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Characterization of Extracted Flavonoids from Cabbage and Study its Biochemical Effect on Alloxan- Induced Diabetic Female Rats

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KEYWORDS

Brassica oleracea L. var. capitata, flavonoids, HPLC, diabetic rats, lipid profile, GOT & GPT activity.

A B S T R A C T

The present study include two parts: part one was aimed to characterize the extracted flavonoids from cabbage (*Brassica olearacea* Var-Capitata) and part two include studying the effect of extracted flavonoids on some biochemical parameters in alloxan-induced diabetic female rats. High performance liquid chromatography (HPLC) was employed to identify and quantify the five important flavonoids components (Gallic acid, Catechin, Epicatechin, Epicatechin-3-O- gallate and Anthocyanins). The concentration of Gallic acid (34.776 µg/ml), Catechin (122.739 µg/ml), Epicatechin (110.434 µg/ml), Epicatechin-3-O- gallate (30.07 µg/ml) and Anthocyanins (27.532 µg/ml) were detected in the extract from cabbage. Fifteen female albino rats equally divided into three experimental groups were used. Two groups served as normal and diabetic control and the third group received (40mg/Kg) flavonoid treatment. Oral administration of plant extracts on alloxan induced diabetic rats showed a marked significant increase($P < 0.05$) in the levels of biochemical parameters such as blood glucose, triglycerides, cholesterol, LDL cholesterol, VLDL cholesterol and GOT & GPT activity, whereas significant decrease ($P < 0.05$) in the level of HDL. Finally the results showed that treatment with flavonoid extract significantly reduced the blood glucose, triglycerides, cholesterol, LDL cholesterol, VLDL cholesterol and GOT & GPT activity, there were significant increase in the level of HDL

Introduction

White cabbage (*Brassica oleracea* L. var. capitata) crops are grown worldwide and known for their high content of health beneficial (Marie Grønbæk *et al*., 2014).

It is an herbaceous and leafy plant which belongs to the Brassicaceae family (Carvalho *et al*., 2008). Many

epidemiological studies have indicated that a diet rich in these vegetables is associated with reduced risk of a several type of cancers, cardiovascular and diabetes mellitus diseases (Byers *et al*., 1992; Faller *et al*., 2009; Verhoeven *et al*., 1996). The beneficial health properties of Brassica crops are due to the presence of health-promoting compounds such as vitamins, carotenoids, phenols, flavonoids, minerals, and glucosinolates (Aires *et al*., 2011; Bellostas *et al*., 2004; Singh *et al*., 2007; Campbell *et al*., 2012). The presence of flavonoids in *Brassica oleracea* species is reported by several authors (Rashed *et al*., 2010; Asadujjaman *et al*., 2011). The flavonoids constitute one of the most important group, and they can be further subdivided into 13 different classes, with more than 5000 compounds described, they are products of plant metabolism and have different phenolic structures (Kuhnau, 1976). Most of the flavonoids are low molecular weight and are soluble depending on their polarity and chemical structure (Bravo, 1998). They are effective antioxidants because of their free radical scavenging properties, thus, they may protect tissues against free oxygen radicals and lipid peroxidation (Rice-Evans *et al*., 1996). Flavonoids can exert their antioxidant activity by various mechanisms, e.g., by scavenging or quenching of free radicals, by chelating of metal ions, or by inhibiting enzymatic systems responsible for free radical generation. The mechanism of protective actions of flavonoids remains little known. Experimental diabetes mellitus in animals is one of the well-defined conditions which include free radical damage. Several researchers have demonstrated that flavonoids may reduce hyperglycaemia and exert protective effect against non-enzymatic glycation of proteins in animals. Alloxan, a chemical diabetogen, in the presence of glutathione is reduced via the alloxan radical into dial uric acid. During this redox cycling process, reactive oxygen species are formed that destroy β-cells in islets of Langerhans. Moreover, it is suggested that transitional metals such as iron, zinc and copper may be involved in alloxan toxicity. It is known that alloxan administration causes severe necrosis of pancreatic β- cells (Rice-Evans, 2001). It has been suggested that alloxan induces the production of H_2O_2 and of some free radicals such as O2 •− (superoxide) and OH• which first damage and later lead about the death of the cells (Soto *et al*., 1994). Therefore, the above model was considered adequate for the study of a pathology, such as diabetes mellitus. The current study was therefore designed to extract five important flavonoid components (Gallic acid, catechin, epicatechin, Epicatechin-3-O- gallate and Anthocyanins) from cabbage and study their effect on some biochemical parameters in alloxan-induced diabetic female rats.

