

Antihyperglycemic Activity of *Carthamus oxyacantha* growing in Saudi Arabia; an *in vitro* and *in vivo* Study

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Aldossary *et al.*: Pharmacological effects of *Carthamus oxyacantha*

The main objective of the current study was to investigate the *in vitro* and *in vivo* antidiabetic activity of methanol extracts *Carthamus oxyacantha* leaves and corollas. *In vitro* antidiabetic assays such as inhibition of α -amylase enzyme, non-enzymatic glycosylation of hemoglobin and glucose uptake in yeast cells were carried out. The results of α -amylase inhibition assay revealed that the IC₅₀ of methanol extracts of leaf and corolla were almost same (58.5 and 57.1 μ g/ml, respectively) when compared with positive control acarbose (45.3 μ g/ml). Glycosylation of hemoglobin was inhibited and the methanol extract of corolla showed an IC₅₀ value of 61.7 μ g/ml, while methanol extract of the leaf showed 93.9 μ g/ml when compared to the positive control, α -tocopherol (35.4 μ g/ml). On the other hand, the percent glucose uptake in yeast cells caused by methanol extracts of leaf and corolla was distinctly greater when compared to that of metformin. *In vivo* antidiabetic study revealed that the two methanol extracts at a dose of 200 mg/kg reduced the fasting plasma glucose level (94.6 \pm 4.4 and 102.1 \pm 5.5 mg/dl, respectively) when compared to that of glibenclamide (79.4 \pm 3.4 mg/dl, p<0.001). The results from this study indicated that *Carthamus oxyacantha* leaf and corolla methanol extracts exhibited considerable *in vitro* and *in vivo* antidiabetic activities, which would make *Carthamus oxyacantha* a potential antidiabetic agent.

Key words: *Carthamus*, antidiabetic, α -amylase, hemoglobin, yeast cells, streptozotocin

Natural products have aided humans since long ages as plants are considered untapped resources of medicinally essential bioactive constituents^[1]. On the other hand, the usage of synthetic drugs may cause several side effects such as vomiting, diarrhea, fluid retention and allergic reactions^[2] as well as could bring about possible resistance of many microbes. Moreover, synthetic drugs are usually more expensive. Hence, natural products could serve to play a significant role in the development of cheaper and effective alternate drugs. The constituents present in medicinal plants are used therapeutically as antidiabetic, chemotherapeutic and for a plethora of other applications^[3]. Diabetes mellitus (DM) is a metabolic disease manifested by elevated levels of glucose in blood. It can be generally

divided into diabetes mellitus type I and diabetes mellitus type II (T2DM). T2DM represents about 90-95 % of all cases of diabetes^[4]. T2DM is considered one of the main international health concerns. T2DM affects around 422 million people all over the world^[5]. Prediabetes and diabetes prevalence and complications are growing in a bothersome way. By 2035, it is anticipated that about 592 million people will suffer from DM^[6]. The treatment of T2DM is

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currently achieved through conventional drugs that are effective in controlling diabetes to some extent but are accompanied with some undesirable effects^[7]. There are some enzymes like α -amylase and other glucose uptake mechanisms by cells could manage the level of blood glucose in our body and maintain the blood sugar level at physiological level^[8].

Asteraceae is one of the largest families of flowering plants. It includes more than 1000 genera and about 10 000 species distributed over all parts of the world^[2]. The genus *Carthamus*, one of family Asteraceae, includes 25 species. Reviewed literature showed that many of *Carthamus* species have been used to treat many diseases and complaints such as, cough, typhoid fever, throat disorders, cardiovascular problems, swelling, menstrual disorders, antiinflammatory, antioxidant, calcium antagonist and anticoagulant, and also used in folk medicine as sedative and anticancer herbal product^[9]. The flowers, seeds and oils of *Carthamus oxyacantha* (*C. oxyacantha*), have been reported to possess a diversity of biological activities ranging from local to systemic actions^[8]. The seeds of *C. oxyacantha* are used for treating diabetes in some parts of the Pakistan. However, there is no detailed record available about this important potential effect of this plant^[10]. Therefore, the current study was conducted to assess the *in vitro* antidiabetic potential of leaves and corollas of *C. oxyacantha* by studying their α -amylase inhibitory activity, effects on inhibition of glycosylation of hemoglobin and glucose uptake by yeast cells. A confirmatory *in vivo* study was also conducted with these extracts of *C. oxyacantha* in streptozotocin (STZ)-induced diabetic rats to evaluate their potential hypoglycaemic effects.

