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Research article

Moderate effect of phenolic and alkaloid compounds extracted from *Brassica oleracea* var. capitata leaf on blood glucose level in alloxan-induced diabetic rabbits

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ABSTRACT

Diabetes mellitus is common metabolic disease and present all over the world with high ration. Herbal drugs against this disease are highly interesting and effective in recent years. In current study, we try to evaluate the effect of phenolic and alkaloid compounds on blood glucose level in experimental animals. Here, the water extracted of *Brassica oleracea* var. capitata leaf was ran in GC-Mass to identify the chemical compounds of water extract. The results of GC-Mass identified 18 compounds, from these compound one phenolic compound (Hydroquinone, acetate) and one alkaloid compound [Pyrrolidine, 1-(1-cyclohexen-1-yl)-] was found. In present study, alloxan-induced diabetic rabbits were prepared by injecting rabbit with three doses of alloxan. The alloxan-induced diabetic rabbits were administrated orally with either 0.3 gm/kg phenolic compound or 0.3 gm/kg alkaloid compound. The best results of blood glucose reduction were found when the diabetic rabbits administrated with phenolic compound. The significant reduction of glucose was observed post 4 h. While, the significant reduction of glucose was observed post 24 h in diabetic rabbits that administrated with alkaloid compound. It can be concluded from present study, the phenolic compound that extracted from *Brassica oleracea* var. capitata leaf was highly effective to reduce glucose as compared with alkaloids.

Keywords: Alkaloid [Pyrrolidine, 1-(1-cyclohexen-1-yl)-], Alloxan, Diabetic rabbits, Phenol (Hydroquinone, acetate).

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INTRODUCTION

Diabetes mellitus is now becoming a common metabolic disorder, resulting from the inability of our body's response to high blood glucose levels [1]. Remarkable progress has been achieved in development of synthetic drugs, but investigations are being carried out to discover natural and cost-effective food sources for managing hyperglycemia. These plant foods consist of basic nutrients such as vitamins, minerals, dietary fibers and more important bioactive compounds such as polyphenols and carotenoids [2, 3] that can have specific structure-function benefits [4]. Many plant foods and specific varieties of specific species contain hypoglycemic compounds, acting as antimetabolites to help block specific disease pathway, including the oxidation pathway of fatty acids [5]. Natural plant plays a part in offering techniques to control postprandial hyperglycemia [4] with minimum side effects.

Herbs of many plant sources are being evaluated to measure their levels of phenolic phyotchemicals, containing high antioxidant activity [6]. Phenolic phytochemicals are used by plants to protect them from abiotic and biotic stresses, but are equally also beneficial to preventing and combating human chronic diseases linked to oxidative stress [7]. The presence of certain phenolic compounds into plant play a role in the treatment of managing hyperglycemia and related complications of hypertension [4,6]. Previous study focused on the effects of ethanol extract of Trigonella foenum-graecum (Fenugreek) seeds on the blood glucose levels in alloxan-induced diabetic rats at different doses. It was found the extract of Fenugreek composed of alkaloids, steroids and carbohydrates and this extract played a good role in reduction of glucose levels in blood of alloxan-induced diabetic rats [8].

Cruciferous vegetables such as cabbage are among the most important dietary vegetables consumed in Iraq owing to their availability in local markets, cheapness and consumer preference. It is playing a good role in improving the immune system by supporting the host with multi important vitamins that helped to eradicate different infectious diseases resident in studied area (Iraq) such as enter viruses, which resident in the south of Iraq [9-11]. In the recent study of Al-Jawadi showed in the diabetic rats, the protein extracted from *B. oleracea* in a dose of 75mg/kg body weight showed a significant decrease in serum glucose, cholesterol and total lipids levels [12].

In present study, the alcoholic extract of *B. oleracea* var. capitata leaf was ran in GC-Mass to identified the composition of this extract and find out the role of alkaloid extract in reduction of glucose level in blood of alloxan-induced diabetic rabbits.

MATERIALS AND METHODS

Study Plant

Leaves of *B. oleracea* var. capitata were collected from *B. oleracea* var. capitata planted in in local market and college of agriculture, University of Basrah, Basrah. The plant was classified in the Herbarium of Basrah, College of Education, University of Basrah. The leaves were dried at 25°C, then ground by a blender (rotel coffee grinder type 24) and kept in plastic containers at room temperature until they were used.