Materials and Methods

Collection of plant sample and preparation of extract

The basic plant material of *Brassica oleracea* L. var. capitata used for the investigation were bought from a local market in Samara state, Iraq and identified by a qualified plant taxonomist. The leaves were picked and left to dry for more than three days at room temperature. The grinding process to the dried leaves was conducted using house hold electric grinder to obtain a fine powder pass through a sieve with holes diameter (1) mm. The powdered plant materials were subjected to continuous hot extraction in soxhlet apparatus with 80% ethanol for three days. Ethanol evaporated using rotary vacuum evaporator (Bu¨chi Rotavapor, CH-9230; Bu¨chi Labortechnik GmbH, Essen, Germany) under reduced pressure at low temperature (40-50°C), then

50 ml of hot methanol 50 C° with the rapid shaking and after obtaining a dry extract, the residue was weighed and the total dry yield of extract was calculated, which ranged from 73 to 95 mg/g sample for the presently analyzed samples. Finally the residue used for diagnosing flavonoid compounds by HPLC.

High performance liquid chromato-graphic identification and quantification of flavonoid compounds in plant extract

The alcoholic extract of sample were separated on fast Liquid chromatographic column, using HPLC Koyoto Japan (6AVP) Shimadzu Under the following conditions: 3 µm particle size (50×2.0 mm I.D) CN (propyl cyanide) column, Mobile phase were 0.05% acetic acid De-ionized water, pH 2.5 solvent A, methanol solvent B, using linear gradient from 0% B to 100% B in 10 minutes. Detection UV set at 370 nm, Flow rate 1.0 ml\min. The sequences of the eluted material of the standard were as Curcumin, Quercetin, Galangin, Demethoxy Curcumin, Bisdemethoxy curcumin and Germacrone., each standard was 25 µg\ml.

Sample Preparation

The extract were filtered on disposable filters 0.2 µm then 20 µl of the extract were injected on HPLC column. The concentration for each compound were quantitatively determined by comparison the peak area of the standard with that of the sample (Soto *et al*., 1994).

Calculations

$$\text{Conc. of sample} = \frac{\text{area of sample} \times \text{conc. of standard} \times \text{dilution factor}}{\text{area of standard}} \ \mu g\backslash ml$$

Experimental Animals

Fifteen female wistar albino rats adults (weeks), weighing (320-325 g) were obtained from the animal house of the General Company for the pharmaceutical industry in Samarra city. Before and during the experiment, rats were fed with standard diet.

Animals are divided into 3 groups, each comprising 5 rats as:

Group I: Normal control (saline).

Group II: alloxan (50 mg/kg).

Group III: Alloxan (50 mg/kg.)+ *Brassica oleracea* L. var. capitata extract flavon (40 mg/kg.).

Before initiation of experiment, the rats were acclimatized for a period of 7 days under standard environmental conditions of temperature, relative humidity and dark/light cycle. They were kept in well ventilated and clean cages at an average room temperature of (22±3) and their beddings changed every two days.

The rats were allowed free access to tap water and fed a standard rat chow throughout the period of the experiment. All the processes involved in the handling and experiment were carried out according to standard protocols approved by the animal ethics committee of the Department of Biological sciences, Covenant University, Ota.

Induction of Diabetes

After 24 h fasting rats alloxan monohydrate of 50 mg/kg body weight (dissolved in 0.9% sterile NaCl solution of pH 7) (Habib *et al*., 2005; Lenzen, 2008; Osinubi *et al*., 2008) was administered intra peritoneal to rats in group II to III to induced diabetes, of which their blood glucose level have been previously determined by Blood Glucose Test Strips. Thereafter, blood was collected from the neck Jugular vein of the rats with serum glucose levels between (250–400 mg/dl), showing clear signs of polyuria, polyphagia and polydipsia after one day were considered diabetic and used for the experiment (Eyo *et al*., 2011; Nafisa *et al*., 2007).

Sample collection

After one week of alloxan dosage for rats, the rats were starving for 24 hours and blood pull by cutting a Alodji Jugular vein in the neck, (6-8) ml of blood were collected from each rat, put in approximately 1 ml of blood in the test tube without coagulation agents. Blood samples were left for about 15mins at room temperature, then separated by centrifuging blood samples on a Humax 14 K (Germany) at 3000 rpm for 10 min, finally, keep on (-20) C° in plastic tubes while conducting biochemical tests.

Biochemical evaluation

Glucose determination

This test was carried out using a glucose enzymatic-colorimetric test kit (GOD-POD), produced by Cypress diagnostics (Belgium). The test principle is based on the oxidation of glucose by glucose oxidase (GOD) to gluconic acid and hydrogen peroxide. The hydrogen peroxide (H_2O_2) forms a red violet color with a chromogenic oxygen acceptor, phenol amino phenazone in the presence of peroxidase (POD). The color intensity is proportional to glucose concentration in the sample.

