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Isolation and Studying the Effect of Proteinous Fractions of Unripe Banana Fruit (*Musa paradisiaca L.)* on Some Biochemical Parameters in Experimental Diabetic Rats

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ABSTRACT

The research was an attempt to isolate and study the active proteinous compounds from the aqueous extract of unripe banana (*Musa paradisiaca L.)*, using different biochemical techniques. One major compound was isolated using gel filtration chromatography for the precipitate produced by ammonium sulphate treatment. The comparative molecular weight of the isolated proteinous compound was found to be 12566 Dalton. The study also included the effect of the aqueous extract and the proteinous compound in two doses (75, 100 mg/kg body weight) on certain blood constituents in normal and alloxan induced diabetic rats. In normal rats, the blood glucose level was significantly decreased by crude aqueous extract and proteinous compound in both doses (75, 100 mg/kg body weight) compared with control group. In addition, the proteinous compound in a dose (100 g/kg body weight) showed a significant decrease in serum cholesterol and total lipids level. In diabetic rats, the proteinous compound in a dose of (100mg/kg body weight) showed a significant decrease in serum glucose, cholesterol and total lipids levels.

عزل و دراسة تأثير الأجزاء البروتينية لثمرة الموز غير الناضجة على بعض المتغيرات الكيموحيوية في الجرذان المصابة بداء السكر التجريبي

الملخص

تضمن البحث عزل المركبات البروتينية من المستخلص المائي لثمرة الموز غير الناضجة، إذ تم فصل مركب أساسي واحد بتقنية الترشيح الهلامي للراسب البروتيني الناتج من المعاملة بكبريتات الامونيوم وتم تحديد الوزن الجزيئي التقريبي له من خلال المقارنة مع مركبات معلومة الوزن الجزيئي وكان بمدى 12566 دالتون. كما تم دراسة فعالية المستخلص المائي الخام والمركب البروتيني المعزول بجرعتين(75 و 100 ملغم/كغم من وزن الجسم) لعدد من المتغيرات الكيموحيوية في مصل دم الجرذان السليمة و المصابة بداء السكر التجريبي المستحدث بالالوكسان. أظهرت النتائج انخفاضا معنويا في مستوى كلوكوز الدم للجرذان السليمة المعاملة بالمستخلص المائي الخام والمركب البروتيني ولكلا

الجرعتين مقارنة مع مجموعة السيطرة. كما أظهر المركب البروتيني بجرعة(75 ملغم/كغم من وزن الجسم) فاعلية في خفض مستوى الكوليستيرول والدهون الكلية في مصل الدم وبشكل معنوي. أما بخصوص الجرذان المصابة بداء السكر المستحدث بالالوكسان فقد اظهر المركب البروتيني بجرعة (100 ملغم/كغم من وزن الجسم) انخفاضاً معنوياً في مستوى الكلوكوز و الكوليستيرول و الدهون الكلية في مصل الدم مقارنة مع مجموعة السيطرة.

INTRODUCTION

Bananas are a favorite food for everyone from infants to elders. Sports enthusiasts appreciate the potassium-power delivered by this high-energy fruit (Bazzano et al., 2003). The green unripe banana (*Musa paradisiaca L.*) is mostly starch. As it ripens this gets progressively hydrolyzed to soluble sugars with the result that the total sugar in a green banana becomes 15.20% when ripe(Rao et al., 1991; Dunjic et al., 1993; Delbourg et al., 1996). Some banana cultivars are also rich in provitamin A carotenoids, which have been shown to protect against chronic diseases, including certain cancers, cardiovascular disease, and diabetes(Englberger et al., 2003; Rashidkhani et al., 2005). Hypoglycemic effect of the proteinous fraction from unripe banana fruit on normal and alloxan induced diabetic rats was also reported (Pari and Maheswari, 1999; Pari and Umamaheswari, 2000)

The aim of the study is to investigate the effect of the proteinous compounds isolated from the aqueous extract of the unripe banana 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

Unripe bananas (*Musa paradisiaca L.*) from local market (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 temperature 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 (Ahmad and Al-Chalabi, 2002). Total protein was determined by modified Lowry method (Schacterle and Pollack, 1973).

Precipition of the Proteins

Proteinous materials were separated from the cold extract using ammonium sulfate precipitation (Robyt and White, 1987). 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 proteinous precipitate was dried by lyophilization then kept in a tight sample tube in a freezer for the next step.

