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The Effect of Proteinous Compounds from *Myrtus communis* L. Fruit on Some Biochemical Parameters in Mice

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ABSTRACT

Three compounds (A, B and C) were isolated by gel filtration chromatography of the precipitate produced by acetone precipitation of the aqueous extract of *Myrtus communis* L. fruits. Comparative molecular weights determination by gel filtration using standard proteins gave a value of 74990, 6310 and 620 dalton respectively. A single intraperitoneal injection of the isolated compounds (A, B and C) at a does of 77.5 mg/Kg was given to normal mice and some biochemical parameters were determined. The results of injection showed a significant decrease of serum glucose, cholesterol, triglycerides, total lipids level and glycogen content in liver tissues for both component A and B at different variation as compared to control group. However, compound C showed only a significant decrease in glycogen content in liver tissues.

تأثير المركبات البروتينية من ثمرة الآس على بعض المتغيرات الكيموحيوية في الفئران

الملخص

تم فصل ثلاثة مركبات (A و B و C) بتقنية الترشيح الهلامي للراسب البروتيني الناتج من الترسيب بالاسيتون للمستخلص المائي لثمرة الآس. كذلك تم تعيين الوزن الجزيئي التقريبي للمركبات الثلاثة بتقنية الترشيح الهلامي من خلال مقارنة حجوم الروغان للمركبات المفصولة مع مركبات معروفة الوزن الجزيئي وكانت بمدى (620 و 6310 و 74990 دالتون) على التوالي.

وشملت الدراسة ايضاً تأثير جرعة واحدة من المركبات البروتينية المعزولة وبتركيز (77.5 ملغم/كغم) في الفتحة البروتينية للفئران السليمة على بعض المتغيرات الكيموحيوية حيث ادت الجرعة الى حدوث انخفاض معنوي في مستوى الكلوكوز والكوليسترول والكلسيريدات الثلاثية والدهون الكلية في مصل الدم ومحتوى الكلايكوجين في انسجة الكبد لكلا المركبين (A و B) مقارنة مع مجموعة السيطرة . اما المركب (C) فقد أظهر إنخفاضاً ملحوظاً لكمية الكلايكوجين في أنسجة الكبد فقط.

INTRODUCTION

Myrtus communis is a species belonging to *Mytraceae* family grown in Iraq and many other countries as shrubs (Jawad et al., 1988). Locally, the plant known as Yas and its fruits (berries) has some time been called Hamablas, Haimush and Fats (Townsend and Evan Guest, 1980). The berries which contain essential oil, citric acid, maliec acid, tannin and resin are used in flavouring wines and foods (Chakravarty, 1976). The leaves and berries were used as antidiabetic, in eczema, epilepsy, wounds and ulcers (Degtyarova, 1962). Pharmacological investigations of Myrtle revealed antimicrobial (Twaij et al., 1988a), hypotensive and cardiac depressant (Twaij et al., 1988b), antihyperglycemic effect (Elfellah et al., 1984). Hypoglycemic effect of the proteinous compounds from Myrtle leaves on normal and alloxan-induced diabetic mice was also reported (Ahmad and Al-Ghalabi, 2002).

The present aim of the study is to investigate the effect of the proteinous compounds from the aqueous extract of the berries of *Myrtus communis* L. on some biochemical parameters in experimental animals. Hopping to isolate an active compounds having insulin-like action and or structure.

MATERIALS AND METHODS

Plants Material:

Myrtus communis L. fruits were collected from the garden of the University of Mousl. They were classified according to plants taxonomy and classification (Core, 1964; Rendle, 1975).

Preparation of Crude Extract:

The aqueous crude extract was prepared by freezing and thawing the fruits of the plant (1000g) with liquid nitrogen several times after homogenized for five minute using a blender to rupture the cell membrane.