Preparation of aqueous extracts of *B. oleracea* var. capitata leaf

Fifty grams of dried ground leaves of *B. oleracea* var. capitata were refluxed in 250 ml of distilled water for 24 hours, the precipitate was removed by filtration, through filter paper no.1, and then filtrate was concentrated in rotary evaporator [13].

Gas chromatography–mass spectrometry (GC-MS) analysis

The GC-MS analysis of the alcoholic extract of seeds was performed using Shimadzu GCMS-QP2010 Ultra system having automatic sampler CTC analysis CombiPAL robotic arm. The inlet temperature was set at 260°C. The specification of the capillary column used was Agilent 19091S-433: 1548, 52849 HP-5MS 5% Phenyl Methyl Silox 30 m × 250 µm x 0.25 µm HP-5MS. The oven temperature was programmed from 50°C to 260°C. The diluted samples (1/100, v/v, in Hexane) of 2 µL were injected.

Isolation of phenols from *B. oleracea* var. *capitata* leaves

Fifty gram of leaf powder (defatted powder) was dissolved in 250 ml of (2%) hydrochloric acid and the mixture was put in water bath for 8 h at 60^{o}C. The mixture was filtered by Buchner funnel and precipitate was removed. Equal volume of diethyl ether was added to the filtrate. The mixture was in water bath at 32^{o}C for 50 minutes. Then, the mixture was concentrated and dried under vacuum rota-evaborator at 70^{o}C. The yielded weight of phenolic product was 3.01 gm [13].

Isolation of alkaloids from *B. oleracea* var. capitata leaves

Fifty gm of leaves powder (defatted powder) was mixed with 250 ml of (10 %) ethanolic acetic acid and put on magnetic stirrer for 24 h. Then the mixture was filtered and precipitate was removed. The filtrate was concen-trated to quarter of previous volume by using vacuum rota-evaborator at 70^{o}C and the pH was adjusted to 9 with ammonium hydroxide to precipitate

the alkaloids. The mixture was put in separation funnel, 20 ml of chloroform was added and the mixture was mixed well. The organic layer was collected. This step was repeated three times and dried by vacuum rota-evaborator at 70^{o}C. The product weight was 1. 8 gm [13,14].

Quantization of Phenols and alkaloids in seeds extracts

The standard method of Al – Maliki, (2012) was followed to estimate the presence and amount of phenols and alkaloids extractions [13].

Animals

Rabbits weighing 2-2.5 kg were procured from central animal house of Basrah University, Basrah, Iraq. Animals were kept in clean polypropylene cages and fed on standard antibiotic free diet. All animals were kept in fast for 24 h before staring the experiments. The study was conducted following approval from the animal ethics committee of Basrah University, Basrah, Iraq.

Diabetes Induction of Rabbits

The diabetes was induced in rabbits using three injections of alloxan monohydrate dissolved in sterile normal saline. Alloxan was used immediately after preparation and administrated intravenously at a period of 48 hrs in a dose of 150 mg/Kg body weight [13], then 20 % of glucose dissolved in drinking water was given to rabbits orally and they were kept in fast for 18 hrs after seven days from last administration. Glucose concentrations were measured in blood of alloxan-induced diabetic rabbits by using glucose oxidase peroxidase enzymatic colorimetric GOD-PAP Method [13].

Experiment

Effect of phenols and alkaloids on glucose level in alloxan-induced diabetic rabbits

Twelve alloxan-induced diabetes rabbits were divided into two equal groups. The first group was given 3 ml of normal saline and considered as a control group. While, the second group was given 0.3 gm/kg body weight of phenolic extract dissolved in 3 ml of normal saline, this group considered as a test group. Blood samples were collected at different time intervals (0, 2, 4, 6, 24 h). The glucose concentrations were measured at each time point [15]. Similar procedure was followed but instead of phenols the alkaloids in same concentration was used.

