

BJP

Bangladesh Journal of Pharmacology

Research Article

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Article Info

Received: 6 June 2015
Accepted: 14 July 2015
Available Online: 13 October 2015
DOI: 10.3329/bjp.v10i4.23600

Cite this article:

Ahmed S, Mothana RA, Al-Rehaily AJ. *In vivo* hypoglycemic and anti-diabetic study of *Oncocalyx glabratus*. Bangladesh J Pharmacol. 2015; 10: 830-35.

Abstract

The present study was designed to investigate the hypoglycemic and anti-diabetic potential of *Oncocalyx glabratus*. The extracts and fractions of plant were administrated orally to mice at a dose of 400 mg/kg. The hypoglycemic effect, glucose tolerance test and lipid profile were assessed in treated mice, compared with normal, diabetic and standard drug treated mice. In the hypoglycemic and anti-diabetic study the extracts and fraction reduced blood glucose level and achieved the significant value ($p < 0.001$). The glucose tolerance test was performed in mice loaded with 3 g/kg glucose solution. *n*-Hexane extract and water fraction reduced the glucose level significantly ($p < 0.001$) after 60, 90 and 120 min conversely ethyl acetate and methanol extracts showed the significant ($p < 0.001$) reduction after 90 and 120 min. Ethyl acetate and methanol extracts showed substantial changes in biochemical parameters, compared to the standard drug. So, the study suggested the hypoglycemic and anti-diabetic activity of the title plant.

Introduction

Diabetes mellitus is considered as a metabolic disorder characterized by fast rising of blood sugar level. It is a chronic disease that occurs either by the deficiency in production of insulin by β cells in pancreas (type 1) or by the incompetence of produced insulin (type 2) (WHO, 1999). Even though substantial work has been done in the treatment of diabetes by administration of oral hypoglycemic agents, exploration of new drugs is still required as the existing drugs have many restrictions (Kavishanker et al., 2011). Hence search for new anti-diabetic agents has continued to be an important area of concern. Since time immemorial diabetes has been treated orally by numerous medicinal plants (Gupta et al., 2005).

Family *Loranthaceae* is one of the largest flowering parasitic plants having about 70 genera and 1000 species (Calvin and Wilson, 2006). This family comprises epiphytic and hemiparasitic plants, known as

mistletoe (Loranzi, 2000). Mistletoes are known as "cure all" and have been found beneficial for more than twenty health problems (Adodo, 2004), including diabetes (Obatomi et al., 1994).

Oncocalyx glabratus belongs to family *loranthaceae* is fairly common only locally in Kingdom of Saudi Arabia (Colenette, 1999). In view of the remedial property associated with mistletoes, the title plant was investigated for its hypoglycemic and anti-diabetic potential.

Materials and Methods

Plant material

O. glabratus was collected in February, 2014 from Dos Village, Saudi Arabia and was identified by a taxonomist Dr. M. Yusuf and a voucher specimen of the plant (No. 16320) was deposited at the herbarium of the College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.



Chemicals

Solvents used for the extraction and fractionation were *n*-hexane, dichloromethane, ethyl acetate, methanol, *n*-butanol (Sigma Chemicals, USA) and distilled water. All solvents except *n*-butanol were distilled in the laboratory prior to use. Alloxan was purchased from Sigma (USA) and glibenclamide was purchased from Spimaco (Saudi Arabia). Kits used in the estimation of different biochemical parameters were purchased from different sources.

Extraction process

The dried and coarsely grinded material of the aerial part (691 g) was extracted in sequence with *n*-hexane, dichloromethane, ethyl acetate, methanol and water (2 L × 3) at room temperature for 72 hours (24 hours × 3). The extract was filtered through filter paper (Whatman No. 1) and solvent was evaporated to dryness at 40°C under reduced pressure using Buchi rotavapour which gave *n*-hexane extract (18.9 g), dichloromethane extract (4.6 g), ethyl acetate extract (2.3 g), methanol extract (88.0 g) and water extract (51.0 g). The methanol extract (50.0 g) was dissolved in water and extracted with ethyl acetate (29.3 g), *n*-butanol (12.2 g) and water (7.8 g).

