/10/2012
Accepted on: 12/11/2012
Available online: 29/11/2012

Key words:
OGTT, Diabetic, Jathakkai, Flavonoid, *Cynoglossum zeylanicum.*

ABSTRACT

The effect of ethanol extract of whole plant of *Cynoglossum zeylanicum* on Oral Glucose Tolerance was determined. Ethanol extract of *Cynoglossum zeylanicum* were evaluated for Oral Glucose Tolerance Test (OGTT) in normal and alloxan induced diabetic rats. Oral administration of ethanol extract at doses of 100 &150mg/kg body weight shows that the extract promote glucose uptake. These results suggest that the ethanol extract of *Cynoglossum zeylanicum* whole plant will be useful in the treatment of impaired oral glucose tolerance.

INTRODUCTION

The Oral Glucose Tolerance test (OGTT) measures the body's ability to use a type of sugar, called glucose that is the body's main source of energy. OGTT, a test of immense value and sentiment, in favor of using fasting plasma glucose concentration alone was seen as a practical attempt to simplify and facilitate the diagnosis of diabetes Hyperglycemia is an important factor in the development and progression of the complications of diabetes mellitus (Luzi, 1998).Diabetes mellitus is recognized as one of the leading causes of morbidity and mortality in the world. About 2.5-3% of the world's population suffers from this disease, a proportion which in some countries can reach 7% or more. Hyperglycemia leads to metabolic disorders and various complications (Seghrouchni *et al.,* 2002). According to W.H.O projections, the prevalence of diabetes is likely to increase by 35% currently there are over 150 million diabetic people worldwide and this is likely to increase to 300 million or more by the year 2025. Statistical projections about India suggest that the number of diabetes will rise from 15 million in 1995 to 57 million in the year 2025 making it the country with the highest number of diabetic people in the world (King *et al.,* 1998 & Boyle *et al.*,2001). Moreover, the World Health Organization (WHO) estimates that 80% of people in developing countries depend on traditional medicine for their health needs, and 85% of traditional medicine involves the use of plant extracts. In other words, about 4 billion people in the world rely on the plants as source of drugs. This has led researchers to continue their search for the "miracle drug" for treatment of diabetes from plants. *Cynoglossum zeylanicum* belongs to Boraginaceae family. It is commonly known as "Jathakkai". Decoction prepared from the whole plant is used to arrest vomiting by Badaga community in Nilgiri Biosphere Reserve, Tamil Nadu (Sathyavathi *et al.,* 2011).

However, no systematic attempts have been made to establish scientific basis of beneficial effects of *Cynoglossum zeylanicum* whole plant extracts. To our knowledge no report on the effect of *Cynoglossum zeylanicum* whole plant on experimental diabetic. This study was undertaken to evaluate the effects of ethanol extract of whole plant of *Cynoglossum zeylanicum* on Oral Glucose Tolerance Test in normal rats and diabetic induced rats.

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MATERIALS AND METHODS

Plant material

Whole plant of *Cynoglossum zeylanicum* was collected from Kothagiri, Nilgiri Biosphere Reserve, Western Ghats, Tamil Nadu. With the help of local flora, voucher specimens (VOCB-1048) were identified and preserved in the Ethnopharmacology Unit, Research Department of Botany, V.O.Chidambaram College, Tuticorin, Tamil Nadu for further references.

Preparation of plant extract for phytochemical screening and antidiabetic studies

The whole plant of *Cynoglossum zeylanicum* was shade dried at room temperature and the dried whole plant were powdered in a Wiley mill. Hundred grams of powdered *Cynoglossum zeylanicum* whole plant was packed in a Soxhlet apparatus and extracted with ethanol for 48hrs. The ethanol extracts were concentrated in a rotary evaporator. The concentrated extract was subjected to qualitative test for the identification of various phytochemical constituents as per the standard procedures (Brinda *et al.*, 1981; Lala, 1983) and concentrated ethanol extracts were used for Oral Glucose Tolerance test.

Animals

Normal healthy male Wistar albino rats (180- 240g) were housed under standard environmental conditions at temperature (25±2º C) and light and dark (12/12 h). Rats were fed with standard pellet diet (Goldmohur brand, MS Hindustan lever Ltd., Mumbai, India) and water *ad libitum*.