Statistical Analysis

All values have been taken as mean value and standard error calculated. The differences between test and control were analyzed by using Student's t test employing origin 8 version Software. A value of $P < 0.05$ was considered to be statistically significant.

RESULTS

Identification of chemical compounds of leaves extract by GC-MS

The alcoholic extract of dried leaves was ran in GC-MS to identify the number, amount and type of chemical compound of this extract. **Fig. 1** showed that the leaf extract composed of 18 types of compound with different amount.

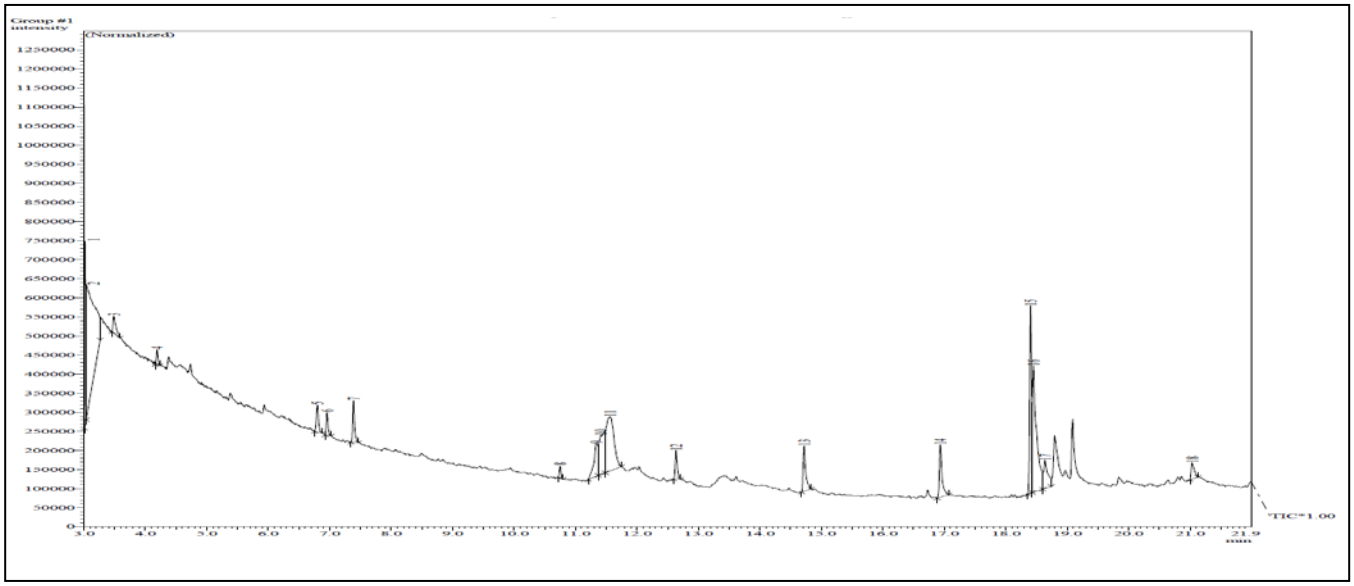

Fig. 1 Leaf extract of *B. oleraceae* var. capitata shows 18 peaks which indicate that 18 compounds are present in the leaf extract.

Table 1 showed clearly the percentage of each compound. From the 18 compounds one phenolic compound (Hydroquinone, acetate) and one alkaloid compound [Pyrrolidine, 1-(1-cyclohexen-1-yl)-] was found. These two compounds were used in further experiments.

Table 1 Chemical compositions of leaf extract of *B. oleracea* var. capitata total components of the extract 100 %

Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	Name
1	3.020	595938	5.66	481714	17.27	1,2-Propadiene-1,3-dione
2	3.045	2784185	26.46	343670	12.32	Propanedioic acid
3	3.487	141038	1.34	44411	1.59	1,2-Cyclopentanedione
4	4.190	70477	0.67	40776	1.46	Dimethyl trisulfide
5	6.800	174785	1.66	72176	2.59	1,4-Dioxane-2,5-dione, 3,6-dimethyl-, (3S-c
6	6.955	120996	1.15	60683	2.18	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy
7	7.387	233453	2.22	110532	3.96	1,4-Dioxane-2,5-dione, 3,6-dimethyl-, (3S-c
8	10.750	57104	0.54	32360	1.16	Pyrrolidine, 1-(1-cyclohexen-1-yl)-
9	11.335	409079	3.89	85709	3.07	2-Oxiraneethanol, 2-t-butyldimethysilyloxy
10	11.410	694672	6.60	103156	3.70	Propanoic acid, di(tert-butyl)silyl ester
11	11.561	1251346	11.89	142456	5.11	Sucrose
12	12.635	171552	1.63	75341	2.70	3',5'-Dimethoxyacetophenone
13	14.719	344824	3.28	119248	4.28	Hydroquinone, acetate
14	16.935	412615	3.92	137844	4.94	Pentadecanoic acid
15	18.404	1059063	10.07	491272	17.61	9,12-Octadecadienoic acid (Z,Z)-
16	18.451	1488248	14.15	331537	11.89	6-Octadecenoic acid, (Z)-
17	18.640	359990	3.42	73471	2.63	Octadecanoic acid
18	21.031	151679	1.44	42972	1.54	Erucic acid
		10521044	100.00	2789328	100.00	

Effect of phenols on glucose level in alloxan-induced diabetic rabbits

In present study, rabbits were injected with alloxan to create alloxan-induced diabetic rabbits. The level of glucose was measured in blood of alloxan-induced diabetic rabbit and high level of glucose was reported (360 mg/100ml) as compared with healthy control (128 mg/100 ml). Alloxan-induced diabetic rabbits were administered with 0.3 gm/kg phenolic compound that extracted from *B. oleracea* var. capitata leaves, blood (sera) glucose was measured at different time intervals (0, 2, 4, 6, 24 h) post administration. The results were compared with alloxan-induced diabetic rabbits that administrated with normal saline orally. Significant decrease ($P<0.05$) of glucose was observed after 4 h of administration with phenolic compound (Hydroquinone, acetate). The decrease of glucose was decrease with time post administration. Thus, maximum difference (decrease) in glucose level in blood of diabetic rabbit was observed at 24 h (**Fig. 2**).

Effect of alkaloid compound on glucose level in alloxan-induced diabetic rabbits

Alloxan-induced diabetic rabbits were administrated orally with 0.3 gm/kg alkaloid [Pyrrolidine, 1-(1-cyclohexen-1-yl)-] that extracted from *B. oleracea* var. capitata leaves**.** The level of glucose was measure at different time intervals post administration with alkaloid (**Fig. 3**). The result of glucose level in this group of diabetic rabbits was compared with level of glucose in sera of diabetic rabbits that administrated orally with normal saline. Significant decrease of glucose level was observed only at 24 h.

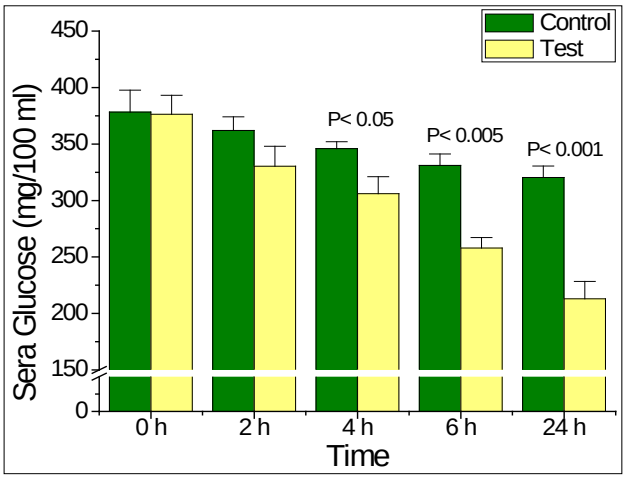

Fig. 2 Level of blood glucose (sera) in alloxan-induced debetic rabbit that administrated with 0.3 gm/kg phenolic compound (Hydroquinone, acetate) extracted from *B. oleracea* var. capitata leaves (yellow bars, test). Green bars represent level of glucose in sera of alloxan-induced debetic rabbits administrated with normal saline (control).