Animals

Male Swiss albino mice (20-25 g) roughly of same age group were procured from the Experimental Animal Care Center, College of Pharmacy, King Saud University, Riyadh. The animals were kept at constant temperature (22 ± 2°C), humidity (55%) and light-dark conditions (12/12 hours light/dark). They were provided with purina chow and free access to drinking water *ad libitum*. The experimental animals were acclimatized for seven days prior being used for the studies.

Evaluation of hypoglycemic and anti-diabetic activity

The dose of 400 mg/kg body weight was given to each animal. Tween 80 solvent (1 mL) was added to each extract/fraction. The experiment was performed according to the procedure described (El Tahir, 2007). Briefly for hypoglycemic study, we prepared eight groups of animals (each group consists of four mice). One group was given normal saline, second was given tween 80, third was given standard hypoglycemic drug, Daonil® (glibenclamide) at a dose of 1 mg/kg body weight and rest of the groups were given extracts/fractions being examined. The extracts/fractions and drugs were given orally to animals after 24 hours fasting. Blood samples were taken before giving the drug (zero time) and 2 hours after giving drugs and glucose blood level was measured using reflotron® instrument. For anti-diabetic screening, diabetes was induced in the overnight fasted experimental animals by injecting alloxan intraperitoneal (150 mg/kg). After 72 hours of injection the animals became diabetic and the experiment were performed as described in hypoglycemic experiment.

Glucose tolerance test in normal mice

Mice were divided into seven groups comprising of four animals each. All animals were fasted before the experiment. Group I was kept as control given 1 mL normal saline, Group II was kept as vehicle control which received 1 mL tween 80, Group III was given glibenclamide (1 mg/kg) and rest of the four groups were given *n*-hexane, ethyl acetate, methanol extracts and water fraction at a dose of 400 mg/kg body weight respectively. The animals were loaded with glucose (3 g/kg p.o) (Hemant et al., 2009) and the blood samples were collected just prior to drug administration and at 30, 60, 90 and 120 min time interval. Serum glucose level was determined immediately by means of glucose estimation kit Refretron (Roche, Germany) to observe the hypoglycemic effects of the tested extracts/fractions relative to control and standard group.

Biochemical parameters

Animals were also treated at a dose of 400 mg/kg body weight for seven days. Then the animals were given alloxan 1 mg/kg and after 72 hours, cholesterol, triglycerides (TG), high density lipoprotein (HDL-C), very low density lipoprotein (VLDL-C) and low density lipoprotein (LDL-C) were measured by using diagnostic kit Refretron (Roche, Germany).

Statistical analysis

Data were expressed as arithmetic means ± standard deviation of the mean (SD) and statistically analyzed by using the one-way analysis of variance (ANOVA) followed by Student's t-test.

Results

Hypoglycemic activity

The effect of different extracts of the plant at a dose of 400 mg/kg body weight on fasting blood sugar level was assessed in normal mice and the results are summarized in Table I. The decrease in glucose level by the *n*-hexane, ethyl acetate and methanol extracts were found to be 41%, 40% and 42% respectively, which was significant reduction in glucose level at the given dose as compared to standard drug. While slight increase in glucose level was observed for the dichloromethane and water extracts in the experimental animals.

Anti-diabetic activity

Changes in blood glucose level in diabetes-induced mice on treatment with different extracts (*n*-hexane, dichloromethane, ethyl acetate, methanol and water) at a dose of 400 mg/kg body weight are shown in Table I. The significant reduction of glucose level by the treatment of *n*-hexane, ethyl acetate and methanol extracts are found to be 48%, 43% and 47% respectively as compared to standard drug.

