------ Raf. J. Sci., Vol. 25, No.1 pp. 82-100, 2014------

The Effect of Isolated Obestatin Hormone from Plasma on some Biochemical Parameters in Normal and Diabetic Rats

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(Received 2 / 4 / 2013 ; Accepted 10 / 6 / 2013)

ABSTRACT

The study included an attempt to isolate and purify obestatin from healthy human plasma using different biochemical techniques. Two proteinous peaks had been isolated by gel filtration chromatography (Sephadex G-50) from the precipitate produced by precipitation using cold acetone. It was found that the second peak (peak B) only had an obestatin. Two proteinous components had also been isolated by the same technique using Sephadex G-25 from the peak B. It was found that the second peak (peak D) had a high concentration of obestatin .

Furthermore, the purity of the isolated obestatin (peak D) had been identified by the reversed phase high performance liquid chromatography and by SDS-polyacrylamide gel electrophoresis technique. The results obtained from the RP-HPLC showed that there was a good identity in retention time between the standard and the isolated obestatin (peak D).

The approximate molecular weight of partially purified obestatin was (2573 ± 100 Da) and (2660 Da) using gel filtration chromatography by Sephadex G-25 and (SDS-PAGE) technique respectively.

The effect of the isolated obestatin (peak D) on some biochemical parameters in normal and alloxan induced diabetic male rats had been studied. After one week of treatment, the results showed that the obestatin at the dose (1 μmol / kg of body weight / d) caused a significant decrease in the levels of glucose, total lipids, total cholesterol, triglycerides, LDL-C, VLDL-C and MDA, in a addition to a significant increase in the levels of HDL-C in normal and alloxan induced diabetic rats and GSH in alloxan induced diabetic rats.

It was concluded that the isolated obestatin from plasma had a an important role in metabolism of glucose and lipid profile in normal and alloxan induced diabetic rats.

Keywords: Obestatin hormone, biochemical parameters, diabetes.

تأثير هرمون الأوبستاتين المعزول من بلازما الدم على بعض المتغيرات الكيموحيوية لذكور الجرذان السليمة والمصابة بداء السكر

الملخص

تضمن البحث عزل وتنقية هرمون الأوبستاتين من بلازما شخص سليم باستخدام التقنيات الحياتية المختلفة. حيث فصلت حزمتين بروتينيتين بتقنية كروماتوغرافيا الترشيح الهلامي باستخدام هلام السيفدكس (G-50) للراسب البروتيني الناتج من الترسيب بالأسيتون البارد. وقد احتوت الحزمة الثانية (الحزمة B) فقط على هرمون الأوبستاين. فضلا عن إلى ذلك عزلت حزمتين بروتينيتين بنفس التقنية وباستخدام هلام السيفدكس (G-25) للحزمة البروتينية (الحزمة B) الناتجة من تقنية الترشيح الهلامي (Sephadex G-50) وأظهرت الحزمة الثانية (الحزمة D) وجود تركيز عالٍ من هرمون الأوبستاتين.

فضلا عن ذلك، قدرت نقاوة الهرمون (الحزمة D) باستعمال تقنية الهجرة الكهربائية و كروموتوغرافيا السائل عالي الأداء. ولوحظ وجود تطابق في وقت الاحتباس لهرموني الأوبستاتين القياسي والمعزول (الحزمة D).

كما حدد الوزن الجزيئي التقريبي لهرمون الأوبستاتين باستخدام الترشيح الهلامي بهلام السيفدكس G-25 والهجرة الكهربائية باستخدام SDS وكان (2573 ± 100 Da) و(2660 Da) على التوالي.

درس تأثير هرمون الأوبستاتين المعزول (peak D) على بعض المتغيرات الكيموحيوية في الجرذان السليمة والمصابة بداء السكر المستحدث بالالوكسان. وأظهرت النتائج، بعد أسبوع من المعاملة بهرمون الاوبستاتين (الحزمة D) وبجرعة ((1) مايكرومول / كغم من وزن الجسم/ يوم)، انخفاضا معنويا في مستوى الكلوكوز، الدهون الكلية، الكولسترول الكلي، الكليسيريدات الثلاثية، كولسترول البروتين الدهني واطئ الكثافة، كولسترول البروتين الدهني واطئ الكثافة جدا والمالوندايالديهايد. بينما اظهرت ارتفاعا معنويا في مستوى كولسترول البروتين الدهني عالي الكثافة في الجرذان السليمة والمصابة بداء السكر المستحدث بالالوكسان، وكذلك مستوى الكلوتاثايون في الجرذان المصابة بداء السكر المستحدث بالالوكسان.

