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Original Article

Antidiabetic and Hypolipidaemic Effects of *Citrus aurantifolin* Leaves on Diabetic and Hyperglycaemic Rats

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ABSTRACT

Objective:This study aimed to investigate the hypoglycaemic and hypolipidaemic effects of Aqueous and methanol extracts of the leaves of C.aurantifolin inhyperglycaemic (type II) and in Streptozotocin diabetic rats (type I). Method: Type I was induced using Streptozotocin and type II, using a glucose loadingdose. Glucose tolerance test (GTT) was adopted.Result: Both doses of aqueous extract and dose 400 mg/kg of the methanol extract,revealed a significant (P<0.001) persistent hypoglycaemic effect, in type II. Regarding blood cholesterol, aqueous extract showed an early, less persistent significant (P<0.001) hypocholesterolaemic effect in type II. Dose 400 mg/kg of the methanol extract, reduced cholesterol level significantly (P<0.05), at the 2nd and 4th hours, dose 200 mg/kg at the 1st and 2nd hours. In type I, dose 400 mg/kg and Insulin, showed a significant (P <0.05) hypocholesterolemic effect at the 4th hour and 8th hours. Dose 200mg/kg of the methanol extract revealed a significant (P <0.001) reduction at the 4th hour. Concerning blood triglycerides, both extracts, reduced blood triglycerides significantly (P <0.001) and (P<0.05), at the 2nd and 4th hours, respectively. In Type I, the effect of the aqueous extract was highly significant (P <0.001) and persistent. The methanol extract, reduced blood triglycerides significantly (P<0.05) 8 and 12 hours, while the effect of Insulin was significant (P<0.05) and persistent. Conclusion: Based on the findings of this study, it can be concluded that the leaves of C.aurantifolin, proved to be potent hypoglycaemic and hypolipidaemic medicinal plants. This confirms its traditional use.

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1. Introduction

Diabetes mellitus is a common disturbance of the endocrine system and disorder ofmetabolism. Its microvascular (retinopathy, neuropathy, and nephropathy) and macrovascular (heart attack, stroke and peripheral vascular disease) complications) complications have led to significant morbidity and mortality [1].According to World Health Organization the diabetic population is likely to increase up to 300 million or more by the year 2025 [2]. The commonly used therapies for diabetes mellitus include insulin and oral hypoglycaemic agents such as sulfonylureas, biguanides and glinides. Many of them revealed different serious adverse effects; the reason which supported search for more effective and safer hypoglycemic agents [3]. Several plants investigated for their hypoglycaemic effects, confirmed to be effective antidiabetic remedies. Traditional medicines is rich with medicinal plants, thus it encourages the discovery of new antidiabetic drugs [2].

Citrus aurantifolin (Family Rutaceae) is a spine scent tree up to 6 m in height. It is cultivated in various areas of the world. It contains volatile oils, terpeins, hesperidin and vitamin B, citricacid, vitamin C, potassium and calcium citrate and flavonoids. Traditionally, its stem is used as an antiseptic mouth cleanser, the juice is used for treatment of rhinitis and cold and can be added to coffee and tea for abdominal pain [4].

2. Materials and Methods

2.1 Plants

The leaves of Citrus aurantifolinwere brought from a home garden in Omdurman city.

2.1.1 Preparation of the aqueous extract

50 grams of the leaves of C.aurantifolin were weighed, immersed in cooled boiling distilled water and then incubated in a water bath at 60°c for four hours after which they were filtered. The filtrate was freeze dried [5].

2.1.2 Preparation of the methanol extract

60 grams of the bark of C. aurantifolin were weighed and packed in Soxhlet apparatus using 500 ml of petroleum ether followed by chloroform, as solvents to separate lipids and terpenoides. The sample was then extracted using methanol as a solvent to get the polar constituents of the plants. The extract was evaporated till dryness using a rotatory evaporator [5].

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1.2 Animals

In this study adult male Wistar albino rats weighing 70-300 grams, were used. They were obtained fromthe Faculty of Pharmacy, University of Khartoum. Rats were divided into controls, standards and subgroups of samples, each group with ten rats. They were supplied with a standard pellet diet and tap water ad libitum.All rats received humane care according to the guidelines outlined by the Committee for the Purpose of Control and Supervision on Experiments on Animals [6]

2.3 Experimental type II diabetes mellitus:

Rats were subjected to eighteen hours - fast, after which blood samples were obtained from the retro orbital plexus of rats [7] using heparinized capillary tubes (0) time sample. Then all groups of were over- loaded with (2g/kg) of 50% glucose intraperitoneally; the control was given distilled water, the standard was given (10 mg/kg) of Glibenclamide while the tested groups were given (400 and 200 mg /kg) of the aqueous and methanol extracts orally. The 1, 2 and 4 hours samples, were collected for determinationplasma glucose, cholesterol and triglycerides [8].

