Journal of Pharmaceutical and Biological Research

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Research Article **Open Access**

Effect of Hydroalcoholic Extract of *Orthosiphon Thymiflorus* Leaves on Glycemia of Diabetic Rats

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A B S T R A C T

The present study was aimed to investigate the antihyperglcemic activity of hydroalcoholic extract of *Orthosiphon thymiflorus* leaves (HAEOT) on high fat diet (HFD) - streptozotocin (STZ) induced diabetic rats. Male wistar albino rats were fed with a high fat diet for a period of two weeks prior to the administration of streptozotocin (50mg/kg) intraperitonially. The long term effect of the extract was studied by treating diabetic rats with vehicle (0.5% CMC) and HAEOT (200 and 400 mg/kg b wt) for a period of 28 days. The effects of HAEOT on fasting blood glucose and serum insulin levels were studied. In addition, body weight changes, lipid profile and serum biomarkers of liver and kidney function were also studied. Results revealed that HAEOT-400mg shows excellent anti hyperglycemic activity when compared to lower dose of the extract 200mg and also shows better anti hyperlipidemic activity. The elevated serum urea and creatinine levels also decreased by the treatment with extract. Hence the results justify that *Orthosiphon thymiflorus* possess antidiabetic activity.

Keywords: *Orthosiphon thymiflorus*; Anti-diabetic activity; Streptozotocin

A R T I C L E I N F O

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Article History: Received 24 August 2016, **Accepted** 05 October 2016, **Available Online** 21 December 2016

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Manuscript ID: JPBR3368

PAPER-QR CODE

Citation: *Riyazunnisa D, et al*. Effect of Hydroalcoholic Extract of *Orthosiphon Thymiflorus* Leaves on Glycemia of Diabetic Rats. *J. Pharm. Bio. Res*., 2016, 4(2): 81-85.

1. Introduction

Diabetes is a chronic metabolic disorder of carbohydrates, proteins and fats due to absolute or relative decrease in insulin resistance (Jarald E et al., 2008). It is an epidemic disease with a frequency of 5% around the world (Turben H et al., 2002). In India more than 30 million people are suffering with diabetes mellitus and the frequency is accelerating (Shankar P et al., 2001). As the incidence is alarming and due to adverse effects of synthetic medicine usage, there is a need for the development of indigenous, inexpensive botanical source of antidiabetic crude drug. (Venkatesh S et al., 2003). In traditional system of medicine, plants specified for the treatment of diabetes mellitus have been tested on experimental animals. (Grover JK et al., 2002). One among such ethnomedicinal plants is *Orthosiphon thymiflorus.*

Orthosiphon thymiflorus (Family: lamiaceae) is a medicinal plant, slightly aromatic subshrub commonly seen in India, It is grown in Hills above 600m on the slopes, in crevices of rocks; more numerous by arable lands, etc (Mathew K.M et al., 1983) . Many species of this genus possesses several pharmacological properties like antidiabetic, diuretic, antihepatotoxic antibacterial, hypertensive and antitumor activity. *Orthosiphon thymiflorus* has good antioxidant activity enriched with terpenoids (Sundarammal et al., 2012) and shows inhibitory effect on skeletal muscle contraction (Kavimani S et al., 1998). In the present investigation the hydroalcoholic extract of *Orthosiphon thymiflorus* leaves (HAEOT) were screened for antidiabetic activity in high fat diet (HFD) - streptozotocin (STZ) induced diabetic rats.

2. Material and methods

Plant material

The leaf of *Orthosiphon thymiflorus* were collected from Tirumala hills, Tirumala, Chittoor DT, A.P, India. The plant was identified and authenticated by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati, A.P, and India.

