International Journal of Enhanced Research in Science, Technology & Engineering
ISSN: 2319-7463, Vol. 7 Issue 2, February-2018, Impact Factor: 4.059

# Isolation and Studying the Effect of Protein Fractions of **Red chili pepper (Capsicum annuum L.)** on Some Biochemical Parameters in Experimental Diabetic Rats

Zena A.M. Aljawadi

Chemistry Department, Science College, Mosul University, Iraq

**ABSTRACT**

**The research was an attempt to Isolate and study the active protein compounds from the aqueous extract of red chili pepper (Capsicum annuum L.), 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 24000 Dalton, also it 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. and 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**.

**Key words: red chili pepper; (Capsicum annuum L.); diabetes disease**

**HOW TO CITE THIS ARTICLE**

Zena A. M. Aljawadi, "Isolation and Studying the Effect of Protein Fractions of Red chili pepper (Capsicum annuum L.) on Some Biochemical Parameters in Experimental Diabetic Rats", International Journal of Enhanced Research in Science, Technology & Engineering, ISSN: 2319-7463, Vol. 7 Issue 2, February-2018.

## INTRODUCTION

The red chili pepper (Capsicum annuum L.) is the fruit of plants from the genusCapsicum. Red chilies contain a substance called capsaicin, which gives peppers their characteristic pungencies, producing mild to intense spice when eaten. In addition, peppers are a good source of vitamin C and small amounts of carotene (provitamin A) and B vitamins (vitamin B6 in particular). They are very high in potassium, magnesium, and iron. Their very high vitamin C content can also substantially increase the uptake of non-heme iron from other ingredients in a meal, such as beans and grains[1,2].

Red chili peppers(Capsicum annuum L.), such as cayenne, have been shown to reduce blood cholesterol, triglyceride levels, and platelet aggregation, while increasing the body's ability to dissolve fibrin, a substance integral to the formation of blood clots. Cultures where hot pepper is used liberally have a much lower rate of heart attack, stroke and pulmonary embolism[3,4], and a very large study found some indications that humans who consume spicy foods, especially fresh chili peppers, were less likely to die of cancer or diabetes[5] Fig.(1).

**Fig.(1): Red chili pepper(Capsicum annuum L.)**

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

## MATERIALS AND METHODS

### Preparation of the Crude Aqueous Extract:

Red chili pepper(Capsicum annuum L.) from local market in Iraq (0.75 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 shahs 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. It was added to cold crude aqueous extract in a ratio (75:100w/v) with slow stirring at $0^{\circ}_{C}$. The mixture was left in a refrigerator 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 (150-170 gm weight),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 (125 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, Cholesterol and Total Lipids:

Serum blood glucose and cholesterol level was measured according to the enzymatic methods using Randox kit for glucose, U. K.[6, 7]. Serum blood total lipids level was measured by Chabral and Chardonnetmethod[8].

**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[9].

## RESULTS AND DISCUSSION

**Precipitation of the Protein:**

Precipitation of total proteins from the crude aqueous extract was accomplished by ammonium sulfate technique[10].The protein content of the precipitate was determined and found to be 54.19% in the crude extract. The efficiency of the precipitation of the protein is 30.12%.

**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 411 ml, Fig.(2).

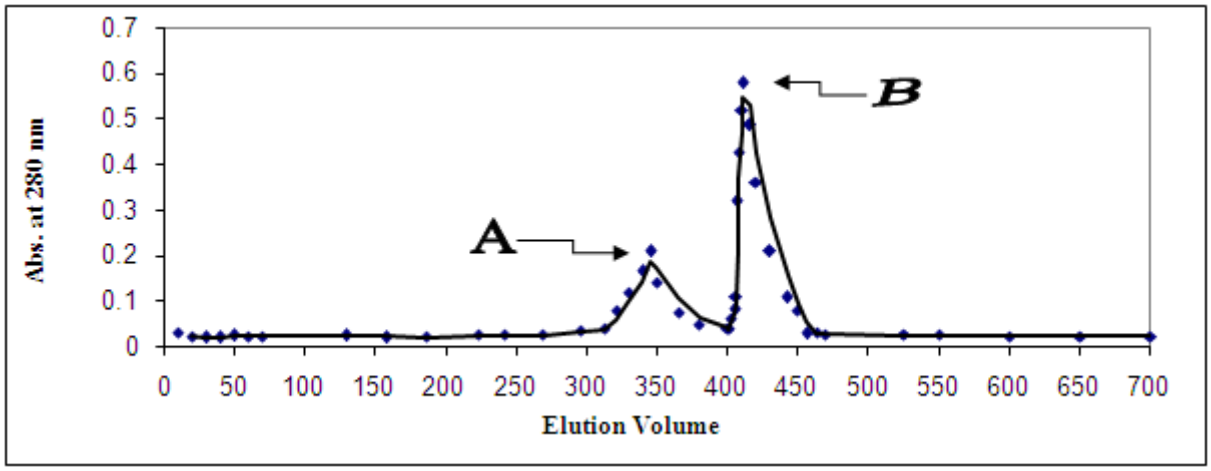


**Fig.(2): Elution profile of total protein precipitate from red chili pepper(Capsicum annuum L.) berry on Sephadex G75 column with a dimension (2.56x87cm). Distilled water was used as eluent, each fraction is 10 ml at flow rate 40 ml/h.**

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 15.9% and (peak B) was found to be 46.1%.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 42000 Dalton while the peak B approximately equal to 24000 Dalton were shown in Fig.(3).