1.3 Experimental type I diabetes mellitus:

Anintraperitoneal injection of Streptozotocin (STZ), at a dose of (60 mg / kg b wt.), dissolved in citrate buffer at a concentration of (20 mg / ml) to provide a pH of 4.5 [9], was used to induce type I diabetes mellitus. Soluble insulin at a dose of (3U/kg diluted 100 times) was used as standard (reference drug) and samples were collected at 0, 4, 8 and 12 hours. Samples were then analyzed biochemically for glucose, cholesterol and triglycerides.

2.5 Statistical analysis

Data were expressed as means ± standard error of means using paired student's t – test [10]

3. Results and Discussion

Results revealed a significant (P <0.001) and persistent hypoglycaemic effect by both doses of the aqueous extract and dose 400 mg⁄kg of the methanol extract, while dose 200 mg⁄kg of the methanol extract reduced blood glucose significantly (P<0.05) throughout the experiment. In type I diabetic rats, the hypoglycaemic effect of both extracts was slow but highly significant (P <0.001) as it only started at the 4th hour post dosing. Regarding the effect of C.aurantifolin on blood cholesterol, the aqueous extract showed a significant (P <0.001) hypocholesterolaemic effect at the 1st hour in type II hyperglycaemic rats. The effect of the methanol extract occurred at the 1st hour only while dose 400 mg/kg of the methanol reduced cholesterol level significantly (P<0.05), at the 2nd and 4th hours and dose 200 mg/kg at the 1st and 2nd hours post dosing. In type I diabetic rats, dose 400 mg/kg and Insulin, showed a significant (P <0.05) hypocholesterolaemic effect at the 4th hour post dosing, the effect of the extract continued to the 8th hour. The highest significant reduction (P <0.001) was exhibited by dose 200mg/kg of the methanol extract at the 4th hour post dosing. Concerning blood triglycerides, both aqueous and methanol extracts, reduced blood triglycerides significantly (P <0.001) and (P<0.05), at the 2nd and 4th hours, respectively. In Type I diabetic rats, the aqueous extract revealed early onsetand highly significant (P <0.001), persistent reduction, which started since the 1st hour and continued throughout the experiment. The methanol extract, reduced blood triglycerides significantly (P<0.05) 8 and 12 hours post dosing while the effect of Insulin was significant (P<0.05) since the 1st hour and continued throughout the experiment.

Table [3.1]: Effects of the aqueous extract of C.aurantifolin on the blood glucose, cholesterol and triglycerides of hyperglycaemic rats:

Name Of Group	Blood Glucose (mg/dl)			
	Time (hours)			
	0	1	2	4
Control (water)	110±8.9	166.8±7.51	129.4±17	110.4±8.5
Glibenclamide (10 mg/kg)	105±5.8	141.7±32.8	87.38±2.89	86.7±10.45*
C.aurantifolin (400 mg/kg	90±10.6	149.5±9.1*	76±10.5**	75.9±6.9**
C.aurantifolin (200mg/kg)	87.8±2.7	127±11.3*.	87.2±4.8**	100.9±0.73**
Cholesterol (mg/dl)				
Control (water)	75.8±6.7	104.2±16.8	107.2±6.4	83.5±6.4
Glibenclamide (10 mg/kg)	88±8.87	87±8.2	83.6±2.8*	86.4±21.2
C.aurantifolin (400mg/kg)	66.6±4.8	67.8±9.6**	107.2±13.2	83.5±6.4
C.aurantifolin (200mg/kg)	57.4±4.5	71.8±5.6**	68.6±4.4**	67.7±6.8**
Triglycerides (mg/dl)				
Control (water)	108±13.6	118.5±11.2	135.2±19.4	122.2±16.5
Glibenclamide (10 mg/kg)	132.2±10.5	140.6±10.2	134.8±16.3	144.4±9.4
C.aurantifolin (400mg/kg)	11.4 ± 16.7	135.2±19.4	87±22.4**	74±20**
C.aurantifolin (200mg/kg)	131±10.5	115.2±9.7	118.4±5.3	108.5±14.9

(Data are expressed in mean ± standard error of mean)
* = (P<0.05),
** = (P<0. 001)

Table [3.2]: Effects of the methanol extract of C.aurantifolin on the blood glucose, cholesterol and triglycerides of hyperglycaemic rats