Table I

Hypoglycemic study of *O. glabratus* on normal and diabetic mice

Treatment	Dose (mg/kg) orally	Glucose levels in normal mice (mg/dL)		% Inhibition	Glucose levels in diabetic mice (mg/dL)		% Inhibition
		Before treatment	2 hours after treatment		Before treatment	2 hours after treatment	
Normal saline		100.1 ± 6.5	108.5 ± 3.6	↑8	307.0 ± 6.0	309.7 ± 6.4	
Vehicle (Tween-80)		108.3 ± 4.1	111.0 ± 6.7		325.3 ± 14.1	316.2 ± 7.9	
Glibenclamide	1	114.0 ± 4.1	45.3 ± 3.0 ^b	↓60	319.8 ± 11.6	155.8 ± 4.9 ^b	↓52
<i>n</i> -Hexane extract	400	102.0 ± 5.4	60.7 ± 2.0	↓41	301.3 ± 9.1	156.0 ± 4.1 ^b	↓48
Dichloromethane extract	400	116.2 ± 3.1	132.0 ± 3.2 ^a	↑14	317.3 ± 5.1	329.2 ± 12.1	↑4
Ethyl acetate extract	400	105.0 ± 6.4	62.9 ± 3.1 ^b	↓40	294.7 ± 12.4	168.2 ± 6.1 ^b	↓43
Methanol extract	400	114.3 ± 4.5	66.5 ± 4.2 ^b	↓42	301.7 ± 7.4	160.8 ± 11.8 ^b	↓47
Water extract	400	106.7 ± 5.3	136.7 ± 5.7 ^a	↑28	306.2 ± 13.6	400.2 ± 10.2 ^b	↑31
Ethyl acetate fraction	400	112.8 ± 2.5	94.5 ± 23.3	↓16	300.0 ± 7.2	293.0 ± 9.8	
<i>n</i> -Butanol fraction	400	110.8 ± 5.6	100.3 ± 7.6	↓10	285.5 ± 20.3	277.5 ± 14.1	
Water fraction	400	116.0 ± 4.4	61.7 ± 2.9 ^b	↓47	316.0 ± 9.4	170.3 ± 8.2 ^b	↓46

Data are mean of 4 male in each group ± SD; ^ap<0.01; ^bp<0.001 student's t-test

While significant increase in glucose level 31% by water extract was observed, on the other hand, dichloromethane extract showed negligible increase in the glucose level.

Based on above results methanol extracts was further fractionated into ethyl acetate, *n*-butanol and water fractions and tested for hypoglycemic and anti-diabetic activity and results are summarized in Table I. From the results it was found that out of three fractions only water fraction showed significant reduction in hypoglycemic and anti-diabetic activity (47% and 46%) respectively.

The hypoglycemic and anti-diabetic results of the tested extracts/fractions were significant and prompted us to investigate the effects of these active extracts/fraction on glucose tolerance test and different biochemical parameters.

Glucose tolerance test

Significant increase in blood glucose level was observed after one hour of administration of glucose (3 g/kg) in normal mice (Figure 1). No significant change in blood glucose level was observed after 30 min of the drug administration. After 60 min *n*-hexane extract and water fraction showed the 36% and 39% reduction respectively in glucose level. On the other hand, after 90 min all the extracts/fraction (*n*-hexane, ethyl acetate, methanol and water) showed the significant reduction

of 41%, 29%, 50% and 54% in glucose level respectively. After 2 hours the reduction in the glucose level in *n*-hexane (46%), ethyl acetate extract (42%), methanol extract (52%) and water fraction (58%) treated groups were observed.

Biochemical parameters

Significant differences were observed in serum lipid profile by the ethyl acetate and methanol extracts (Table II). The percent decrease in cholesterol and triglycerides level by the ethyl acetate extract was 22% and 32% respectively and while percent decrease in cholesterol and triglycerides level by the methanol extract was 32% and 36% respectively. Ethyl acetate and methanol extracts increase the HDL-C percentage by 42% and 80%. Conversely ethyl acetate extract decrease VLDL-C and LDL-C percentage by 32% and 30% respectively and methanol extract decrease VLDL-C and LDL-C percentage by 36% and 50% respectively.