Fractionation of the Proteinous 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 proteinous 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(Voet and Voet, 1990).

Intrapertioneal Injection

Group of healthy adult rats (155-173 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 (125 mg/kg) to induce diabetic rats (Ahmad and Al-Chalabi, 2002). Each group was then sub divided into four 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. (Passing and Bablok, 1983).

Determination of Cholesterol

Serum blood cholesterol level was measured according to the enzymatic method using Randox kit for Cholesterol, U. K.(Tietz,1999).

Determination of Total Lipids

Serum blood total lipids level was measured by Chabral and Chardonnet method (Chabral and Chardonnet, 1937).

Statistical Analysis

The statistical methods used to analyze 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 (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Precipitation of the Protein:

Precipitation of total proteins from the crude aqueous extract was accomplished by ammonium sulfate technique (Robyt and Whit,1987).The proteinous content of the precipitate was determined(Schacterle and Pollack,1973) and found to be 77.1% in the crude extract.The efficiency of the precipitation of the protein is 70.1%.

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 427.7ml (Fig.1).

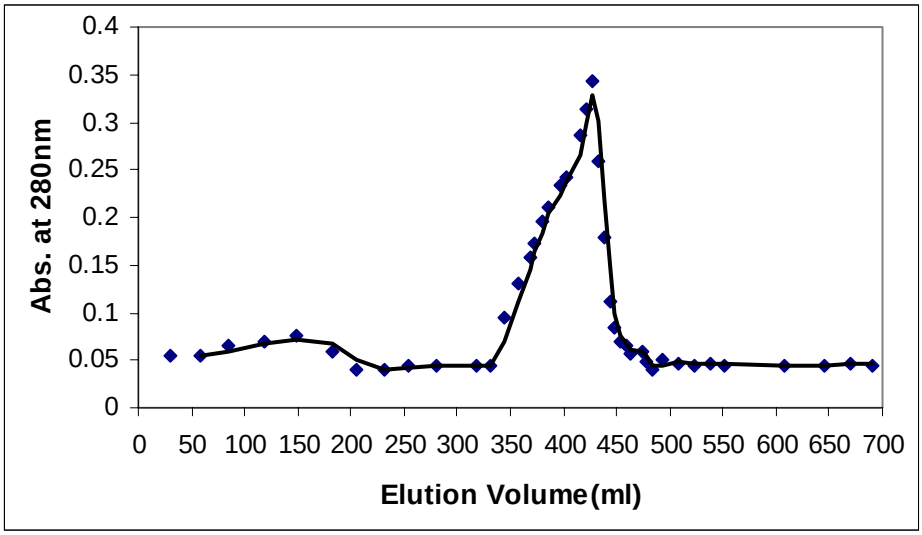

Fig.1: Elution profile of total protein precipitate from unripe banana(*Musa paradisiaca L.)* berry on Sephadex G75 column with a dimension (2.56x87cm). Distilled water was used as eluent, each fraction is 9 ml at flow rate 36 ml/h.

Quantitative determination of the protein in the peak after gel filtration chromatography was performed and then the percent of the component (peak) was found to be 18%.

Comparative molecular weight of the isolated proteinous compound was determined by gel filtration chromatography on a pre-calibrated column using known molecular weight proteins as shown in Table (1), and found to be 12566 dalton Fig.(2).

Table 1: Molecular weights and their volumes of different proteinous compounds on Sephadex G 75.

Compounds	**Molecular weight (Dalton)**	**Elution volume (ml)**
Blue dextran	2000000	270
Bovine serum albumin (BSA)	67000	349
α- amylase	58000	379
Eggs albumin	45000	399
Pepsin	36000	449
Insulin hormone	5750	475
Tryptophan	204	490

Effect of crude aqueous extract and the isolated proteinous compound on glucose, cholesterol and total lipids in normal rats

The results in Table(2) showed the effect of crude aqueous extract and protein products on glucose, cholesterol and total lipids in normal rats.