Acute Toxicity Study

Acute oral toxicity study was performed as per OECD – 423 guidelines (acute toxic class method), albino rats (n=6) of either sex selected by random sampling were used for acute toxicity study (OECD, 2002). The animals were kept fasting for overnight and provided only with water, after which the extracts were administered orally at 5mg/kg body weight by gastric intubations and observed for 14 days. If mortality was observed in two out of three animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for higher doses such as 50,100 and 2000 mg/kg body weight.

Induction of Diabetes in Experimental animal

Diabetes induced rats were administrated by the simple intraperitoneal dose of alloxan monohydrate (150 mg/kg) (Nagappa *et al.*, 2003). Two days after alloxan injection, rats screened for diabetes having glycosuria and hypoglycemia with blood glucose level of 200-260 mg/100ml were taken for the study. All animals were allowed free access to water and pellet diet and maintained at room temperature in plastic cages.

Experimental design

In the present investigation, a total 45 rats were taken and divided into nine groups, the four groups for Oral Glucose Tolerance Test in normal rats (each group 5 rats) and five groups for Oral Glucose Tolerance Test in diabetic induced rats (each group 5 rats).

Oral Glucose Tolerance Test (OGTT) in normal rats

Normal rats were tested for the Oral Glucose Tolerance Test (OGTT) of the various concentrations of *Cynoglossum zeylanicum* extracts and standard drug glibenclamide. Albino Wistar rats of either sex weighing 150-200gm were divided into 4 groups consisting of 5 rats in each group.

Groups:

Group I - Normal control received 0.9% saline

Group II - Ethanol extract of *Cynoglossum zeylanicum* whole plant (100mg/kg p.o)

Group III - Ethanol extract of *Cynoglossum zeylanicum* whole plant (150mg/kg p.o)

Group IV - Standard drug glibenclamide (600µg/kg p.o)

Oral Glucose Tolerance Test (OGTT) in diabetic induced rats

OGTT is carried out in the diabetic induced rats.

Groups:

Group I - Normal control received 0.9% saline

Group II - Diabetic control received 0.9% saline

Group III - Diabetic rats treated with 100mg/kg of ethanol extract of *Cynoglossum zeylanicum* whole plant

Group IV - Diabetic rats treated with 150mg/kg of ethanol extract of *Cynoglossum zeylanicum* whole plant

Group V - Diabetic rats treated with (600µg/kg) of Standard glibenclamide

After 60minutes of drug administration, the rats of normal and diabetic groups were orally treated with 2g/kg of glucose. The blood samples were collected through femoral vein at 0, 30, 60, 90, 120 minutes. Blood glucose level was estimated at various time intervals.

RESULTS AND DISCUSSION

Phytochemical screening of ethanol extract of *Cynoglossum zeylanicum* whole plant revealed the presence of alkaloids, catechin, coumarin, tannin, phenols, saponins, steroid, flavonoid, glycoside and xanthoprotein. Acute toxicity study revealed the non-toxic nature of the ethanol extract of *Cynoglossum zeylanicum* whole plant.

The blood glucose concentration of control and different doses of *Cynoglossum zeylanicum* (100 and 150 mg/kg) extracts were estimated 0, 30, 60, 90 and 120 minutes respectively are shown in Table-1. Drug treated rats suppress its rise in blood glucose level with 100mg/kg and 150mg/kg as compared with vehicle control. Glibenclamide (10mg/kg) showed suppress in blood glucose at 3rd and 4th (Table-1).

Table. 1: : Oral Glucose Tolerance Test in normal rats after treatment with *Cynoglossum zeylanicum* whole plant extract.

Treatment	Dose	Blood glucose level (mg/dl)				
		0-hour	30 minutes	60 minutes	90 minutes	120minutes
Group-I	0.9%Saline	91.85±2.88	134.72±3.83*	152.31±3.29*	121.42±2.19*	82.53±1.53
Group-II	100mg/kg	84.62±1.54	103.14±2.39	123.91±2.63*	92.17±1.72	69.23±1.02
Group-III	150mg/kg	76.29±1.35	118.28±1.66	137.32±2.39*	109.64±1.54	78.27±1.14
Group-IV	600µg/kg	73.72±1.22	109.48±1.54	128.37±1.07*	102.98±1.04	72.53±1.11

Values are mean ±SEM (N=5):*P<0.05, statistically compared to 0 min to other respective groups.

