International Research Journal of Medical Sciences _____ ISSN 2320 –7353
Vol. **1(11),** 17-22, December **(2013)** *Int. Res. J. Medical Sci.*

Isolation and Studying the Effect of Protein Fractions of White Cabbage (*Brassica oleracea var. capitata)* on Some Biochemical Parameters in Experimental Diabetic Rats

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Available online at: www.isca.in, www.isca.me
Received 2nd November 2013, revised 14th November 2013, accepted 27th November 2013

Abstract

The research was an attempt to isolate and study the active protein compounds from the aqueous extract of white cabbage (Brassica oleracea var. capitata) from local market in Iraq, using different biochemical techniques. Two compounds (A and B) were isolated using gel filtration chromatography for the precipitate produced by ammonium sulphate precipitation. The results predicted that, glucose level was lowered by intraperitoneally administration of the concentrated aqueous extract and the protein compound (B) compared with oral administration. The comparative molecular weight of the isolated protein active compound (peak B), was found to be 4797.34 Dalton. The study was also included the effect of the aqueous extract and the protein compound (B) in four doses (50, 75, 100 and 125 mg/kg body weight) on certain blood constituents in normal and alloxan induced diabetic rats. In normal rats, the blood glucose level was significantly lowered by crude aqueous extract and the protein compound in a dose (75 mg/kg body weight) compared with control group. In addition, the protein compound in a same dose showed a significant decrease in serum cholesterol and total lipids level. In diabetic rats, the protein compound in a dose of (75mg/kg body weight) showed a significant decrease in serum glucose, cholesterol and total lipids levels.

Keywords: White cabbage, *Brassica oleracea var., capitata*; diabetes disease.

Introduction

Brassica or cruciferous vegetables are the "Wonder Kids" of the vegetable world. This includes cabbage, cauliflower, broccoli and kholrabi. They are known as Knol-Khol in Indian vernacular languages. 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. The mechanism of chemopreventive action of cruciferous vegetables is still not fully clarified, these vegetables are rich in the antioxidant vitamins C, E and carotene and are good sources of dietary fibre. They also contain sulphoraphanes and other isothiocyanates, which are believed to stimulate the production of protective enzymes in the body[1-3]. Reactive oxygen species (ROS) are an important part of the defence mechanisms against infection, but excessive generation of free oxygen radicals may damage tissue[4, 5]. The role of ROS in tissue damage in various human diseases such as cancer, ageing, neurodegenerative disease, diabetes and atherosclerosis has been recognized. *Brassica oleracea var. capitata* (Brassicaceae) has similar composition as other Brassica vegetables. Aqueous extract of Knol-Khol is reported to have antidiabetic activity[6].

The aim of the study is to investigate the effect of the protein compounds isolated from the aqueous extract of the white cabbage on some biochemical parameters in experimental animals. Hopping to isolate an active compounds having insulin-like action and / or structure.

Material and Methods

Preparation of the Crude Aqueous Extract: White cabbage (*Brassica oleracea var. capitata*) from local market in Iraq (0.5 kg weight) which were used in the study were cut into small pieces, mixed with cold distilled water in a ratio 1:3 w/v, and then homogenized for five minute using a blender. The crude homogenate was stirred for additional two hours in ice bath, and then allowed to stand in a refrigerator overnight. The mixture was then filtered through several layers of shash to remove all residual materials. Finally, the filtrate or the mixture was then centrifuged at a refrigerated centrifuge for 15 minutes at 8000 xg to obtain the supernatant. The volume of the resulting supernatant was reduced to about 1/3 by lyophilization and kept for further investigation. Total protein was determined by modified Lowry method.

Precipition of the Proteins: Protein materials were separated from the cold extract using ammonium sulfate precipitation. Ammonium sulfate was added to cold crude aqueous extract in a ratio (75:100w/v) with slow stirring at 0˚C. The mixture was left in a refrigarator for 24h and the precipitated protein was isolated by centrifugation for 15 minutes at 8000xg. The protein precipitate was dried by lyophilization then kept in a tight sample tube in a freezer for the next step.