Preparation of extract

After shade drying, leaves of *Orthosiphon thymiflorus* were then blended in to fine powder with the blender and used for the preparation of hydro alcoholic extract. 500 gms of coarsely ground powder of leaves was taken separately and were placed in a large glass chamber. 1550 ml of water and 850 ml of ethanol (95%) was added in a 70:30 ratio to get hydro alcoholic extract of leaf. The glass chamber was closed with a glass lid to prevent evaporation of the menstrum and this system was allowed to stand for 7 days with occasional stirring. The liquid i.e. the menstrum was then strained and the solid residue, called marc, was pressed to recover as much occluded solution as possible. The strained and expressed liquid thus obtained were mixed and clarified by filtration. The filtration was carried out in a beaker using a Whatman's filter paper no 1. 2000 ml of menstrum was obtained for the extract which was stored in a refrigerator at 4°C in a beaker. China dish was used for evaporation of the menstrum. This china dish containing the menstrum was placed on a water bath. After evaporation of the menstrum a yellowish brown colored sticky mass was obtained as the hydro-alcoholic extract. This extract [HAEOT] was stored in a dark colored pre-sterilized airtight container. The same procedure was performed for the remaining menstrum. It was then stored in a refrigerator at 4° C in a dark colored pre-sterilized airtight container until its further use (Sukhdev Swami Handa et al 2008).

Drugs and chemicals

Streptozotocin (STZ) was purchased from Sigma Ul- drich, USA and pioglitazone was a gift sample from Dr. Reddy's Laboratories. All the other chemicals were of analytical grade.

Experimental animals

Male Wistar Albino rats (200-250 gm) were used in the study. Animals were housed individually in polypropylene cages in a ventilated room under ambient temperature of 22 ± 2° C and 45-65 % relative humidity, with a 12 hour light followed by 12 hour dark. All the animals were acclimatized for at least 7days to the laboratory conditions prior to experimentation. Tap water and food pellets were provided ad libitum. Food pellets were with held overnight prior to dosing.

Induction of diabetes mellitus

Wistar albino rats of male sex were fed with high fat diet (HFD) that consists of 20% fat, 46% carbohydrate and protein (w/w). Two weeks later HFD fed rats were administered streptozotocin at a dose of 50mg/kg b wt intraperitonially and allowed to free access to food and water (Reed MJ et al., 1999). Fasting blood glucose levels were measured 3days after STZ administration. The rats with fasting glucose ≥ 200mg/dl were considered diabetic and selected for the study.

Experimental design

Normal and HFD fed –STZ-diabetic rats were divided in to five groups of six in each. Group I: Normal rats treated with 0.05 %CMC (p.o). Group II: Diabetic rats treated with 0.05 % CMC (p.o) .Group III: Diabetic rats administered with pioglitazone (2mg/kg b wt p.o). Group IV: Diabetic rats treated with HAEOT 200mg/kg b wt p.o. Group V: Diabetic rats treated with HAEOT 400mg/kg b wt p.o. The above dosage schedule should be given for 28 days. Blood glucose levels and body weights were monitored on day 1, 7, 14, 21 and 28.

Estimation of biochemical parameters

At the end of the experimental period, rats were fasted overnight and blood was collected by cardiac puncture. The serum samples were analyzed for various bio- chemical parameters lipid profile and serum biomarkers of liver and kidney. The serum insulin was measured by ELISA kit. The rats were sacrificed by cervical dislocation and samples of pancreas, liver and kidney were collected immediately, stored in 10% formalin and send for histological assessment.

Statistical analysis

The statistical analysis were carried out by one way ANOVA followed by Tukey's multiple comparison test for all groups using Graph Pad prism 5.0. The results

were expressed as the mean ± S.E.M.to show variations in a group. Differences are considered significant when *p* value < 0.05.

3. Results and discussion

Effect of HAEOT on body weights of rats

All the rats did not show any significant changes in their body weights and the results were not statistically significant at p<0.05 as illustrated in figure 1.Treatment with pioglitazone and extract did not improve the body weight of diabetic rats.

Effect of HAEOT on fasting blood glucose

The fasting blood glucose levels are in normal range in non diabetic rats until the end of experimental period and is significantly (p<0.05) high in untreated diabetic rats when compared to all other groups. Diabetic rats treated with HAEOT 200 & 400 mg/kg b wt for 28 days period exhibited a significant decrease in fasting blood glucose on day 28 as compared to that of untreated diabetic rats. The results are depicted in table 1.

Effect of HAEOT on serum insulin

In the present study the insulin levels in diabetic rats are almost similar to that of normal rats. Diabetic rats treated with pioglitazone, HAEOT 200 & 400 mg/kg showed decrease in serum insulin levels, while HAEOT 400 mg treated rats exhibited significantly (p<0.05) reduced serum insulin levels similar to that of standard group. The details are shown in figure 2.