Discussion

In present study, different extracts of *O. glabratus* were examined for hypoglycemic and anti-diabetic activity. Administration of the *n*-hexane, ethyl acetate and methanol extracts out of five extracts showed significant reduction in blood glucose level in both normal and diabetic induced mice at a given dose of

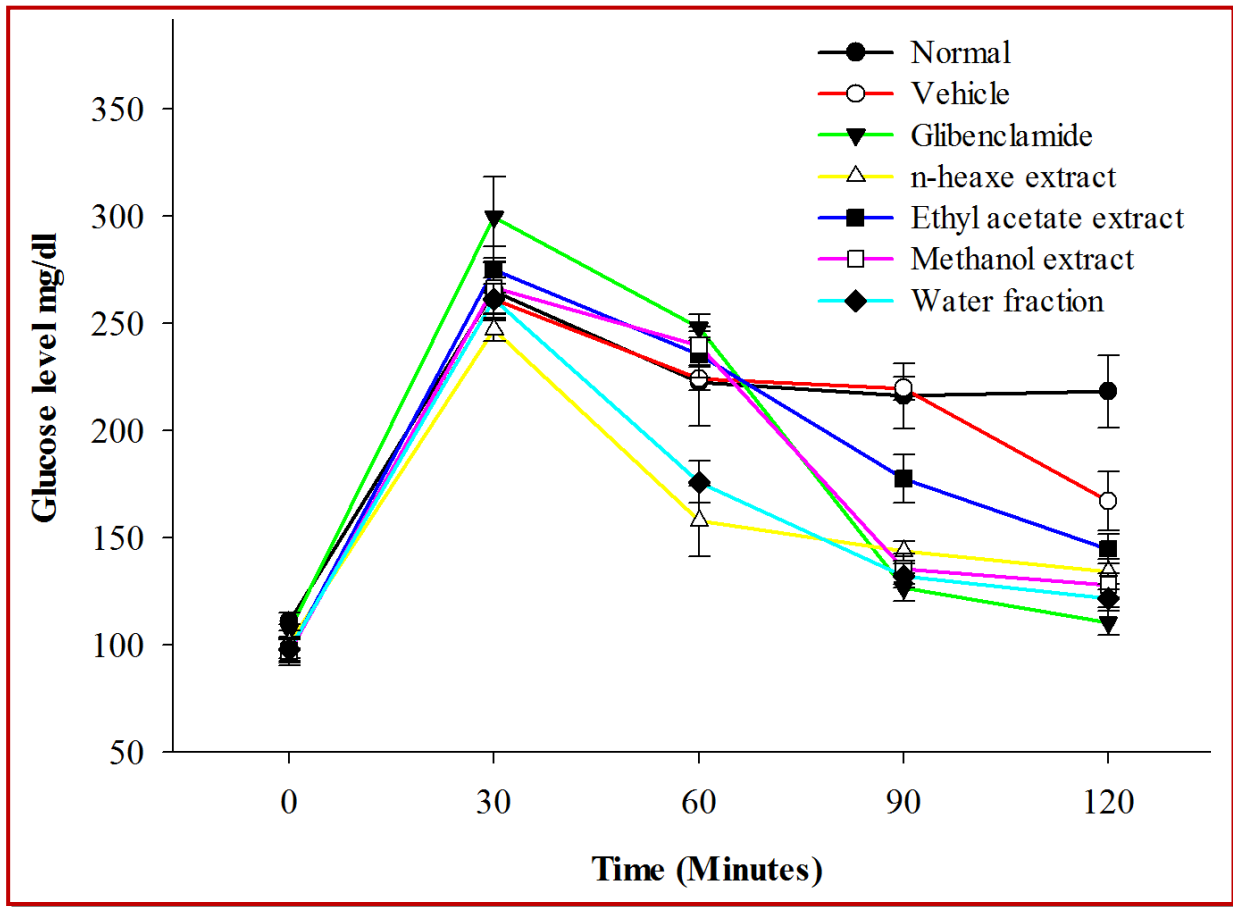


Figure 1: Effects of *O. glabratus* on glucose tolerance test

Treatment	Cholesterol (mg/dL)		Triglycerides (mg/dL)		HDL-C (mg/dL)		VLDL-C (mg/dL)		LDL-C (mg/dL)	
	Mean (SE)	% Change	Mean (SE)	% Change	Mean (SE)	% Change	Mean (SE)	% Change	Mean (SE)	% Change
Normal	94.3 (4.4)		92.8 (6.8)		54.1 (2.3)		18.5 (1.4)		21.7 (1.6)	
Diabetic	195.3 (10.1) ^c		185.8 (6.5) ^c		22.6 (1.6) ^c		37.2 (1.3) ^c		133.5 (9.4) ^c	
Vehicle	189.3 (11.4)	3↓	175.5 (11.6)	6↓	24.8 (1.9)	10↑	34.9 (2.3)	6↓	129.6 (8.6)	4↓
Glibenclamide	113.3 (4.8) ^c	42↓	109.5 (4.2) ^c	41↓	42.8 (2.5) ^c	89↑	21.9 (0.8) ^c	41↓	48.5 (5.6) ^c	64↓
n-Hexane extract	166.0 (7.8) ^a	15↓	163.5 (3.9) ^a	12↓	26.8 (1.2)	19↑	32.7 (0.8) ^a	12↓	106.5 (9.2)	21↓
Ethyl acetate extract	152.0 (5.0) ^b	22↓	126.5 (5.1) ^c	32↓	32.2 (1.5) ^b	42↑	25.3 (1.0) ^c	32↓	94.6 (4.4) ^b	30↓
Methanol extract	132.8 (5.7) ^c	32↓	119.0 (3.3) ^c	36↓	40.7 (2.5) ^c	80↑	23.8 (0.7) ^c	36↓	68.2 (8.2) ^c	50↓
Water fraction	176.0 (7.8)	10↓	166.0 (7.4)	11↓	24.3 (1.5)	8↑	33.2 (1.5)	11↓	115.5 (9.9)	13↓

Data are mean of 4 male in each group ± SD; ^ap<0.01; ^bp<0.05; ^cp<0.001 student's t-test

400 mg/kg body weight which was comparable to that of glibenclamide. Further fractionation of the methanol extracts into ethyl acetate, *n*-butanol and water fraction revealed that water fraction was the active fraction. In glucose tolerance test the active *n*-hexane extract and water fraction reduced the glucose level significantly after 60, 90 and 120 min conversely ethyl acetate extract and methanol extract showed the significant reduction after 90 and 120 min over all maximum reduction in glucose level by the active extracts and fraction was observed after 120 min which was comparable with glibenclamide. As far as the biochemical parameters are concerned the maximum increase (80%) in HDL-C level was observed by methanol extract as compared to other tested extracts/fraction, it also altered