**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 |

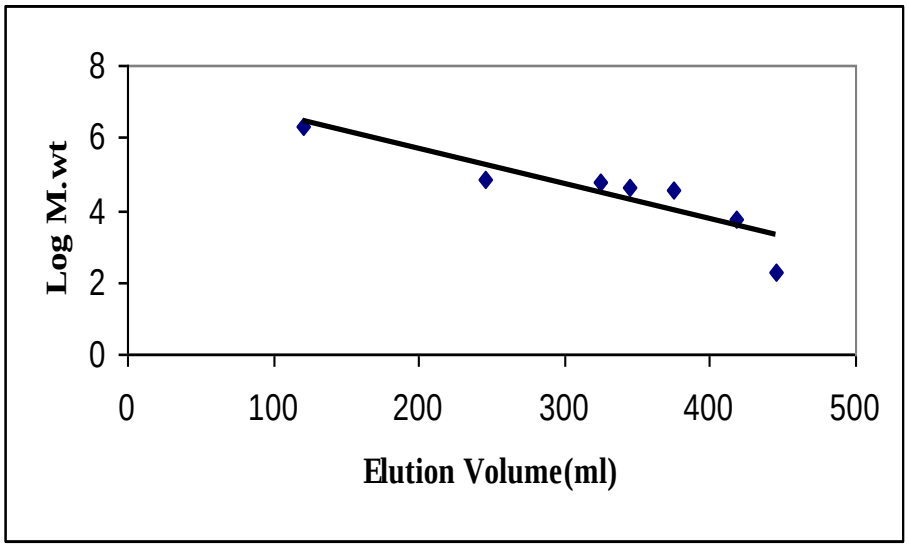


**Fig.(3): A plot of the logarithm molecular weights of known proteins versus elution volumes on a Sephadex G 75.**

**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(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 | 3.50±0.506* | 2.44±0.20 | 403.8±99.97 |
| Protein(peak A) at (125 mg/kg) | 3.94±0.19 | 2.65±0.10 | 413.5±78.90 |
| Protein(peak A) at (100 mg/kg) | 3.90±0.25 | 2.50±0.13 | 409.5±78.78 |
| Protein(peak A) at (75 mg/kg) | 3.76±0.39 | 2.21±0.21 | 393.9±70.30 |
| Protein(peak A) at (50 mg/kg) | 3.75±0.32 | 2.16±0.29 | 383.8±73.53 |
| Protein(peak B) at (125 mg/kg) | 3.76±0.53 | 2.61±0.14 | 377.5±51.77* |
| Protein(peak B) at (100 mg/kg) | 3.71±0.54 | 2.58±0.17 | 371.5±31.66** |
| Protein(peak B) at (75 mg/kg) | 3.53±0.23* | 1.85±0.52** | 261.3±72.22** |
| Protein(peak B) at (50 mg/kg) | 3.77±0.23 | 1.97±0.53 | 387.3±63.46* |

*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 intraperitonealinjection of the rats with crude aqueous red chili pepper(Capsicum annuum L.) extract compared with control[10].When chili-containing meals are a regular part of the diet, insulin requirements drop even lower. Plus, chili's beneficial effects on insulin needs get even better as body mass index (BMI, a measure of obesity) increases[4]. In overweight people, not only do chili-containing meals significantly lower the amount of insulin required to lower blood sugar levels after a meal, but chili-containing meals also result in a lower ratio of C-peptide/ insulin, an indication that the rate at which the liver is clearing insulin has increased[11].The amount of C-peptide in the blood pointer how much insulin is being produced by the pancreas. The pancreas produces proinsulin, which splits into insulin and C-peptide when secreted into the bloodstream. Each molecule of proinsulin breaks into one molecule of C-peptide and one molecule of insulin, so less C-

peptide means less insulin has been secreted into the bloodstream. The results also showed lowering level in cholesterol and total lipids in the blood compared with control group,the lower cholesterol levels maybe by reducing accumulation of cholesterol in the body and increasing its breakdown and excretion in the feces[4, 10].

The results also showed that the protein compound (peak B) in red chili pepper(Capsicum annuum L.)at a dose of (75 mg/kg) led to maximum depression (64%) 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 red chili pepper(Capsicum annuum L.)[11], 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[12].