Fractionation of the Protein Extract: A concentrated sample 5 ml (clear aqueous solution obtained by dissolving a sample of

150 mg in 5 ml distilled water and centrifuged) of the protein material from plant was fractionated by gel-filtration chromatography using Sephadex G-75(2.56x87cm) column. Distilled water was used as eluent in the separation.

Intrapertioneal Injection: Group of healthy adult rats (153-170 gm weight) were obtained from the animal house of the Veterinary Medicine College, University of Mosul. The rats were fasted for (16h) and divided randomly into two main groups. The first group was normal while the second group was injected intraperitoneally with the alloxan (127 mg/kg) to induce diabetic rats. Each group was then sub divided into eight group(each containing 4 rats). Group one in the sub group was kept as a control group while the remaining subgroups were injected intraperitoneally with the crude aqueous extract and the fractionated proteins (75,100mg/kg). After two hours of injection blood samples were collected for analysis by the orbital sinus puncture under ether anesthesia using non-heparinized micro-hematocrit capillary tubes.

Determination of Glucose: Serum blood glucose level was measured according to the enzymatic methods using Randox kit for glucose, U.K.[7]

Determination of Cholesterol: Serum blood cholesterol level was measured according to the enzymatic method using Randox kit for Cholesterol, U.K.[8]

Determination of Total Lipids: Serum blood total lipids level was measured by Chabral and Chardonnet method.[9]

Statistical Analysis: The statistical methods used to analize the data including mean, standard deviation, minimum and maximum, while student T-test was used to compare between control and diabetic rats at $p \leq 0.05$ level[10].

Results and Discussion

Precipitation of the Protein: Precipitation of total proteins from the crude aqueous extract was accomplished by ammonium sulfate technique[11]. The protein content of the precipitate was determined and found to be 55.08% in the crude extract. The efficiency of the precipitation of the protein is 30.76%.

Fractionation of Total Protein: Fractionation of total Protein was accomplished by gel filtration chromatography using Sephadex G75 to give mainly one major peak with elution volume of 406 ml, figure-1.

Quantitative determination of the protein in the peak after gel filtration chromatography was performed and then the percent of the component (peak A) was found to be 7.5% and (peak B) was found to be 22.5%.

Comparative molecular weight of the isolated protein compound were determined by gel filtration chromatography on a pre-calibrated column using known molecular weight proteins as shown in table-1. Peak A which approximately 1675.14 Dalton while the peak B approximately equal to 4797.34 Dalton were shown in figure-2.