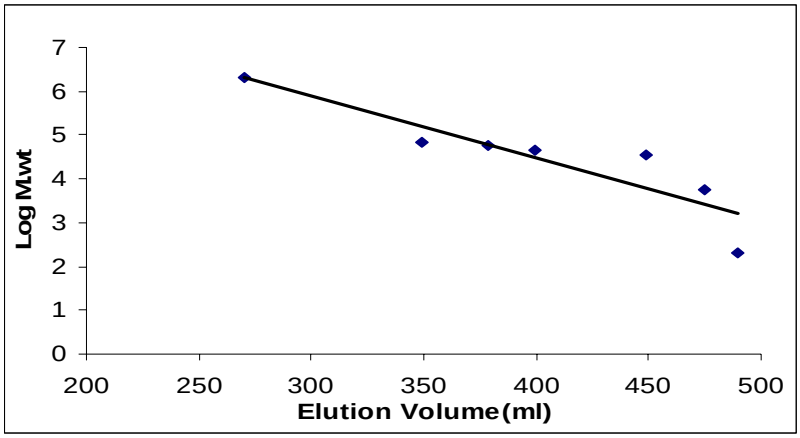

Fig. 2: A plot of the logarithm molecular weights of known proteins versus elution volumes on a Sephadex G 75.

Table 2:Effect of crude aqueous extract and isolated proteinous compound on serum glucose, cholesterol and total lipids in normal rats.

Groups	Glucose (mmol/l)	Cholesterol (mmol/l)	Total lipids (mg/dl)
Control	4.82±0.36	2.41±0.29	634.9±38.49
Crude aqueous extract	3.89±0.46*	2.01±0.10	485.7±122.08
Protein (100 mg/kg)	3.49±0.71**	2.02±0.67	442.83±123.50
Protein (75 mg/kg)	2.95±0.72**	1.93±0.15*	406.96±145.73*

*Significant difference at P=0.05
**Significant difference at P<0.05

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 unripe banana extract compared with control. This finding agreed with the result obtained from other investigators (Chithra and Leelammay, 1999). Hypoglycemic effect on blood glucose might be due to unripe banana contained an insulin like action and / or other products which stimulate insulin secretion from pancreatic beta cells or increase the rate of entrance of various sugars via glucose transporters in the plasma membrane(Ashcroft and Ashcroft, 1992; Gray and Flatt, 1999; Jachak, 2002). 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 (Chithra and Leelammay, 1999). Decrease in cholesterol might due to inhibition of ß-hydroxy-ß-methyl glutaryl-CoA reductase responsible for cholesterol synthesis (Ingebritsen et al., 1979; Maechller et al.,1992). The decrease in total lipids might be due to the insulin like action which activates lipase enzyme and facilitates lipolysis of lipids (Murray et al., 1996).

The results also showed that the protein compound in unripe banana at a dose of (75 mg/kg) led to maximum depression (39%) 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 unripe banana (Gray and Flatt, 1999; Jachak, 2002), or might be due to insulin like structure of the protein product that binds with insulin receptors and lower blood glucose level (Ahmad and AL- Chalabi, 2002). The protein compound 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 the inhibition of 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 (Khan et al., 2003).

Effect of crude aqueous extract and the isolated proteinous compound on glucose, cholesterol and total lipids in diabetic rats

To test the effect of crude aqueous extract and the protein compound from unripe banana 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 (Nammi et al., 2003) thus diabetes will occur (Holm, 1997). The results of intraperitoneal injection into diabetic rats were listed in Table 3.

Table 3:Effect of crude aqueous extract and isolated proteinous compound on serum glucose, cholesterol and total lipids in diabetic rats.

Groups	Glucose (mmol/l)	Cholesterol (mmol/l)	Total lipids (mg/dl)
Control	32.93±4.05	4.04±0.02	679.36±33.74
Crude aqueous	27.74±1.24	3.17±0.11*	505.76±32.91**
Protein (100 mg/kg)	18.82±1.09***	1.97±0.27*	389.43±23.47***
Protein (75 mg/kg)	21.65±2.98**	2.68±0.39	432.79±80.82**

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

The results in Table (3) showed that the protein compound injection caused a maximum depression of glucose, cholesterol and total lipids in diabetic rats in the same fasnion as for normal rats. However, the protein product at a dose of 100 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 (Ahmad and Tohala 2005). 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 a further evidence that the proteinous compound possessing insulin-like action mechanism that facilitates the interance of glucose inside the cells and increases its metabolism (Platel and Srinivasan, 1997; Jachak, 2002).

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