Assay for Liver enzymes

Alanine aminotransferase (ALT/GPT) and Aspartate transferase (AST/GOT) tests were carried out using ultra violet (UV) kinetic test kits produced by Cypress diagnostics. The test is based on photometric determination of rate of nicotinamide adenine dinucleotide (NADH) consumption by pyruvate and oxaloacetate which is directly related to GPT and GOT activities, respectively.

Determination of lipid profile

Cholesterol, High density lipoprotein cholesterol (HDL-c) and triglycerides were determined by enzymatic method using Randox kits. Low density lipoprotein cholesterol (LDL- c) and very low density lipoprotein cholesterol (VLDL- c) were obtained by deduction using Friedwald equation (Friedewald *et al*., 1972).

Statistical analysis

All values were compared with the control by taking mean and standard error to the mean using one sample t-test. Values of $P < 0.05$ were considered as significant and $P < 0.01$ as highly significant. All statistical methods were performed using SPSS version 17.

Results and Discussion

The chromatographic conditions were optimized to obtain chromatograms with good resolution. Table (1) and figure (1) represents the HPLC profile of five standard flavonoid: Gallic acid, Catechin,

Epicatechin, Epicatechin-3-O- gallate, Anthocyanins.

The alcoholic extract of *Brassica oleracea* L. var. capitata analyzed by fast Liquid chromatographic column allowed the identification of five flavonoids, namely (Gallic acid, Catechin, Epicatechin, Epicatechin-3-O- gallate and Anthocyanins) when comparing the retention time of the isolated sample with the retention time of the standard flavonoid (Table 2, Figure 2):

The results showed the presence of five peaks which indicated that the *Brassica oleracea* L. var. capitata extract contain 27.532µg/cm^3 from Anthocyanins, 30.07 µg/cm^3 from Epicatechin-3-O-gallate, 34.776 µg/cm^3 from Gallic acid, high concentrations 122.739 µg/cm^3 from Catechin and 110.434 µg/cm^3 from Epicatechin,table (1), in addition to uncharacterized compound because not available of standard compounds.

Table (3) shows the levels of serum glucose and the activity of GOT and GPT in the studied groups:

The results indicated that there were significant increase in the levels of glucose and (GPT & GOT) activity in the alloxan-induced diabetic female rats and significant decrease in the sera of rats treated with flavonoids extracted from *Brassica oleracea* L. var. capitata when compared with control group.Table (4) showed the lipid profile levels in the sera of the studied groups.

From table (4) it's clear that the levels of cholesterol, TG, VLDL and LDL were significantly increased in the alloxan-induced diabetic female rats and significant decrease in the sera of rats treated with extracted flavonoids when compared with control group, while HDL level were significantly decreased in the alloxan-induced diabetic female rats and significant increase in the sera of rats treated with extracted flavonoids when compared with control group.

Diabetes mellitus is considered to be one of the five leading causes of death worldwide and has risen dramatically over the past 2 decades. Diabetes mellitus is a disease condition characterized by alterations in carbohydrate, lipid and protein metabolism (Das *et al*., 1996). Recently the search for suitable antidiabetic agents has focused on plants used in traditional medicine (Chandel *et al*., 2011). Traditional medicines from medicinal plants are used by about 60% of the world's population because of their natural origin and less side effects. The present study was hence carried out to evaluate the antidiabetic effect of flavonoids extracted from *Brassica oleracea* L. var. capitata on Alloxan induced diabetic rats**.** The alcoholic extract of *Brassica oleracea* L. var. capitata analyzed by HPLC allowed the identification of five flavonoids, namely, Anthocyanins, Epicatechin-3-O-gallate, Gallic acid, Catechin and Epicatechin**.** Catechin & Epicatechin The results of glucose levels on the alloxan induced diabetic rats in this study were agreed with Zainab & Alabadi (Zainab *et al*., 2010) on alloxan– induced diabetic rats and Isa J.k on alloxan-induced diabetic female Albino Mice, increased glucose levels may be attributed to that alloxan is a urea derivative which causes selective necrosis of the β-cells of pancreatic islets.