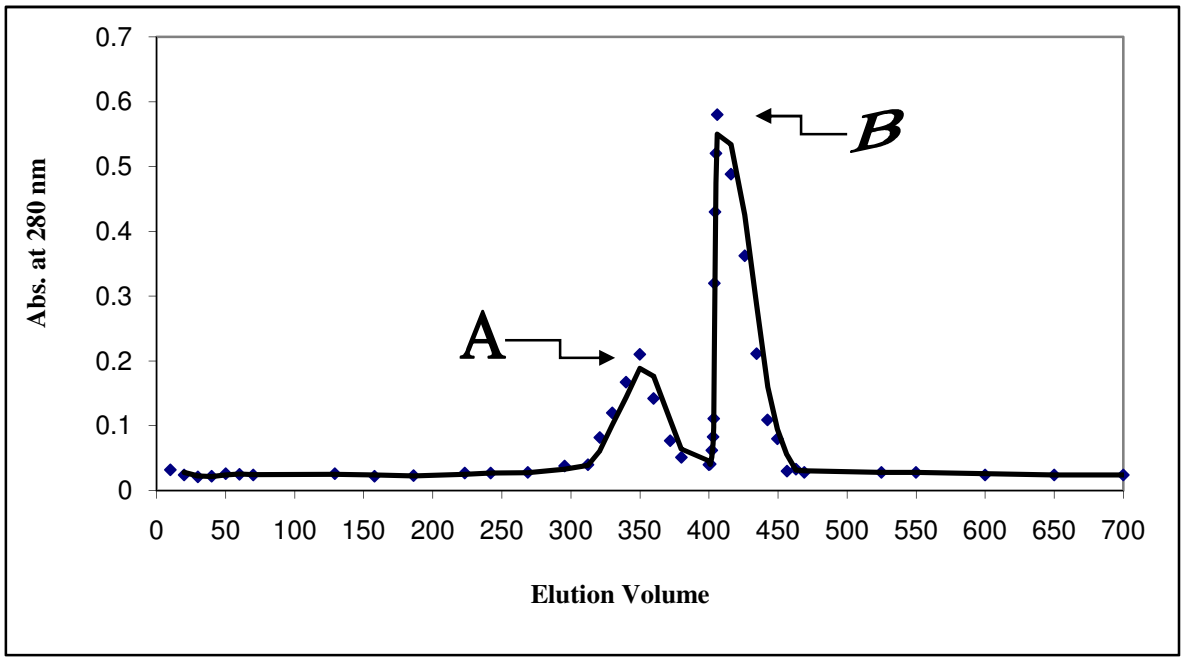

Figure-1
Elution profile of total protein precipitate from white cabbage (*Brassica oleracea var. capitata)* berry on Sephadex G75 column with a dimension (2.56x87cm), Distilled water was used as eluent, each fraction is 10 ml at flow rate 40 ml/h

Effect of crude aqueous extract and the isolated protein compound on glucose, cholesterol and total lipids in normal rats after intraperitoneally administration: The results in table-2 showed the effect of crude aqueous extract and protein products on glucose, cholesterol and total lipids in normal rats.

Results indicated not significant decrease of serum glucose in normal rates for protein fraction (Peak A).

Table-1
Molecular weights and their elution volumes of different protein compounds on Sephadex G 75

Compounds	Molecular weight (Dalton)	Elution volume (ml)
Blue dextran	2000000	121
Bovine serum albumin (BSA)	67000	246
α- amylase	58000	324
Eggs albumin	45000	345
Pepsin	36000	375
Insulin hormone	5750	418
Tryptophan	204	446

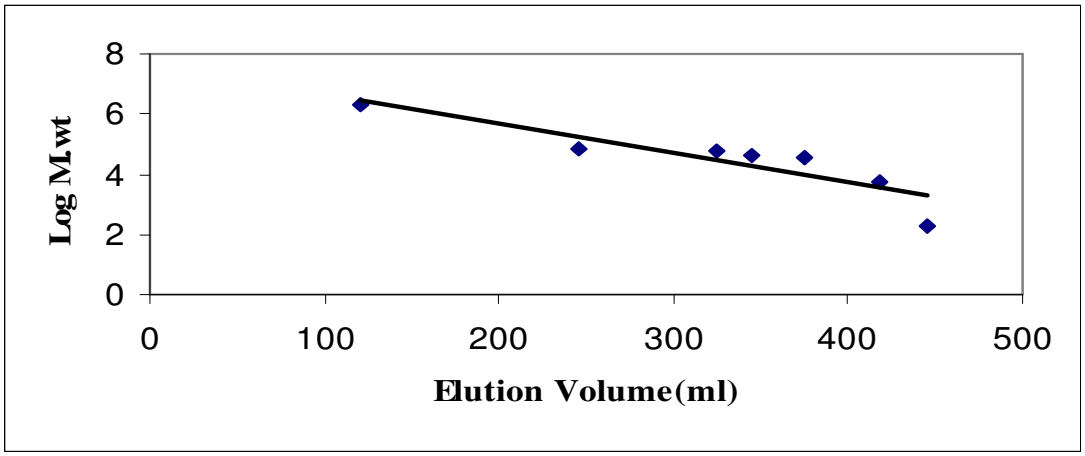

Figure- 2
A plot of the logarithm molecular weights of known proteins versus elution volumes on a Sephadex G 75