Effect of HAEOT on serum lipid parameters

Untreated diabetic rats showed significant hypercholesterolemia, hypertriglyceridemia, elevated LDL-C, VLDL-C and decrease in HDL–C as compared with nor- mal control. Standard dose and test dose of HAEOT 200 & 400mg showed significant results (p<0.001) for all lipid parameters when compared with diabetic group. HAEOT at dose 200mg/kg dose not shown significant decrease in VLDL-C levels compared to that of diseased group as depicted in table 2.

Effect of HAEOT on serum biomarkers of liver and kidney: Serum biomarkers like SGOT and SGPT significantly increased in diseased rats compared to that of normal rats. There was no significant difference among the diabetic groups for SGPT and total proteins after the treatment with standard and test drugs. Extract of both doses 200 & 400 mg shows a very good significant de- crease in creatinine (p<0.001) and urea (p<0.05) levels in diseased group compared to that of standard as shown in table 3.

Discussion:

An immense reservoir of biologically active compounds with various chemical structures and disease preventive properties is the plant kingdom (Builders MI et al., 2012). Hence herbal drugs have received greater attention as an alternative to clinical therapy and the demand for these herbal remedies has greatly increased recently. Their utilization is often based in long term clinical experience.

The present study was aimed to investigate the antihyperglcemic activity of hydroalcoholic extract of *Orthosiphon thymiflorus* leaves (HAEOT) on high fat diet (HFD)- streptozotocin (STZ) induced diabetic rats. Body weights of all the animals were observed on day 1, 7, 14, 21 and 28 of the study period. All the rats including diabetic rats did not show any significant changes in the body weight. There was no mortality or signs of toxic reactions in animals and maintained their health status during the study period. Number of plant have been reported for their hypoglycemic activity and the possible mechanism underlines could be an insulin secretion from β-cell of islets of langerhans or release of bounded insulin or their insulin like actions (Twaij HA et al., 1998).

Hypoglycemic effect of HAEOT may be due one of the above said reasons. In the present study the resultant decrease in insulin levels could probably be due to the insulin sensitizing activity of the extract. An increase in the mobilization of free fatty acids from the peripheral storage area leads to an unusually high concentration of hepatic and plasma lipids in diabetes because hormone sensitive lipase is hindered by the insulin.

The distinct hyperlipidemia that distinguishes the diabetic state is considered as a significant uninhibited measure of lipolytic hormones (glucagon and catecholamine) on the fat storage area (Ravi K et al., 2005). It is stated that a deficiency in lipoprotein lipase activity in diabetics may grant to an important increase of triglycerides in blood with insulin administration; lipoprotein lipase activity is enhanced and leads to reduction of plasma triglyceride concentrations (Lopes- Virella et al., 1983).

HAEOT administration almost reversed these effects as it reduced triglyceride and total cholesterol concentrations, LDL concentration, and enhanced HDL, notably in combination. In this context, HAEOT was found to be as effective as pioglitazone in lowering the plasma lipid profiles in the diabetic rats. Serum urea and creatinine are elevated in diabetic hyperglycemia and are considered as significant markers related to renal dysfunction (Alamdal & Vilstrup et al., 1998). Moreover, the protein glycation in diabetes may lead to muscle wasting and increased release of purine, the main source of uric acid as well as the activity of xanthine oxidase (Anwar & Meki et al., 2003).

Hepatic serum biomarkers like SGOT and SGPT were estimated on day 28 and used for the evaluation of hepatic damage. Standard drug pioglitazone showed elevated levels of SGOT and SGPT which are known to cause hepatic damage. There is no significant differ- ence in total protein content.

4. Conclusion

The present study concealed that *Orthosiphon thymiflorus* is an antihyperglycemic and antihyperlipidemic agent. The root basis for these activities might be due to its enrichment with terpenoids. Further research has to be envisaged on its isolated compounds and on molecular basis.