The toxic action of alloxan on pancreatic beta cells involve oxidation of essential sulphydryl (-SH groups), inhibition of glucokinase enzyme, generation of free radicals and disturbances in intracellular calcium homeostasis (De Carvalho *et al*., 2003), also alloxan is a hydrophilic and

unstable chemical compound which has similar shape as that of glucose, which is responsible for its selective uptake and accumulation by the pancreatic beta cell (Gorus *et al*., 1982). Similarity in the shape allows it to transports into the cytosol by the glucose transporter (GLUT2) in the plasma membrane of beta cell.It is noted that the animals infected diabetic that treated with flavonoids extracted from *Brassica oleracea* L. var. capitata with a concentration of 40 mg / kg every day by injection. Peritoneal for a one week cause significant decrease $P<0.05$ in the level of blood glucose compared to the alloxan induced diabetic rats, this may be attributed to the flavonoids dosage to the animals that improve insulin secretion from beta cells or may act to regenerate beta cells.

Table.1 Retention volume and area under the curve of five standard flavonoids

Standard	**Rt. (min)**	**Area**
Gallic acid	1.43	22169
Catechin	1.95	32583
Epicatechin	3.48	39950
Epicatechin-3-O- gallate	4.07	60621
Anthocyanins	4.99	38813

Table.2 Retention volume and area under the curve of flavonoids isolated from *Brassica oleracea* L. var. capitata

Standard	**Rt. (min)**	**Area**	**Conc.(µg/ml)**
Gallic acid	1.42	30838	34.776
Catechin	1.91	159969	122.739
Unknown	2.93	48968	-
Epicatechin	3.43	176475	110.434
Epicatechin-3-O- gallate	4.16	72931	30.07
Anthocyanins	4.97	42744	27.532

Table.3 Serum glucose levels and liver enzyme activity in the studied groups

Parameter	**Glucose (mg/100ml)**	**GOT IU/ml**	**GPT IU/ml**
Control	59.020±3.133 C	38.667±4.509 B	29.667 ± 2.3094C
Alloxan mg\kg50	265.337±1.996 A	52.333±4.509 A	57.00± 5.00 A
Alloxan + flavonide extract mg/kg40	138.890±6.261B	28.333±2.309 C	41.333±5.5075 C

Table.4 serum lipid profile in the studied groups.

Parameter	Cholesterol mg/100ml	T.G (mg/100ml)	HDL (mg/100ml)	VLDL (mg/100ml)	LDL (mg/100ml)
Control	101.667±7.6376C	44.00 ±1.00 C	96.373±1.791 A	8.8 ± 0.2 C	47.323±1.142 B
Alloxan mg\kg50	158.393±1.3767 A	58.480±1.5476 A	44.133±4.474 C	11.697±0.309 A	74.933±6.697 A
Alloxan + flavonide extract mg/kg 40	114.820±3.8218 B	47.9367±0.6465 B	80.233±3.150 B	9.5867±0.129 B	19.033±2.743 C

Fig.1 HPLC chromatogram of five standard flavonoids.