Table-2
Effect of crude aqueous extract and isolated protein compound on serum glucose, cholesterol and total lipids in normal rats intraperitoneally administration

Groups	Glucose (mmol/l)	Cholesterol (mmol/l)	Total lipids (mg/dl)
Control	4.70±0.435	2.90±0.158	487.1±11.762
Crude aqueous extract	3.63±0.503*	2.46±0.269	400.9±99.986
Protein(peak A) at (125 mg/kg)	3.96±0.29	2.67±0.10	411.5±77.91
Protein(peak A) at (100 mg/kg)	3.90±0.35	2.61±0.11	406.5±89.71
Protein(peak A) at (75 mg/kg)	3.77±0.49	2.19±0.23	397.9±70.43
Protein(peak A) at (50 mg/kg)	3.75±0.44	2.17±0.28	383.8±73.53
Protein(peak B) at (125 mg/kg)	3.79±0.564	2.62±0.165	379.5±51.757*
Protein(peak B) at (100 mg/kg)	3.70±0.557	2.59±0.172	373.5±31.697**
Protein(peak B) at (75 mg/kg)	3.50±0.265*	1.88±0.503**	265.1±72.291**
Protein(peak B) at (50 mg/kg)	3.78±0.245	1.96±0.793	389.1±63.401*

*Significant difference at P<0.05, **Significant difference at P<0.001

The results in table-2 showed a reduction of blood glucose to low significant level after intraperitoneally (IP) injection of the rats with crude aqueous white cabbage extract compared with control. This finding agreed with the result obtained from other investigators[12]. Hypoglycemic effect on blood glucose might be due to white cabbage contained an insulin like action and / or other products which stimulates insulin secretion from pancreatic beta cells or increases the rate of entrance of various sugars via glucose transporters in the plasma membrane. The results also showed lowering level in cholesterol and total lipids in the blood compared with control group and this observation agrees with other study[13]. Decrease in cholesterol might due to inhibition of ß-hydroxy-ß-methyl glutaryl-CoA reductase responsible for cholesterol synthesis. The decrease in total lipids might due to the insulin like action which activates lipase enzyme and facilitates lipolysis of lipids[14].

The results also showed that the protein compound (peak B) in white cabbage at a dose of (75 mg/kg) led to maximum depression (34%) of blood glucose level compared to control group as listed in table-2. This depression might be due to insulin like action of the protein content of white cabbage[15], or might be due to insulin like structure of the protein product that binds with insulin receptors and lower blood glucose level. The protein compound (peak B) at a dose of (75 mg/kg) in the same table showed a significant lower level in cholesterol and total lipids. This might be due to inhibit cholesterol synthesis or increases the rate of cholesterol ejection loss from the body and insulin like action may help to lower the level of cholesterol[16].

Effect of crude aqueous extract and the isolated protein compound on glucose, cholesterol and total lipids in normal rats after orally administration: The results in table-3 showed the effect of crude aqueous extract and protein products on glucose, cholesterol and total lipids in normal rats.

Results indicated not significant decrease of serum glucose in normal rates for protein fraction (Peak A, peak B).

The results indicated that the orally administration of crude aqueous extract white cabbage extract and protein fractions (Peak A, peak B) produced an increase of serum glucose. This might due to the crude aqueous extract which contains many materials or constituents such as polysaccharides, proteins, fats and amino acid. The metabolism of these compounds due to an increase of serum glucose, because the glycolysis, glycogenolysis, glugoneogenesis becomes active. Also, the protein fraction (Peak A, peak B) caused increase of serum glucose in orally administration, the proteins may be destroyed by the gastric juice or easily inactivated by the proteolytic enzymes. For all of these, the intraperitoneally administration of extract and protein fraction of the plant has hypoglycemic effect more than orally administration and it was a preferable route.