Name Of Group	Blood Glucose (mg/dl)			
	Time (hours)			
	0	1	2	4
Control(water)	110±8.9	166.8±7.51	129.4±17	110.4±8.5
Glibenclamide(10mg/kg)	105±5.8	141.7±32.8	87.38±2.89	86.7±10.45*
C.aurantifolin 400mg/kg)	100.2±2.4	82.2±15**	76.4±3.6**	88.5±8.8**
C.aurantifolin (200mg/kg)	105±8.7	138.2±2.1*	119.1±6.7*	91.9±1.6*
Cholesterol (mg/dl)				
Control (water)	75.8±6.7	104.2±16.8	107.2±6.4	83.5±6.4
Glibenclamide(10mg/kg)	88±8.87	87±8.2	83.6±2.8*	86.4±21.2
C.aurantifolin (400mg/kg)	72.6±5.1	104.2±16.8	93.2±21.6*	75.8±4.6*
C.aurantifolin (200mg/kg)	65±3.7	79.8±21.6*	88.2±6.4*	80±6
Triglycerides (mg/dl)				
Control (water)	108±13.6	118.5±11.2	135.2±19.4	122.2±16.5
Glibenclamide(10mg/kg)	132.2±10.5	140.6±10.2	134.8±16.3	144.4±9.4
C.aurantifolin (400mg/kg)	123.8±9.7	131.2±5.6	120.8±9.5	114.4±7.5
C.aurantifolin (200mg/kg)	125.4±19.2	114±22.4	101.4±11.8*	99.4±16.3*

(Data are expressed in mean± standard error of mean)
* = (P<0.05),
** = (P<0. 001)

Table [3.3]:Effects of the aqueous extract of C.aurantifolin on the blood glucose, cholesterol and triglycerides of diabetic rats:

Name Of Group	Blood Glucose (mg/dl)			
	Time (hours)			
	0	4	8	12
Control(water)	286.2±18.2	226.6±12.5	202.8±56.5	277±56.5
Glibenclamide(10mg/kg)	311.8±46.3	221.2±55	259.8±64.9	266.6±49.4
Soluble insulin (3U/kg)	273.6±18.3	286.8±6.6	226±2	222±6.6
C.aurantifolin (400mg/kg)	210.4±19.5	249.6±20.2	184.8±2.2	130.8±2.2**
C.aurantifolin (200mg/kg)	314±44	247.2±52.3	180.6±14.4	160.6±5**
Cholesterol (mg/dl)				
Control (water.)	66±10.2	61±3.7	53±5.4	56.8±5.9
Glibenclamide(10mg/kg)	98.6±18.6	112±17.4	97.4±15	56.8±4.9
Soluble insulin (3U/kg)	40.4±2.5	31.4±3*	34.2±.8	42 ±2.1
C.aurantifolin (400mg/kg)	75.8±12.7	51±4.7*	39.6±3.3*	42.8±1.1
C.aurantifolin (200mg/kg)	65.6±13.7	87.4±13	48.8±.8	47.2±.7
Triglycerides (mg/dl)				
Control (water)	247.2±35.7	227.8±20.3	187±15.3	284±54.3
Glibenclamide(10mg/kg)	123.2±22.5	222.4±52.3	247±55.9	230.4±61.5
Soluble insulin (3U/kg)	57.8±12.8	33.2±4.8*	33.6±2.5*	42±6.5*
C.aurantifolin (400mg/kg)	122.2±14.3	55.8 ±12.8**	43.4 ± 8**	27.4 ± 0.8*
C.aurantifolin (200mg/kg)	143.2 ± 36.6	24.3 ± 37**	32.4 ±37**	44.4 ± 5.3**

(Data are expressed in mean± standard error of mean)
* = (P<0.05),
** = (P<0. 001).

Table [3.4]: Effects of the methanol extract of C.aurantifolin on the blood glucose, cholesterol and triglycerides of diabetic rats:

Name of Group	Blood Glucose (mg/dl)			
	Time (hours)			
	0	4	8	12
Control (water)	286.2±18.2	226.6±12.5	202.8±56.5	277±56.5
Glibenclamide(10mg/kg)	311.8±46.3	221.2±55	259.8±64.9	266.6±49.4
Soluble insulin (3U/kg)	273.6±18.3	286.8±6.6	226±2	222±6.6
C.aurantifolin (400mg/kg)	277.8±22.7	233.2±14.7	179.8±16.6	168.4±8.6**
C.aurantifolin (200mg/kg)	224.8±10	223.6±6.8	201±41.6	171.4±29.8**
Cholesterol (mg/dl)				
Control (water. 10ml/kg)	66±10.2	61±3.7	53±5.4	56.8±5.9
Glibenclamide(10mg/kg)	98.6±18.6	112±17.4	97.4±15	56.8±4.9
Soluble insulin (3U/kg)	40.4±2.5	31.4±3*	34.2±.8	42±2.1
C.aurantifolin (400mg/kg)	58.8±5.9	33±2.7*	49.2±5.9	41±5.1
C.aurantifolin (200mg/kg)	63.4±11	48±5.4**	69±5.4	51.2±5
Triglycerides (mg/dl)				
Control (water)	247.2±35.7	227.8±20.3	187±15.3	284±54.3
Glibenclamide(10mg/kg)	123.2±22.5	222.4±52.3	247±55.9	230.4±61.5
Soluble insulin (3U/kg)	57.8±12.8	33.2±4.8*	33.6±2.5*	42±6.5*
C.aurantifolin (400mg/kg)	210.2 ± 51.2	383.4 ±16.2	165.2 43.7*	156.8±43.7**
C.aurantifolin (200mg/kg)	179.8 ± 6.5	195 ± 57.9	165.2±61.4*	163.2±43.1**