Distilled water (2 lt) was added and the crude homogenate was stirred for additional two hours in ice bath, and then kept in a refrigerator overnight. The mixture was filtered through several layers of moselin (Chees-Cloth) to remove all the residual materials. Finally,the filtrate was then centrifuged using a refrigerated centrifuge for (20) minutes at (4000 xg). The supernatant (crude extract) after reduction its volume to about (1/3) by lyophilization, was kept for further investigation. Total protein concentration was determined by a modified Lowry method (Schacterle and Pollack, 1973).

Precipitation of the protein:

The proteinous material was separated from the crude extract by cold acetone precipitation technique (Robyt and White, 1987). To the crude extract (360 ml) cold acetone was added at a ratio (40:60 v/v) with slow stirring at 0^{o}C. The mixture was left for (24) hours at 0^{o}C and the precipitated protein was isolated by cold centrifugation for (20 min) at (12000 xg). The proteinous precipitate was dried by lyophilization for the next step.

Fractionation of the Total Protein:

The isolated proteinous precipitate was fractionated by gel filtration chromatography using sephadex G 75 on a 4.5 x 92 cm column with distilled water as eluent. Each

peak component (A, B and C) was pooled, freezed then dried by lyophilization in air tight sample tube for further investigations.

Intraperitoneal Injection:

Groups of healthy male adult mice (30-35 g) weight were obtained from the animal house of the Veternary Medicine College, University of Mosul. The mice were fasted for (16 hr) and divided randomly into five groups each containing five mice. Group one was kept as a control group while the other four groups were injected intraperitoneally with the frationated proteins A,B and C (Group 2,3 and 4) and insulin (6.64 iu/Kg, Actrapid 40 iu/ml Nova Nor disk A/S, Denemark) as group 5. After two hours of injection blood samples were collected for analysis by the orbital sinus puncture under ether anaesthesia , using non-heparinized micro-hematocrit capillary tubes (Ahmad and Al-Chalabi, 2002).

Determination of Biochemical Parameters:

Serum blood glucose, cholesterol and triglycerides levels were measured according to the enzymatic methods using Randox Kit for glucose, U.K. (Bahram and Trinder, 1972), bioMe rieux for cholesterol and triglycerides Kits, France (Allain et al., 1974; Fassati and Prencipe, 1982). Total lipids levels were determined by the method of Chabrol and Chardonnet (1937). Glycogen content in liver tissues was estimated by anthrone method (Plummer, 1978).

Statistical Analysis:

Results were expressed as mean $\pm$ S.E., estimation of the significance of difference between control and proteinous compounds, insulin treated groups were analysed by student's t-test (Steel and Torrie, 1980).

The percentage of glycemic variation after two hours of injection for treated groups was calculated by applying the formula:

$$\% \text{ Change of glycemia} = \frac{Gx - Gc}{Gc} \times 100$$

Where Gc and Gx are the values of control and glycemia after two hours (Gonzalez et al., 1992). The above formula was applied for the other parameters.

RESULTS AND DISCUSSION

Precipitation of the Protein:

Precipitation of total proteins from the crude extract was accomplished by cold acetone technique (Robyt and White, 1987). The proteinous content of the precipitate was determined (Schacterle and Pollack, 1973) and found to be 1.24 % in the crude extract. The efficiency of the precipitation of the protein is 8.13 % .

Fractionation of Total Protein:

Fractionation of total proteins was accomplished by gel filtration chromatography using Sephadex G 75 to give three peaks (A, B and C) with elution volumes of 350 ml, 1314 ml and 2229 ml respectively (Fig. 1).

Fig. 1: Elution profile of total protein from *Myrtus communis L.* berry on Sephadex G75 (4.5x92 cm). Distilled water was used as eluent. The arrows A, B and C represent the elution volumes of the top peaks A, B and C. The volume of each fraction is 12.5 ml at flow rate 125 ml/hr.

Quantitative determination of total protein in each peak after gel filtration chromatography was performed and then the percentage of each component (peak) was determined and found to be 0.27 %, 77.98 % and 9.42 % respectively.