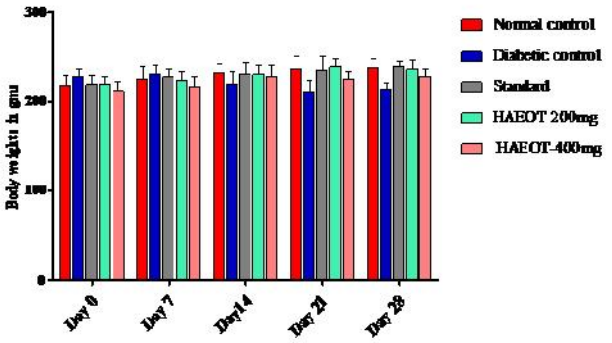

Figure 1: Effect of HAEOT on body weight

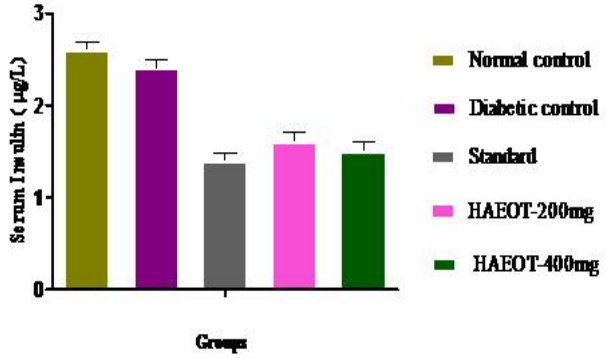

Figure 2: Effect of HAEOT on serum insulin on day 28

Table 1: Effect of HAEOT on blood glucose

Group	Treatment (mg/kg)	Blood glucose (mg/dl)				
		Day 0	Day 7	Day 14	Day 21	Day 28
I	Normal	73.2±1.43	76.02±1.4	75.93±1.84	76.32±1.93	78.83±1.3
II	Diabetic control	264.28±3.04 †	269.29±3 †	264.04±3.09 †	259.34±4.19†	260.16±2.3 †
III	Pioglitazone-2	266.35±3.26	99.32±2.36 ***	88.62±3.27 ***	79.45±2.52 ***	71.81±3.53 ***
IV	HAEOT-200	270.34±1.73	224.75±4.82 **	122.52±3.12 **	99.3±.2.34 ***	94.42±3.07 ***
V	HAEOT-400	269.3±2.52	209.14±4.94 ***	99.03±2.03 ***	86.32±2.12 ***	79.42±1.98 ***

All values are expressed as mean ± SEM; †= p<0.001 compared to normal.*= p<0.05 when compared to diabetic control.

Table 2 Effect of HAEOT on lipid profile

Group	Treatment (mg/kg)	TG (mg/dL)	TC (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	VLDL-C (mg/dL)
I	Normal	78.82±1.92	78.76±2.03	50.13±1.51	15.30±1.49	15.32±1.64
II	Diabetic control	105.21±2.42†	109.14±2.52†	34.28±1.64†	53.57±1.3 †	22.58±1.8 †
III	Pioglitazone-2	84.52±2.06 **	84.54±1.94 **	47.35±1.73 **	23.47±1.68 ***	17.32±1.46 *
IV	HAEOT-200	88.33±2.13 *	88.34±2.43 *	43.25±1.84 *	28.41±2.04 ***	18.39±1.00
V	HAEOT-400	87.24±1.69 **	86.33±2.07 **	46.30±1.22 **	23.56±1.70 ***	18.17±0.99 *

All values are expressed as mean ± SEM; †= p<0.001 compared to normal.*= p<0.05 when compared to diabetic control.

Table 3: Effect of HAEOT on serum biomarkers of liver and kidney

Group	Treatment (mg/kg)	SGOT (IU/L)	SGPT (IU/L)	Urea (mg/DL)	Creatinine (mg/DL)	Total protein (gm/DL)
I	Normal	264.12±4.25	73.56±2.19	64.63±1.56	0.78±0.03	7.72±0.11
II	Diabetic control	296.27±4.23	78.93±2.32	78.28±2.24†	0.97±0.04†	7.02±0.03†
III	Pioglitazone-2	295.38±5.78	81.36±5.73	67.25±3.08	0.76±0.02 **	6.68±0.12
IV	HAEOT-200	189.13±7.36 ***	62.40±4.78	68.09±1.90	0.63±0.04 ***	6.79±0.19
V	HAEOT-400	218.13±6.24**	67.37±2.55	65.13±2.56*	0.58±0.05***	6.70±0.10

All values are expressed as mean ± SEM; †= p<0.001 compared to normal.*= p<0.05 when compared to diabetic control.

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