(Data are expressed in mean± standard error of mean)
* = (P<0.05),
** = (P<0. 001)

Based on the fact that diabetes is a leading cause of morbidity and mortality in the world, there is currently an active search for antidiabetic drugs with greater effectiveness and fewer less adverse side effects [11]. The currently used antidiabetic therapy is mostly depending on synthetic drugs that very often have undesirable effects. Thus, there is a continuous need to develop new and better pharmaceuticals as alternatives for the management and treatment of this disease. Hypoglycaemic compounds of natural sources, can begood alternatives to synthetic drugs or reinforcements to currently used treatments. Their ingestion in everyday diet, is a huge advantage. The study of natural products as potential antidiabetics, is recently being paid more attention [12].

The number of plant species, used ethno-pharmacologically or experimentally to treat symptoms of diabetes mellitus, is more than 1200, they represent more than 725 genera in 183 families. From the review it was suggested that, plant showing hypoglycemic potential mainly belongs to the families Leguminoseae, Lamiaceae, Liliaceae, Cucurbitaceae, Asteraceae, Moraceae, Rosaceae and Araliaceae. The most active hypoglycaemic plants are Allium sativum, Gymnema sylvestre, Citrullus colocynthis, Trigonella foenum greacum, Momordica charantia and Ficus bengalensis [13]. The biologically active components of hypoglycaemic plants include; flavonoids, alkaloids, glycosides, polysaccharides, peptidoglycans, steroids and terpenoides[14]. In this study, Phytochemical screening of Citrus aurantifolin resulted in presence of sterols, alkaloids, flavonoids, saponin, cyanogenic glycosides and coumarins, another study revealed presence of volatile oil, hesperidin, vitamin B, vitamin C, and potassium and calcium citrate. On the basis of the above evidences it is possible that the presence of alkaloids, saponin, sterols, volatile oils, glycosides, coumarins and flavonoids were responsible for the observed anti diabetic effect of C.aurantifólin. These findings agreewith a previous study which reported that,flavonoids, alkaloids, glycosides, polysaccharides, peptidoglycans, steroids and terpenoides are the biologically active components of plants with hypoglycaemic plants [15].

The ethanol extract of G. montanumleaves, indicated a positive therapeutic agent for Diabetes [16].

In studying the hypoglycaemic effect of C.aurantifolin, doses (400 and 200 mg/kg) of the aqueous extract reduced blood glucose of hyperglycaemic rats significantly (P<0.05), (P<0. 001) and (P<0. 001) at hours 1, 2 and 4 post dosing respectively as shown in table (3-1). Regarding the methanolextract, dose (400 mg/kg) resulted in a significant reduction in blood glucose (P<0. 001) and (P<0.05), one and two hours respectively post administration of the extract. Dose (200 mg/kg) showed a significant lowering of blood sugar (P<0.05), one hour after administration of extract as shown in table (3-2). In diabetic rats the two doses of the aqueous extract reduced blood glucose significantly (P<0.05), (P<0.001), 4 and 8 hours after extract administration respectively, table (3-3), while both doses of the methanol extract showed a significant hypoglycaemic effect (P<0.05) 12 hours post dosing. Neither the reference drugs glibenclamide nor insulin reduced blood glucose, as shown in table (3-4).

As indicated previously, the hypoglycaemic effect of Citrus aurantifolin, is attributed to presence of flavonoids, alkaloids, glycosides, polysaccharides, peptidoglycans, steroids and terpenoides.Thus the folk use of this plant may be validated by this study. The leaves of this plant seems to have a promising value for the development of potent phytomedicenes for diabetes.

Conclusion:

The findings of this research revealed pronounced hypoglycaemic effect of Citrus aurantifolin leaves in lowering glucose level of hyperglycaemic and hyperlipidaemic rats. Furthermore, it proved to be a very effective hypolipidaemic agent. Based on these findings, it can be concluded that Citrus aurantifolin is a promising medicinal plants and its traditional use as an antidiabetic and lipid lowering agent, is confirmed experimentally.

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