Comparative molecular weight of each component (A, B and C) was determined by gel filtration chromatography on a pre-calibrated column using known molecular weight proteins and formed to be 74990 , 6310 and 620 dalton respectively.

The effect of isolated compounds on some biochemical parameters:

The results of intraperitoneal injection with the isolated compounds A, B, C and insulin in mice were listed in Table (1).

Table 1: The effect of isolated compounds A, B, C and insulin on serum glucose, cholesterol levels in normal mice.

Group N=5	Sample	Glucose level (mg/dL)		Cholesterol level (mg/dL)	
		MEAN ± SE	% Change	MEAN ± SE	% Change
1	Control	141 ± 33.3	---	193 ± 1.38	---
2	Compound A	97 ± 1.52**	-31.2	185 ± 2.77*	-4.2
3	Compound B	95 ± 5.11***	-32.6	159 ±2.07***	-17.6
4	Compound C	132 ± 1.71	-6.4	189 ± 1.58*	-2.1
5	Insulin	14 ± 1.41***	-90.1	170 ± 2.07**	-11.9

- Blood sample were taken after two hours of intraperitoneal group injection.
- Proteinous compounds A and B doses were 77.5 mg/Kg and insulin dose was 6.64 iu/Kg.
- Significantly different from control to t-test at $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.
- N = Number of mice in each group is five.

Results depicted from table (1) indicated that in comparison to control group there is a significant decrease in the level of glucose in blood serum for group 2 and 3 of fasted mice injected intraperitoneally (77.5 mg/Kg) with each of the isolated compound A or B .

On the other hand, insulin was more effective in lowering serum glucose level for normal mice. The decrease in serum glucose level by insulin was in agreement with several studies in normal individuals (Gerich et al., 1979) , in normal rats (Ercan et al., 1955) and in broiler chicks (Al-Kajafi et al., 1997). The hypoglycemic effect of insulin may be due to increase in the rate of entrance of various sugars and glucose transporters in the plasma membrane (Ashcroft and Ashcroft, 1992).

The results of compounds A, B and C showed a significant decrease at $P <0.001$ in serum blood glucose level for A and B only compared to the control group. These results are in good agreement with the previous work on the hypoglycemic activity of the proteinous compound isolated from the aqueous extract of other local plants (Al-Obaydee, 1996; Ahmad et al., 2000, 2002).

It was suggested that the mechanism of action of the low molecular weight protein isolated from different local plants was similar to insulin in its structure and action (Ahmad and AL-Chalabi, 2002). Also, the decrease in serum glucose level of mice treated with high molecular weight protein compound (compound A) was in agreement with the results obtained by other investigators (Al-Khashab, 1999; Al-Ameen, 2001; Ahmad and AL-Chalabi, 2002). It was proposed that the protein compounds with high molecular weight which were isolated from the aqueous extracted of different plants

might contain sequence of amino acids similar to insulin which binds to specific insulin receptors located on the plasma membrane. Binding might medicate or facilitate the rate of uptake of glucose to inside the cell leading to hypoglycemic activity or may cause an increase secretion of internal insulin by impairing Langerhans cell in normal and diabetic mice. Also this hypoglycemic effect of the proteinous compounds is in agreement with the results obtained for a peptide, polypeptide isolated from fruit, seed and tissue of *Momordica charantia* in patients with juvenile diabetes (Khanna et al., 1981) and in normal and diabetic mice (Lei et al., 1985).

The decrease in cholesterol level for the compounds A, B, C and insulin was also noticed in Table (1). These results were in agreement with normoglycemic chick ingested with the proteinous compounds isolated from *Saliva syriaca* aqueous extract (Ahmad, 2000). The decrease in cholesterol level might be due to the inactivation of the regulatory enzyme β-hydroxy-β-methyl glutaryl CoA reductase responsible for cholesterol biosynthesis (Ingebritsen et al., 1979). The decrease is also in agreement with the hypocholesterolemic properties of fenugreek seeds on mice (Petit et al., 1995).