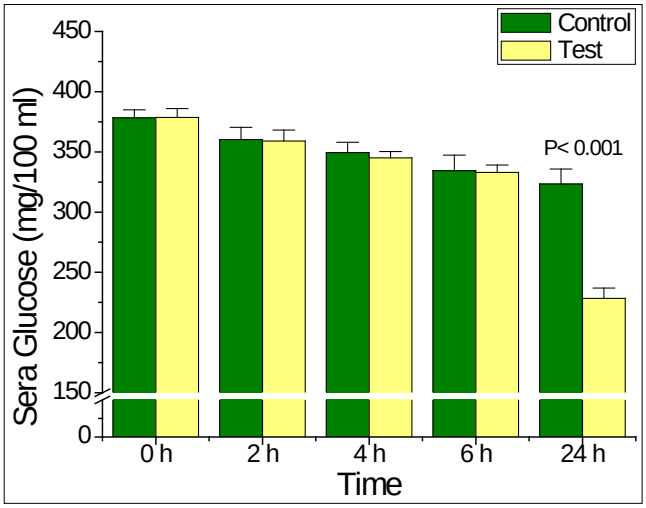

Fig. 3 Level of blood glucose (sera) in alloxan-induced diabetic rabbits administrated with 0.3 gm/kg alkaloid compound [Pyrrolidine, 1-(1-cyclohexen-1-yl)-] extracted from *B. oleracea* var. capitata leaves (yellow bars, test). Green bars represent levels of glucose in sera of alloxan-induced diabetic rabbits administrated with normal saline (control).

Current study proved the role of phenolic compound (Hydroquinone, acetate) that extracted from *B. oleracea* var. capitata leaves to reduce the glucose level in diabetic rabbits and phenolic compound has higher ability to reduce glucose level in experimental rabbits as compared with alkaloid compound [Pyrrolidine, 1-(1-cyclohexen-1-yl)-].

DISCUSSION

Recently, several study focused on using different plants in treating diabetes. The interest in this area increased from time to time as plants are safe drug and also cheaper than artificial drug [16, 17]. In current study, water extract of *B. oleracea* var. capitata leaves was ran into GC-MS to estimate the components of this extract and to know the level of each one. It was found 18 components and only one phenolic compound ((Hydroquinone, acetate) and one alkaloid compound [Pyrrolidine, 1-(1-cyclohexen-1-yl)-] was found. The ability of these two compounds to reduce the levels of glucose in diabetic rabbits was evaluated. Both to compound have anti-diabetic effect but phenolic compound was better than alkaloid in this job.

Alloxan is well known for its selective pancreatic islets B cell cytotoxicity. It produces oxygen free radicals [reactive oxygen species (ROS)] in the body, which causes pancreatic injury and could be responsible for increased blood glucose level [18]. Thus, the blood glucose was increased compared to normal rabbit. The mechanism of cruciferous vegetables (*B. oleracea* var. capitata) to reduce glucose levels in diabetes patients still not fully clarified; these vegetables are rich in the antioxidant vitamins C, E and carotene and are good sources of dietary fiber. They also contain sulforaphane and other isothiocyanates, which are believed to stimulate the production of protective enzymes in the body [19,20]. Sathya and Siddhuraju, (2012) reported the antioxidant activity of phenols that extracted from *Acacia auriculiformis* [21]. Tiong et al., (2013) reported the antidiabetic and antioxidant properties of alkaloids from *Catharanthus roseus* (L.) G. Don [**22**]. Thus these compounds may be affected on the activity of ROS to damage tissue especially in pancreatic cells (beta cells) that is why; these compounds may have anti-diabetic effects. The other sight of antidiabetic affectivity of phenolic compound by induction of pancreatic insulin secretion from B-cell of islets of Langerhans and antioxidant effect and Aldose reductase (AR) inhibitor effect [18].

It can be concluded from present study, the phenolic (Hydroquinone, acetate) and alkaloid compounds [Pyrrolidine, 1-(1-cyclohexen-1-yl)-] that extracted from water extract of *B. oleracea* var. capitata leaves have significant anti-diabetic effect. This strongly gives evidence to the use of this plant as an effective treatment of diabetes and they can be viewed as a lead compound for the development of antidiabetic therapeutics.

Conflict of interest

The authors declare that they have no conflict of interests.

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