the values of cholesterol, triglyceride, VLDL-C and LDL-C in a good way and suggested most active extract. While *n*-hexane, ethyl acetate and methanol extracts and water fraction showed the varying degree of reduction in glucose level this was comparable to that of standard drug. Water fraction showed negligible changes in the cholesterol, triglycerides, HDL-C, VLDL-C and LDL-C as compared to methanol extract itself proven that more activity of methanol extract may be due to synergetic effect.

The significant lowering of blood glucose level in both normal and diabetes induced experimental animals and as well as in glucose tolerance test particularly by the extracts *n*-hexane, ethyl acetate and methanol extract, and water fraction ranges from 40 to 58% was a sign of hypoglycemic activity of the plant. Previous studies (Khan and Shechter, 1991) have suggested that a 25% lowering in blood glucose levels was considered a significant hypoglycemic effect.

A number of mechanisms of action have been proposed for plant extract to exert their effects. Some of the proposed hypothesis related to their effects on the activity of pancreatic β cells, increase in the inhibitory action against insulinase enzyme and increase in insulin sensitivity/insulin like activity. Other mechanism may also be included, increase in peripheral utilization of glucose, increase in the synthesis of hepatic glycogen or decrease of glycogenolysis, inhibition of intestinal glucose absorption, reduction of glycemic index of carbohydrate and reduction of the effect of glutathione (Bnouham et al., 2006).

Moreover, in general, we can say that there are numerous hypoglycemic plants and their chemical structure responsible for the activity varies widely. Therefore, mechanism of action must also be varied. Some of them act by increasing the discharge of insulin and require least number of β cells to exert their action. Conversely other plant extract or their active chemical constituents act by modifying blood glucose metabolism and there are some that seems to correct the complications of diabetes. All are equally important since they potentially can be used for the treatment of different aspects

of diabetes mellitus. So, they are the rich source of new hypoglycemic agents (Ivorra et al., 1989).