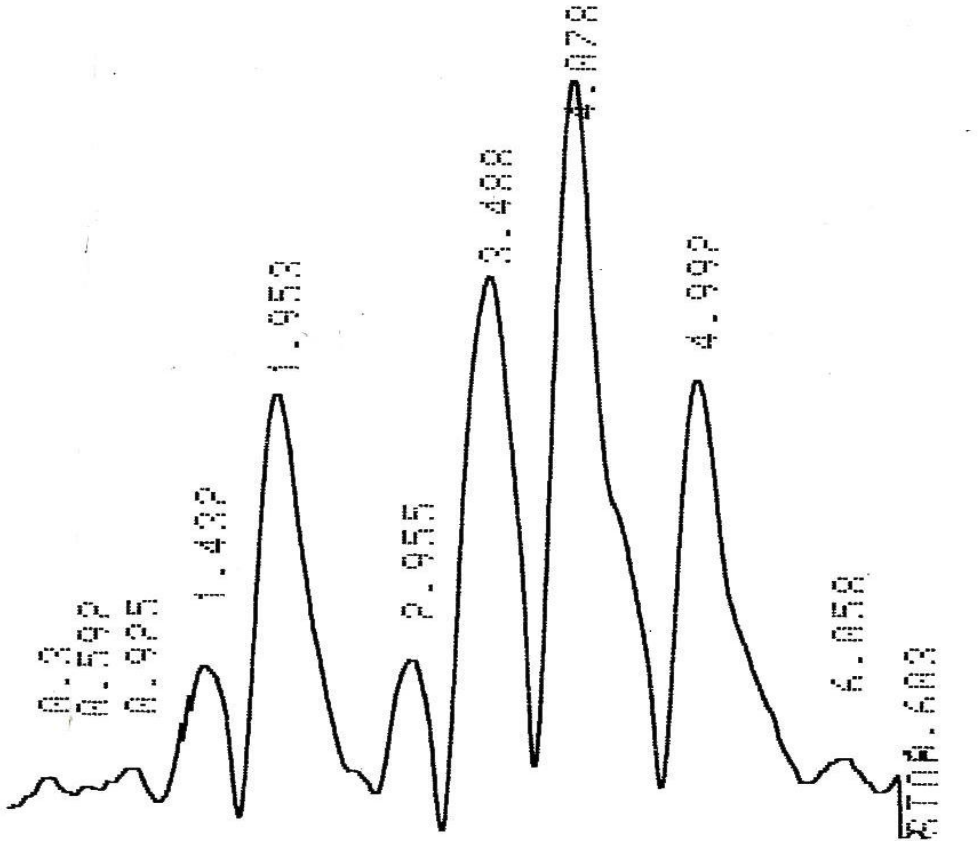
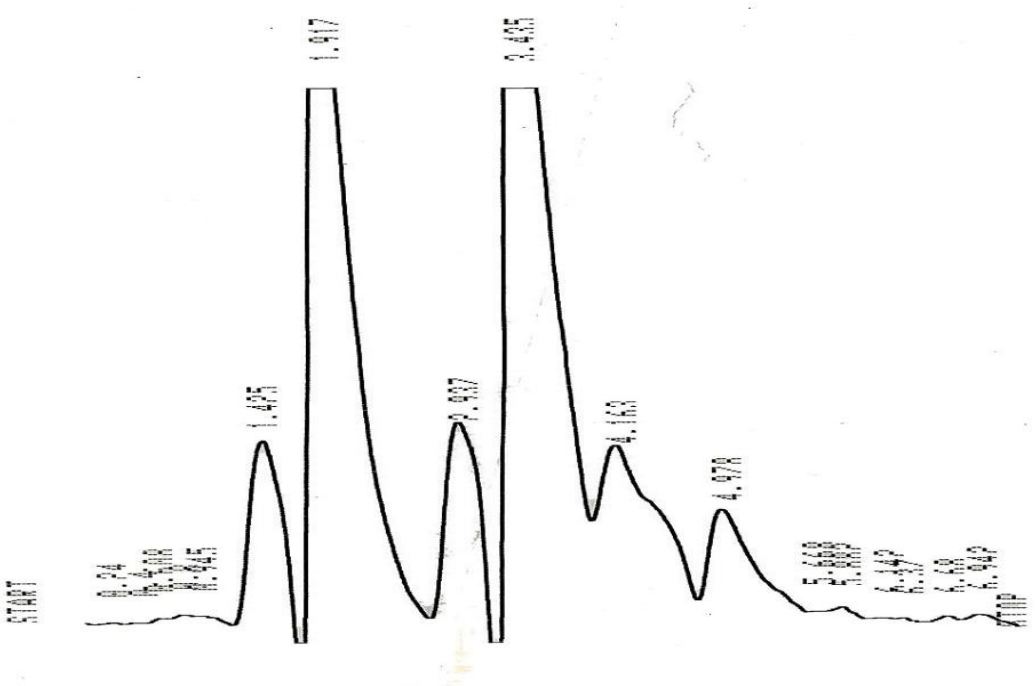


Fig.2 HPLC chromatogram of flavonoids isolated from *Brassica oleracea* L. var. capitata



Increased SGOT and SGPT activities were reported in diabetes and it may be due to liver dysfunction. In this study, increased level of SGOT and SGPT was observed in alloxan-induced diabetic rats which may have occurred by leakage of enzymes from the liver cytosol into the blood stream; it represents the toxicity of alloxan on liver.

Diabetic rats treated with alcoholic extract from *Brassica oleracea* L. var. capitata significantly reduced both enzyme activities which represents the protective action of alcoholic extract from *Brassica oleracea* L. var. capitata in diabetic condition. The abnormal high concentrations of serum lipids in diabetic animals are mainly due to an increased mobilization of free fatty acids from peripheral fat depots. In this study the results of lipid profile indicated that there were significantly increase in the levels of serum cholesterol, TG, VLDL and LDL as well as marked reduction in serum HDL level in in alloxan induced diabetic rats. Administration of doses of alcoholic extract of *Brassica oleracea* L. var. capitata decreased levels of cholesterol, LDL, V-LDL and TG levels as well as increased the level of HDL. Several authors reported that flavonoids are known to be bioactive antidiabetic principles (Anitha *et al*., 2012; Alagammal *et al*., 2012). Studies with flavonoids are underway to further elucidate their mechanism of action. Further research also is needed to determine which combinations might prove efficacious given the large number of persons taking nutriceuticals.

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