Table. 2: Oral Glucose Tolerance Test in diabetic induced rats after treatment with *Cynoglossum zeylanicum* whole plant extract.

Treatment	Dose	Blood glucose level (mg/dl)				
		0-hour	30 minutes	60 minutes	90 minutes	120 minutes
Group-I	0.9%Saline	67.38±0.96	129.56±1.58*	132.38±2.03	103.93±2.03	83.56±0.94
Group-II	0.9%Saline	201.56±4.52	321.48±4.54***	358.27±3.28***	307.43±5.27***	289.18±6.57**
Group-III	100mg/kg	229.68±5.03	358.22±5.88***	49.27±10.84**	296.48±4.28**	221.69±7.29
Group-IV	150mg/kg	247.99±7.35	318.58±6.83	368.36±4.62	279.47±4.53	203.08±5.88

Values are mean ±SEM (N=5):**P<0.01; ***P<0.001, statistically compared to 0 min to other respective groups.

For Oral Glucose Tolerance Test, the blood samples were analyzed for glucose content at 0, 30, 60, 90 and 120 minutes, respectively. The blood sugar levels of *Cynoglossum zeylanicum* (100 and 150 mg/kg) treated groups were found compared to be diabetic control and the effects were dose-dependent. Group III and IV glucose lowering efficiency between 90-120 minutes and were comparable to diabetic standard shown in Table-2.

Diabetes mellitus of long duration is associated with several complications such as atherosclerosis, myocardial infarction, neuropathy, etc. These complications have long been assumed to be related to chronically elevated blood glucose levels. Diabetes mellitus causes disturbances in the uptake of glucose as well as glucose metabolism. Alloxan-induced hyperglycemia has been described as a useful experimental model to study the activity of hypoglycemic agents (Junod *et al.*, 1964).

The single dosed study of ethanol extract of *Cynoglossum zeylanicum*, doses at 100 and 150mg/kg produced no significant hypoglycemic effect in normal rats but *Cynoglossum zeylanicum* extract (100 and 150mg/kg) significantly decreased blood glucose in alloxan induced diabetic rats. In glucose tolerance test, the ethanol extract of *Cynoglossum zeylanicum* (150mg/kg) decreased blood glucose level in normal and alloxan induced diabetic rats. Thus the extract enhanced glucose utilization and improves tolerance in glucose loaded rats.

The experiment showed that Glucose Tolerance Test (GTT) measures the body ability to use glucose, the body's main source of energy (Gold, 1970). This test can be used to diagnose pre-diabetes and diabetes. Glucose lowering effects were found after oral administration of ethanolic extracts in rats. This may be due to the presence of hypoglycemic flavonoids, terpenes or saponins that also requiring further investigation.

The antihyperglycemic effect of *Cynoglossum zeylanicum* may result from the potentiation of insulin from existing β-cells of the islets of langerhans. The blood glucose lowering effect was compared with Glibenclamide has been used for many years to treat diabetes and stimulates insulin secretion from pancreatic β-cells (Tian *et al*., 1998).

CONCLUSION

It is concluded that administration of ethanol extract of *Cynoglossum zeylanicum* whole plant promotes glucose tolerance. *Cynoglossum zeylanicum* whole plant is gaining much importance in diabetic control, since the phytochemical analysis has shown the presence of potent phytochemicals like flavonoids, glycosides, steroids, tannins, saponin and phenols. Several authors reported that flavonoids, steroids, terpenoids, phenolic acids are known to be bioactive antidiabetic principles (Oliver, 1980). Flavonoids are known to regenerate the damaged beta cells in the alloxan induced diabetic rats and acts as insulin secretagogues (Chakravarthy *et al*., 1980). This evidence suggests that the whole plant of *Cynoglossum zeylanicum* could be beneficial for the protection of allevation of diabetic complications. Further studies need to be carried out to define the active principles present in the ethanolic extract.

ACKNOWLEDGEMENT

The authors are thankful to Dr.R.Sampathraj, Honorary Director, Dr.Samen Clinical Research Laboratory, Thiruppur for providing necessary facilities to carry out this work.

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How to cite this article:

M.Anitha, G.Sakthidevi, S,Muthukumarasamy, V.R.Mohan. Effect of *Cynoglossum zeylanicum* (Vehl ex Hornem) Thunb. Ex Lehm on Oral Glucose Tolerance in rats J App Pharm Sci. 2012; 2 (11): 075-078.