O. glabratus have been reported to contain reducing sugar, terpenoids, steroids, flavonoids and tannis, (Waly et al., 2012). Thus hypoglycemic and anti-diabetic activity of the *O. glabratus* may be attributed due the presence of these secondary metabolites which could act as synergistically or independently.

Conclusion

The plant has potential hypoglycemic and anti-diabetic activity beside this it increases the HDL-C level and decrease the cholesterol, triglycerides, VLDL-C, LDL-C and glucose level, may recommended as a potential source of anti-diabetic agent.

Financial Support

King Saud University project No. (RGP-VPP-073)

Ethical Issue

The conduct of experiments and the procedure of sacrifice (using ether) were approved by the Ethics Committee of the Experimental Animal Care Society, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

Acknowledgements

The authors extended their appreciation to the Deanship of Scientific Research at King Saud University for funding the work through the research group project No. (RGP-VPP-073). The assistance of Mr. Malik Sawood Ahmed is thankfully acknowledged.

References

- Adodo A. Nature power: A christian approach to herbal medicine. Benedictine Publication Nigeria, 3rd ed. Lagos, Generation Press Ltd, Surulere, 2004, pp 103-11.
- Bnouham M, Ziyat A, Mekhfi H, Tahri A, Legssyer A. Medicinal plants with potential anti-diabetic activity: A review of ten years of herbal medicine research (1990-2000) Int J Diabetes Metab. 2006; 14: 1-25.
- Calvin CL, Wilson CA. Comparative morphology of epicortical roots in Old and New World Loranthaceae with reference to root types, origin, patterns of longitudinal extension and potential for clonal growth. Flora (Jena). 2006; 201: 51-64.
- Collenette S. Wild flowers of Saudi Arabia. National Commission for Wild Life Conservation and Development, Kingdom of Saudi Arabia, 1999, p 543.
- El Tahir K. A Guide to drug discovery: Directions for pharmacological screening for new synthetic and natural compounds leading to discovery of new medicines. Riyadh, El

- Tahir Publication, 2007, pp 100-05.
- Gupta RK, Kesari AN, Murthy PS, Chandra R, Tandon V, Watal G. Hypoglycemic and anti-diabetic effect of ethanolic extract of leaves of *Annona squamosa* L. in experimental animals. *J Ethnopharmacol.* 2005; 99: 75-81.
- Hemant P, Sharma S, Khajja BS, Jain K, Jain GC. Evaluation of hypoglycemic and antihyperglycemic potential of *Tridax procumbens* Linn. *BMC Complement Altern Med.* 2009; 9: 48.
- Ivorra MD, Paya M, Villar A. A review of natural products and plants as potential anti-diabetic drugs. *J Ethnopharmacol.* 1989; 27: 243-75.
- Kavishankar GB, Lakshmidivi N, Murthy SM, Prakash HS, Niranjana SR. Diabetes and medicinal plants: A review. *Int J Pharm Biomed Sci.* 2011; 2: 65-80.
- Khan CR, Shechter Y. Oral hypoglycemic agents and the pharmacology of the endocrine pancreas. In: Goodman and Gilman's the pharmacological basis of therapeutics. Theodore WR, Alan SN, Taylor P, Gilman AG (eds). 8th edn, New York, McGraw-Hill, 1991, pp 1463-95.
- Lorenzi H. Weeds of Brazil: Terrestrial, aquatic, parasitic and toxic herbs. 3rd ed. Brazil, Nova Odessa, 2000.
- Obatomi DK, Bikomo EO, Temple VJ. Anti-diabetic properties of the African mistletoe in streptozotocin-induced diabetic rats. *J Ethnopharmacol.* 1994; 43: 13-17.
- Waly NM, Ali AE El Din, Jrais RN. Botanical and Biological studies of six parasitic species of family Loranthaceae growing in Kingdom of Saudi Arabia. *Int J Environ Sci.* 2012; 4: 196-205.
- WHO. Expert committee on diabetes mellitus: Diagnosis and classification of diabetes mellitus. Technical report series. Part 1. Geneva, World Health Organization, 1999.
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