C. oxyacantha was collected from Al-Hasa, eastern region of Saudi Arabia. Leaves and corollas of plant were collected and air-dried according to the standard protocols. The plant under investigation was authenticated at the El-Zohria Botanical Garden, Giza, Egypt. A voucher specimen was kept in the Department of Pharmaceutical Sciences, College of Clinical Pharmacy, King Faisal University, Al-Hasa, Saudi Arabia (02-16-Apr-CO).

The powdered air-dried leaves and corollas of *C. oxyacantha*, 300.0 and 100.0 g, respectively were exhaustively extracted twice (each for 5 d) using 3 l of 70 % MeOH/H₂O applying cold maceration technique at room temperature to protect the potential active ingredients from being destroyed. The solvent mixture

was removed through distillation under vacuum using a rotavapor and the dried extracts were directly freeze-dried to give the total methanol extracts of the leaf and the corolla weighting 22.4 and 4.1 g, respectively, which were stored at -20° for the next steps^[9].

Male Wistar rats weighing 150-210 g were kept in quarantine for 2 w under standard husbandry conditions (27°, relative humidity 65±10 %) under 12 h dark and light cycle and were given standard food and water *ad libitum*^[11]. All of the experiments done in this study were approved by the Animal Ethics Committee of King Faisal University (KFU-REC/2018-10-03). Acarbose, glibenclamide, STZ, metformin, gentamycin, α -amylase from porcine pancreas, hemoglobin porcine and α -tocopherol were purchased from Sigma Aldrich (ST. Louis. Mo, USA). Solvents used for extraction and assays were all of analytical grade.

The assay mixture was prepared to contain 0.02 M sodium phosphate buffer (200 μ l), α -amylase enzyme (20 μ l, 2 unit/ml) together with different plant extracts in the range of concentrations 20-100 μ g/ml. Then, it was incubated for 10 min at room temperature followed by the addition of 200 μ l of 1 % starch suspension to all the tubes containing reaction mixture. The reaction was later terminated by the addition of 400 μ l of 3,5-dinitro salicylic acid colour reagent. Then the tubes were kept in boiling water bath for 5 min, cooled to room temperature and diluted with 15 ml of distilled water. The absorbance of each reaction mixture was measured at 540 nm. Control mixture reactions were also prepared accordingly without addition of extracts of plant under investigation and were compared with the test samples containing concentration of different plant extracts (20-100 μ g/ml) freshly prepared in dimethyl sulfoxide. The results were expressed as % inhibition of activity calculated using the following Eqn., % inhibition = (Abs(control)–Abs(extract))/Abs(control)×100, where, Abs (control) is the absorbance of the control reaction (containing all reagents except the test sample) and Abs (sample) is the absorbance of different plant extracts^[12,13]. The IC₅₀ values (concentration causing 50 % inhibition of the enzyme activity) of the plant extracts were determined. Acarbose, which is used to treat T2DM, was employed as a positive control in the concentrations range of 20 to 100 μ g/ml^[14]. Experiments were performed in triplicate.

Solutions of glucose (2 %), hemoglobin (0.06 %) and gentamycin (0.02 %), were freshly prepared in phosphate buffer (0.01 M, pH 7.4). One milliliter of

each of above mentioned solution was mixed. One milliliter of each concentration of different plant extracts (20-100 µg/ml) was added to the prepared mixture. Then, the reaction tubes were incubated in a dark place at room temperature for three days. The degree of glycosylation of hemoglobin was obtained colorimetrically at 520 nm where the percent inhibition was calculated applying the formula, % inhibition = (Abs(control)–Abs(extract))/(Abs(control))×100. The IC₅₀ values of the plant extracts were determined. α-Tocopherol was used as a standard drug^[12-14]. Experiments were carried out in triplicate.