يستنتج من هذا أن هرمون الأوبستاتين المعزول من بلازما الدم له دور كبير في عملية أيض الكلوكوز والدهون في الجرذان السليمة والمصابة بداء السكر المستحدث بالالوكسان.

الكلمات الدالة: هرمون الأوبستاتين، متغيرات كيموحيوية، داء السكر.

INTRODUCTION

In 2005, it was discovered that 23 amino acids amidated peptide was called obestatin hormone. It was firstly isolated and purified from the rat stomach using high-performance liquid chromatography (HPLC) of Sep-Pak C18 and fractionated using a sephadex G50 gel filtration column (Zhang *et al*., 2005). The most obestatin producing cells in the stomach were distributed in the basal part of the oxyntic mucosa (Jahan *et al*., 2011). As well as, it is present in plasma (Mondal *et al*., 2008), adipose tissue (Granata *et al*., 2012) and different peripheral tissues (Ren *et al*., 2009).

Obestatin is a peptide hormone that is derived from the C-terminal part of the mammalian preproghrelin gene that also encodes ghrelin. It was found to interact with the orphan receptor G-protein coupled receptor 39 (GPR 39) (Zhang *et al*., 2005; 2008; Dong *et al*., 2009) and to oppose the stimulatory effect of ghrelin on food intake and gastrointestinal motility (Zhang *et al*., 2005; Ishitobi *et al*., 2012). It was also circulated in blood as an endocrine hormone (Semi *et al*., 2011)

Many studies showed that obestatin promoted the survival of β-cells and regulated the endocrine pancreatic function (Volante *et al*., 2009; Granata *et al*., 2010), insulin biosynthesis, and antiapoptic actions in pancreatic β-cells (Granata *et al*., 2008). It was also inhibited glucose induced insulin secretion, prevented lipolysis (Ren *et al*., 2008; Granata *et al*., 2012) and regulated energy balance (Zhang *et al*., 2005).

The aim of this research is to isolate and purify obestatin from human plasma, and study its effect on diabetes induced in the experimental animals.

MATERIALS AND METHODS

Isolation and purification of obestatin from plasma

- Sample:

A human fresh plasma (50 ml) was obtained from only one healthy male person, his age (30 years), weigh (70 kg) with the assistance of blood bank in Mosul city.

Obestatin hormone was purified from plasma as following:

I- Organic solvent precipitation:

Proteinous materials were precipitated by using acetone (Zhang *et al*., 2005). Gradual addition of cold acetone (60:40 v/v) to plasma with slowly stirring at 4 °C for 60 min. The mixture was left in the refrigerator for 24 h and the precipitated protein was isolated by centrifugation for 30 minutes (10000 × g) in a cooling centrifuge. Protein concentration was estimated after dissolving the precipitate in a lowest volume of distilled water. The solution of proteinous precipitate was kept in a tight sample tube for next step.

II- Gel filtration chromatography:

Two columns were used in this research

A- The first: It has a dimension of (2 × 100 cm) which filled by a sephadex G-50 gel. The proteinous precipitate solution prepared in section (I) was added to this column and eluted with distilled water. The fractions was collected at flow rate (88 ml/h).

B- The second: It has a dimension of (1.21 × 110 cm), was filled by sephadex G-25 gel. The proteinous material which was obtained from the first column (peak B which contains obestatin hormone) was added to this column and eluted with distilled water. The fractions were collected at 68 ml/hour. Protein and obestatin concentrations were estimated at each step of isolation.

The comparative molecular weight of proteinous peaks (B and D) which contains obestatin hormone was obtained from its elution volume under the same conditions of known molecular weight in both columns. Peak B (from the first column) and peak D (from the second column) were dried by freeze dryer technique.

III- Electrophoresis

A sample from (peak D) which contains a high obestatin concentration was applied on SDS-PAGE (Laemmli, 1970) using slab electro apparatus (Mediphor Heidelberg Dosage, Germany). This analysis was performed in the Department of Chemistry / College of Science.

IV- High performance liquid chromatography (HPLC)

A sample from (peak D) and standard obestatin (CUSBIO, China) were applied on C18 RP-HPLC (Agilent 1200 series, USA). This analysis was performed in the state Company for Drug Industries and Medical Appliances in Nineveh.

Obestatin assay

Obestatin concentration was determined by enzyme linked immunosorbent assay (ELISA) technique (Cuping and Dongmei, 2009) using CUSBIO kit (China). This analysis was performed in the immunity laboratory in Al-Salam Hospital in Mosul City, BIO-TEK INSTRUMENTS, USA.