Table-3
Effect of crude aqueous extract and isolated protein compound on serum glucose, cholesterol and total lipids in normal rats after orally administration

Groups	Glucose (mmol/l)	Cholesterol (mmol/l)	Total lipids (mg/dl)
Control	4.70±0.435	2.90±0.158	487.1±11.76
Crude aqueous extract	4.27±0.354*	2.57±0.276	438±51.63
Protein(peak A) at (125 mg/kg)	4.77±0.09	2.86±0.182	460±63.14
Protein(peak A) at (100 mg/kg)	4.75±0.18	2.80±0.189	457±68.24
Protein(peak A) at (75 mg/kg)	4.78±0.62	2.76±0.173	437±79.13
Protein(peak A) at (50 mg/kg)	4.76±0.68	2.70±0.165	439±80.19
Protein(peak B) at (125 mg/kg)	4.76±0.82	2.72±0.144	431.4±66.10
Protein(peak B) at (100 mg/kg)	4.68±0.87	2.79±0.154	439.4±71.40
Protein(peak B) at (75 mg/kg)	4.75±0.71	2.78±0.281	390±89.13*
Protein(peak B) at (50 mg/kg)	4.74±0.96	2.71±0.178	331±75.16*

*Significant difference at P<0.05, **Significant difference at P<0.001

Effect of crude aqueous extract and the isolated protein compound on glucose, cholesterol and total lipids in diabetic rats after intraperitoneally administration: To test the effect of crude aqueous extract and the protein compound of active peak from white cabbage on blood glucose, cholesterol and total lipids in diabetic rats, alloxan was used to induce diabetic experimental animals. Alloxan can damage the langerhans cells leading to decrease the production and secretion of insulin[17]. thus diabetes will occur. The results of intraperitoneal injection into diabetic rats were listed in table-4.

Table-4
Effect of crude aqueous extract and isolated protein compound on serum glucose, cholesterol and total lipids in diabetic rats

Groups	Glucose (mmol/l)	Cholesterol (mmol/l)	Total lipids (mg/dl)
Control	29.6±1.019	3.02±0.680	657.15±29.747
Crude aqueous	20.8±3.675*	2.16±0.465	480.57±92.941*
Protein (peak B) at (125 mg/kg)	24.3±4.123	2.39±0.442	488.51±16.651*
Protein (peak B) at (100 mg/kg)	22.7±4.215	2.33±0.745	489.31±15.345*
Protein (peak B) at (75 mg/kg)	16.7±8.13*	1.75±0.330*	290.43±33.400**
Protein (peak B) at (50 mg/kg)	27.7±3.83	2.55±0.891	471.45±38.763*

*Significant difference at P≤0.05, **Significant difference at P<0.001

The results in table-4 showed that the protein compound injection caused a maximum depression of glucose, cholesterol and total lipids in diabetic rats in the same fusion as for normal rats. However, the protein product (peak B) at a dose of 75 mg/kg is more effective in lowering the biochemical parameters under investigations in diabetic rats compared to normal. This lowering effect in this study is similar to what was previously reported where higher dose of protein is required for hypoglycemic in diabetic mice[18]. This could be explained on the basis that in diabetic experimental animals the amount of glucose, cholesterol and total lipids is higher than in control which required higher amount of protein to produce hypoglycemic effect. This is also further evidence that the protein compound possessing insulin-like action mechanism that facilitates the interance of glucose inside the cells and increases its metabolism[19, 20].

Conclusion

Novel of this research showed that the White cabbage *(Brassica oleracea var. capitata)* has the potential in the treatment of diabetes as it contain active protein compounds that have the employability to reduce the concentration of blood sugar and lipids.

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