Commercial baker's yeast (*Saccharomyces cerevisiae*) was prepared for experiment by repeated washing and centrifugation (3000×g, 5 min) in distilled water until the supernatant fluids were clear. A concentration of 10 % (v/v) suspension of supernatant fluid was prepared. Concentrations of different plant extracts (20-100 µg/ml) were added to 1 ml of freshly prepared 5, 10 and 25 mM glucose solution and incubated for 10 min in an incubator at 37°. Reaction was initiated by the addition of 100 µl of freshly prepared yeast suspension, vortexed and further incubated at 37° for 1 h. Then the tubes containing the reaction mixture were centrifuged at 2500×g, for 5 min and the remaining glucose was determined in the supernatant. The % increase in glucose uptake by yeast cells was calculated according to the formula, glucose uptake % = (Abs(control)–Abs (extract))/(Abs(control))×100^[12,15-18]. Metformin was used as positive control^[12]. Measurements were carried out in triplicates.

Acute toxicity testing was performed for both leaves and corollas extracts, were studied where the rats received ascending oral doses up to 2000 mg/kg of each extract and signs and symptoms of toxicity were observed for the next 48 h^[19].

Diabetes was induced by an intraperitoneal (ip) injection of STZ dissolved in 0.1 M cold citrate buffer (pH=4.4) at a dose of 60 mg/kg. On the third day after STZ injection, fasted blood glucose levels were measured by hand-held glucose monitoring (Bayer Contour). Only rats with serum glucose levels of 190-200 mg/dl were selected and considered diabetic animals^[20]. The animals were segregated into 5 groups of 6 rats each. Group I served as normal control rats, administered drinking water and 0.1 M cold citrate buffer (pH=4.4) daily for 12 d; group II comprised of diabetic control rats, administered drinking water daily

for 12 d; group III diabetic rats were administered leaf extract (200 mg/kg) for 12 d; group IV diabetic rats were administered corolla extract (200 mg/kg) for 12 d; and group V diabetic rats were administered glibenclamide (0.25 mg/kg) for 12 d. The fasting glucose levels were determined on d 1, 5, and 12 of extracts administration^[19-21].

Data were expressed as mean±SE. To analyse the differences between groups, statistical analysis was performed using the one-way ANOVA followed by post-hoc Tukey's test using a computer soft program SPSS v.20.

The *in vitro* α-amylase activity measurements demonstrated that both leaf and corolla methanol extracts of *C. oxyacantha* has the potential to inhibit α-amylase activity. α-Amylase inhibitory potential was compared using the IC₅₀ values (Table 1). It was found that the α-amylase inhibitory activity of both leaf and corolla extracts were almost the same (IC₅₀ 58.5 and 57.1 µg/ml, respectively). Acarbose, the positive standard showed an IC₅₀ value of 45.3 µg/ml under similar conditions.

Inhibition of non-enzymatic glycosylation of hemoglobin produced by both leaf and corolla extracts of *C. oxyacantha* were compared using the IC₅₀ values (Table 2). Corolla methanol extract showed an IC₅₀ value of 61.7 µg/ml compared to the positive control, alpha-tocopherol's IC₅₀ value of 35.4 µg/ml. Leaf methanol extract inhibited with a IC₅₀ of 93.9 µg/ml.

The effect of both leaf and corolla extracts of *C. oxyacantha* on transportation of glucose through the yeast cell membrane was demonstrated in an *in vitro* model comprising of freshly prepared mixture of yeast cells and glucose solution of different strengths (5, 10, 25 mM) in the presence of extracts at different concentrations ranging from 20 to 100 µg/ml (Table 3).