On the other hand, the decrease of cholesterol level when treated with insulin is in agreement with the results obtained on diabetic rats and rabbits (Mohammad, 1998; Al-Kakey, 1999). This might due to the inhibiting intestinal acyl CoA: cholesterol acyl transferase which is responsible for absorbing cholesterol from the intestine (Maechler et al., 1992).

Triglycerides and total lipids level were also affected by the isolated compounds A, B and C (Table 2) .

Table 2: The effect of isolated compounds A, B, C and insulin on serum triglycerides and total lipids levels in normal mice.

Group N=5	**Sample**	**Triglycerides level (mg/dL)**		**total lipids levels (mg/dL)**	
		MEAN ± SE	**% Change**	**MEAN ± SE**	**% Change**
1	Control	154 ± 2.07	---	722 ± 1.58	---
2	Compound A	132 ± 3.71***	-14.3	548 ± 5.10***	-24.1
3	Compound B	143 ± 2.55**	-7.1	536 ± 1.30***	-25.8
4	Compound C	148 ± 4.81*	-4.1	700 ± 4.62*	-3.1
5	Insulin	139 ± 1.22**	-9.7	629 ± 2.12***	-12.9

- Blood sample were taken after two hours of intraperitoneal group injection.
- Proteinous compounds A , B and C doses were 77.5 mg/Kg and insulin dose was 6.64 iu/Kg.
- Significantly different from control to t-test at $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.
- N = Number of mice in each group is five.

The results in Table (2) showed a significant decrease in both triglycerides and total lipids level. These results are in agreement with the results of decreasing of the total lipids for the proteinous extract of *Olea europaea leaves* (Ahmad et al., 1994) also in agreement with the decrease in cholesterol, triglyceride levels of the aqueous extract and seeds of *Trigonella foenum-graecum* for rats (Sharma, 1984; Riyad et al., 1988) and for rabbits (Al-Hussary, 1993). The decrease might be due to the inhibition of lipase enzyme and preventing lipolysis of stored lipids (Goodman and Gillman, 1985; Al-Kass, 1989).

Finally, glycogen content in liver tissues was decreased when the mice were injected with the isolated compounds A, B and C (Table 3).

Table 3: The effect of isolated compounds A, B, C and insulin on glycogen content in liver tissues in normal mice.

Group N=5	**Sample**	**glycogen content in liver tissues level (mg/dL)**	
		MEAN + SE	**% Change**
1	Control	348 + 1.64	---
2	Compound A	200 + 1.52***	-42.5
3	Compound B	289 + 2.07***	-16.9
4	Compound C	160 + 2.17***	-54.02
5	Insulin	392 + 1.92**	+12.6

- Liver tissues were taken after two hours of intraperitoneal injection.
- Proteinous compounds A , B and C doses were 77.5 mg/Kg and insulin dose was 6.64 iu/Kg.
- Significantly different from control to t-test at $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.
- N = Number of mice in each gropu is five.

The decrease in glycogen content as shown in Table (3) is in agreement with the results obtained by other investigators for aqueous extract of different plants (Al-Salahy and Hassanien, 1993; Ponnachan et al., 1993) and for the protein isolated from *Eminium spiculatum* leaves and *Brassica campestris Linn* var. rapa root (Flayeh et al., 1994; Ahmad et al., 2002). This decrease might stimulate glycogenolysis due to decrease in blood glucose which activate glucagon hormone secretion (Applemann et al., 1973; Amiel et al., 1987).

On conclusion *Myrtus communis L.* berries containing active components having insulin like action and/or structure also having a significant depression of cholesterol, total lipid and glycogen content in live tissues.

Further experiments are know under current investigations in our laboratories to support the hypothesis that hypoglycemic plants may contain a compound having insulin-like action and/or structure.

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