**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).

**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 | 4.25±0.33* | 2.53±0.26 | 435±51.5 |
| Protein(peak A) at (125 mg/kg) | 4.76±0.07 | 2.84±0.16 | 457±63.10 |
| Protein(peak A) at(100 mg/kg) | 4.75±0.21 | 2.82±0.19 | 456±68.00 |
| Protein(peak A) at(75 mg/kg) | 4.77±0.74 | 2.78±0.16 | 435±78.12 |
| Protein(peak A) at(50 mg/kg) | 4.76±0.77 | 2.71±0.17 | 438±79.17 |
| Protein(peak B) at (125 mg/kg) | 4.76±0.90 | 2.71±0.13 | 433.4±76.10 |
| Protein(peak B) at (100 mg/kg) | 4.67±0.86 | 2.78±0.12 | 438.4±71.33 |
| Protein(peak B) at(75 mg/kg) | 4.74±0.74 | 2.77±0.26 | 391±83.11* |
| Protein(peak B) at(50 mg/kg) | 4.73±0.86 | 2.73±0.16 | 337±75.19* |

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

The results indicated that the orally administration of crude aqueous extract red chili pepper(Capsicum annuum L.)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.

**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 red chili pepper(Capsicum annuum L.)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[4, 13, 14]. 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 | 20.6±3.65* | 2.14±0.43 | 478.51±32.92* |
| Protein(peak B) at(125 mg/kg) | 23.2±4.13 | 2.37±0.43 | 485.53±15.62* |
| Protein(peak B) at(100 mg/kg) | 20.5±4.24 | 2.32±0.72 | 487.34±13.32* |
| Protein(peak B) at(75 mg/kg) | 17.6±8.10* | 1.71±0.37* | 294.40±32.38** |
| Protein(peak B) at(50 mg/kg) | 25.6±3.81 | 2.58±0.81 | 473.40±35.73* |

*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 75mg/kg is more effective in lowering the biochemical parameters under investigations in diabetic rats compared to normal. This lowering effect in this study could help reduce the risk of hyperinsulinemia (high blood levels of insulin)a disorder associated with type 2 diabetes[16]. because insulin was secreted after the chilirich diet and meal. This is also further evidence that the protein compound possessing insulin-like action mechanism that facilitates the entrance of glucose inside the cells and increases its metabolism[14, 15].

## CONCLUSION

Novel of this research showed that the red chili pepper(Capsicum annuum L.) has the potential in the treatment of diabetes as it contain active protein compoundsIn addition, the C-peptide/insulin ratio was highest after the chili-containing meal, indicating an increase in the liver's ability to clear insulin. besides capsaicin, chilies contain antioxidants, including vitamin C and carotenoids, which might also help improve insulin regulation. that have the employability to reduce the concentration of blood sugar and lipids.

## REFERENCES

[1]. Hwang, M. K.; Bode, A. M.; Byun, S.; Song, N. R.; Lee, H. J.; Lee, K. W.; Dong, Z., Cancer Research. 70 (17): 6859–6869, 2010.
[2]. Tewksbury, J. J.; Nabhan, G. P.,Nature., 412 (6845): 403–404. 2001.
[3]. Ahuja KD. and Ball MJ., Br J Nutr.,96(2):239-242. 2006.
[4]. Ahuja KD., Robertson IK.andGeraghty DP., Am J ClinNutr., 84(1):63-69. 2006.
[5]. Sharma SK. andVij AS., PharmacolEur J., 720:55-62, 2013.
[6]. Passing H. and Bablok W., J. Clin. Chem. Clin. Biochem.,24: 863-869, 1983.
[7]. Tietz N.W., Text book of clinical chemistry,3rded .C.A. Burtis, E.R. Ashwood, W.B. Saunders, pp.809-856,1999.
[8]. Chabral and Chardonnet , Cited by GelosonTorol, Philip G. Ackermann, Drac. Clin. Chem. Little, Brown and Company, Boston, 1975.
[9]. Steel R.G. and Torrie J.H., Principles and Procedures of Statistics a Biometrical Approach. 2nd ed., McGraw-Hill, Inc., Singapore, 1980.
[10]. Abdel Magied, M.M., SalamaN.A., Ali M.R., Journal of Food and Nutrition Research, 2(11): 850-856, 2014.
[11]. Nordquist L., Brown R., Fasching A., Persson P. and Palm F.,Am. J. Physiol. Renal Physiol., 297(5): F1265–1272, 2009.
[12]. Esayas K., Shimelis A., Ashebir F. andNegussie, R., Bulletin of the Chemical Society of Ethiop, 25(3): 451-454. 2011.
[13]. Liang Y.T., Tian X., Chen J.N., Peng C., Ma K.Y., Zuo Y., Jiao R., Lu Y., Huang Y. and Chen Z., European Journal of Nutrition., 52(1): 379-388, 2013.
[14]. Ozgur1M., OzcanT., Akpinar-Bayizit, A. and Yilmaz-ErsanL., African Journal of Agricultural Research., 6 (25): 5638-5644, 2011.
[15]. Chandini R.,Udayabhaskaran V.,Binoy J.P. andRamamoorthyK.P., International Journal of Diabetes in Developing Countries, 33(2): 83–85, 2013.