TABLE 1: α-AMYLASE INHIBITORY EFFECT OF C. OXYACANTHA EXTRACTS

Concentration (µg/ml)	Percent inhibition		
	Leaf methanol extract	Corolla methanol extract	Acarbose
20	22.7±1.0	23.9±1.1	32.2±1.1
40	37.7±1.3	33.7±1.4	43.8±1.3
60	57.2± 1.1	60.4±1.3	64.9±2.3
80	63.8±1.0	66.8±0.9	75.5±1.4
100	73.5±0.8	75.1±1.1	81.1±1.3
IC ₅₀ (µg/ml)	58.5	57.1	45.3

No toxicity or death was observed in the experimental rats. Hence 200mg/kg (1/10 of the 2000mg/kg) was selected as a maximum safety dose. The effects of both leaf and corolla methanol extracts of *C. oxyacantha* on fasting blood glucose levels of diabetic rats were presented in Table 4. In diabetic rats, as shown in Table 4, both leaf and corolla methanol extracts of *C. oxyacantha* and glibenclamide produced significant time-dependent hypoglycaemic activity, compared with the diabetic control group at each time point ($p < 0.001$).

α -Amylase is one of the enzymes responsible for the hydrolysis of α -oriented bonds of polysaccharides and oligosaccharides such as starch, glycogen and other macromolecules of α -bond linked monosaccharides to disaccharides and finally to glucose^[22-25]. Both leaf and corolla methanol extracts of *C. oxyacantha* showed promising α -amylase inhibitory assay, which suggested that *C. oxyacantha* might be effective in slowing down hydrolysis of starch to reduce glucose availability.

In vitro non-enzymatic glycosylation of hemoglobin method is one of the important assays to judge control of diabetic state. The hemoglobin present in RBCs has an affinity to bind to glucose. The greater the glucose level in blood, more amount of glucose-bound (called glycosylated) hemoglobin will be formed. Such glucose

hemoglobin association is to some extent stable and stays for 1-2 mo (the life-span of red blood corpuscles)^[25,26]. Consequently presence of higher concentration of glycosylated hemoglobin is a sure guide to the higher concentration of glucose in the blood. Normally, the percentage of glycated hemoglobin should not be exceeding 12 %. The current study demonstrated good activity of corollas methanol extract (half that of positive control, α -tocopherol) in preventing such binding of glucose to surface proteins of erythrocytes. On the other hand, leaves methanol extract expressed low IC_{50} when compared to positive control.

In the glucose uptake in yeast cells model, transport of glucose across yeast cell membrane has been used efficiently as *in vitro* screening method for evaluation of hypoglycemic effect of natural derived products^[17,27,28]. After treating the yeast cells with both leaf and corolla methanol extracts of *C. oxyacantha*, the results revealed that corolla methanol extract exhibited activity at all levels of glucose concentrations used. The minimum increase was 15.3 ± 1.1 % at 20 μ g/ml and maximum increase was 49.9 ± 1.6 % at 100 μ g/ml concentration. On the other hand, leaf methanol extract showed lesser activation when compared to that of corolla extract at all glucose concentrations. The minimum increase was 3.7 ± 0.1 % at 20 μ g/ml and the maximum increase was 21.5 ± 1.5 % at 100 μ g/ml concentration. The results clearly indicated that both leaf and corolla methanol extracts had noticeable efficacy in increasing the glucose uptake by yeast cells as compared to the standard drug metformin 19.1 ± 1.0 % at 20 μ g/ml and 91.4 ± 0.7 % at 100 μ g/ml.

The fundamental mechanism underlying elevated blood sugar in DM involves over-production and decreased utilization of glucose by the tissues. In the current study, the difference observed between the initial and final fasting plasma glucose levels of different groups of rats under investigation, revealed a significant elevation in blood glucose in the diabetic control group as compared to normal animals, at the end of the

TABLE 2: EFFECT OF C. OXYACANTHA EXTRACTS ON NON-ENZYMATIC GLYCOSYLATION OF HEMOGLOBIN

Concentration (μ g/ml)	Percent inhibition		
	Leaf methanol extract	Corolla methanol extract	α -Tocopherol
20	14.5 \pm 1.1	28.8 \pm 0.6	38.8 \pm 0.5
40	22.4 \pm 0.9	35.4 \pm 1.0	49.3 \pm 0.6
60	38.9 \pm 1.3	49.5 \pm 1.3	71.6 \pm 0.6
80	43.5 \pm 0.8	62.7 \pm 1.2	81.0 \pm 1.0
100	50.9 \pm 1.1	68.8 \pm 0.7	82.7 \pm 1.6
IC_{50} (μ g/ml)	93.9	61.7	35.4

Values were expressed as mean \pm SE (standard error) of n=3 independent experiments

TABLE 3: EFFECT OF C. OXYACANTHA ON GLUCOSE UPTAKE BY YEAST CELLS

Test concentration (μ g/ml)	Percent glucose uptake at different glucose concentrations								
	Leaf methanol extract			Corolla methanol extract			Metformin		
	5 mM	10 mM	25 mM	5 mM	10 mM	25 mM	5 mM	10 mM	25 mM
20	9.5 \pm 1.1	4.9 \pm 0.9	3.7 \pm 0.1	18.9 \pm 0.8	16.7 \pm 1.4	15.3 \pm 1.1	24.3 \pm 0.3	22.1 \pm 0.7	19.1 \pm 1.0
40	13.5 \pm 1.6	11.7 \pm 0.7	8.5 \pm 0.5	21.9 \pm 0.8	20.3 \pm 1.3	15.8 \pm 1.6	53.1 \pm 0.4	49.1 \pm 0.3	38.0 \pm 0.8
60	16.1 \pm 0.6	12.7 \pm 0.8	10.8 \pm 1.0	30.6 \pm 0.6	20.7 \pm 1.8	16.4 \pm 1.9	78.0 \pm 0.3	74.9 \pm 0.5	61.4 \pm 0.9
80	18.4 \pm 0.4	13.3 \pm 0.3	8.4 \pm 0.6	40.2 \pm 2.0	28.9 \pm 0.7	28.4 \pm 1.0	83.6 \pm 0.6	78.4 \pm 1.4	70.9 \pm 0.7
100	21.5 \pm 1.5	12.4 \pm 1.1	11.8 \pm 1.0	49.9 \pm 1.6	33.2 \pm 1.2	29.7 \pm 2.0	91.4 \pm 0.7	84.9 \pm 0.9	80.2 \pm 0.4

Values were expressed as mean \pm SE (standard error) of n=3 independent experiments

TABLE 4: RESULTS OF THE *IN VIVO* STUDY ON STZ-INDUCED DIABETIC RATS BY *C. OXYACANTHA*

Groups	Fasting plasma glucose concentration (mg/dl)		
	Day 1	Day 5	Day12
I- Normal control	79.9±1.2	80.9±0.8	81.6±1.1
II- Diabetic control (streptozotocin, 55 mg/kg)	196.8±2.4	198.18±1.6	211.3±2.5
III- Diabetic+leaf methanol extract (200 mg/kg)	200.3±2.6	114.2±1.8*	94.56±1.8*
IV- Diabetic+corolla methanol extract (200 mg/kg)	192.4±1.3	130.2±1.9*	102.1±2.3*
V- Diabetic+glibenclamide (0.25 mg/kg)	196.5±1.5	91.38±1.1*	79.4±1.4*

Values were expressed as mean±SE (standard error), n=6, *significantly different from diabetic control (p<0.001)

twelve-day experimental period. When leaf and corolla methanol extracts of *C. oxyacantha* were administered to diabetic rats, a decrease in plasma glucose level was observed after 12 d (Table 4). During the study it was found that both extracts significantly controlled the blood glucose level in STZ-induced diabetic rats as compared to the diabetic control group (Table 4). However, the corolla methanol extract showed greater antidiabetic activity as compared to the leaf methanol extract.

The *in vitro* examinations conducted revealed a substantial α -amylase inhibitory activity, a reduction in the glycosylation of hemoglobin and an increase in the glucose uptake by the yeast cell by both the leaf and the corolla methanol extracts of *C. oxyacantha*. The *in vivo* studies also showed that both extracts significantly controlled the blood glucose level diabetic rats. It could therefore be conclude from these results that *C. oxyacantha* could serve as a therapeutic agent or as a potential source of novel antidiabetic molecules.

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Conflict of interest:

Authors declare no conflicts of interest.

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