Determination of protein concentration

The concentration of protein was determined by the modified of lowery (Schacterle and Pollack, 1973), and using bovine serum albumin as a standard (its extinction coefficient equal to 0.67).

The second part of experimental part focused on studding the effect of obestatin (which was isolated and purified from human plasma by different techniques) on the diabetic rats.

Effect of isolated obestatin hormone (peak D) in normal and alloxan–induced diabetic rats

Animals used

Male albino rats (24) weighing (300±35 g), obtained from animal house, College of Veterinary Medicine, University of Mosul were used in the experiments. They were housed under standard environmental conditions, are the pelleted food and water were available *ad Libitum.*

Induction of diabetes

The animals were fasted for (24 h) and the diabetic was induced by injecting them with alloxan tetrahydrate (180 mg/kg, i.p) dissolved in sterile normal saline (Miura *et al*., 1995). The diabetic state was monitored by hyperglycemia (Colorimetric assay kit, BIOLABO, France) along the next ten days. Rats with blood glucose level more than (250 mg/dl) were considered diabetic and used for study.

Experimental design

The dose of the isolated obestatin hormone was (1 µmol). The dose used for intraperitoneal injection was selected on the basis that was the same as used in several previous in vivo studies (Zhang *et al*., 2005; Green *et al*., 2007; Subasinghage *et al*., 2010; Granata *et al*., 2012). The rats were divided randomly into four groups, each contained six rats:

1. The first group was normal rats injected intraperitoneally with physiological saline solution and served as a control group.
2. The second group was alloxan –induced diabetic rats and considered to be a control diabetic group.
3. The third group was injected intraperitoneally with (1 μmol / kg of body weight/d) of isolated obestatin (peak D).
4. The fourth group (alloxan induced diabetic rats) was injected intraperitoneally with (1 μmol / kg of body weight/d) isolated obestatin (peak D). All the groups were injected for one week.

Collection of blood

Fasting blood samples (16 h) were collected from four groups by orbital sinus puncture technique, using capillary tube without anticoagulant (Tomoda *et al*., 1990). Serum was separated and used to estimate the following biochemical parameters:

Blood glucose was determined immediately by the enzymatic colorimetric method (Trinder, 1969). Total lipids was determined by colorimetric method manually (Chabrol and Chardonnet, 1937). Total cholesterol was determined by enzymatic colorimetric method (Allian *et al*., 1974). Triglycerides was determined by enzymatic colorimetric method (Fossati and Prencipe, 1982). High density lipoprotein-cholesterol (HDL-C) was determined by precipitation method (Lopez-Virella *et al*., 1977), using BIOLABO kit (France), but the low density lipoprotein – cholesterol (LDL-C) was calculated using the following equation.
LDL-C (mmol/L) = Total cholesterol – HDL-C-T.G conc.(mmol/L)/2.2 (Burtis and Ashwood, 1982).

Very low density lipoprotein – cholesterol (VLDL-C) was calculated using the following equation:

VLDL-C conc. (mmol/L): T.G conc. (mmol/L)/2.2 (Fischbach, 2000). Glutathione was determined manually according to the modified method of (Sedlak and Lindsay, 1968), malondialdehyde was determined manually by the modiefied method of (Guidet and Shah, 1989). Alkalin phosphatase and acid phosphatase activity were determined manually according to the (kind and King, 1954) method. Glutamate and pyruvate transaminase and the glutamate oxaloacetate transaminase activity were determined by a colorimetric method manually (Reitman and Frankel, 1957).

Statistical analysis

The data obtained in the current study was analyzed using statistical package for social science (SPSS).

1. Standard statistical method was used to determine the mean and standard error.
2. Independ – sample T-test is used to compare between two parameters.
3. One way anova (Duncan-test) is used to compare between more than two parameters.
4. P-value ≤ 0.05 was considered to be statistically significant (Kirkwood, 1988).

RESULTS AND DISCUSSION

Isolation and purification of obestatin

The precipitate obtained from plasma by cold acetone precipitation high concentration of obestatin (332.34 pg/ml) compared with plasma (301.44 pg/ml) while the filtrate has not any obestatin hormone. So, the filtrate was neglected.

Gel filtration chromatography was applied to separate the protein as a source of obestatin hormone by using two columns as following:

First column: This technique was applied to separate the proteinous materials, which were obtained by acetone precipitation method from human plasma. The results of elution is shown in (Fig. 1) which indicated that there were two main peaks (A and B). The elution volume of peak A and B were (86.3 ml), (255.9 ml) respectively. Only the peak (B) was obtained has a high concentration of obestatin hormone. The peak (B) was broad and represented by two interfered peaks. So, gel filtration (sephadex G-25) was used to purify this peak.

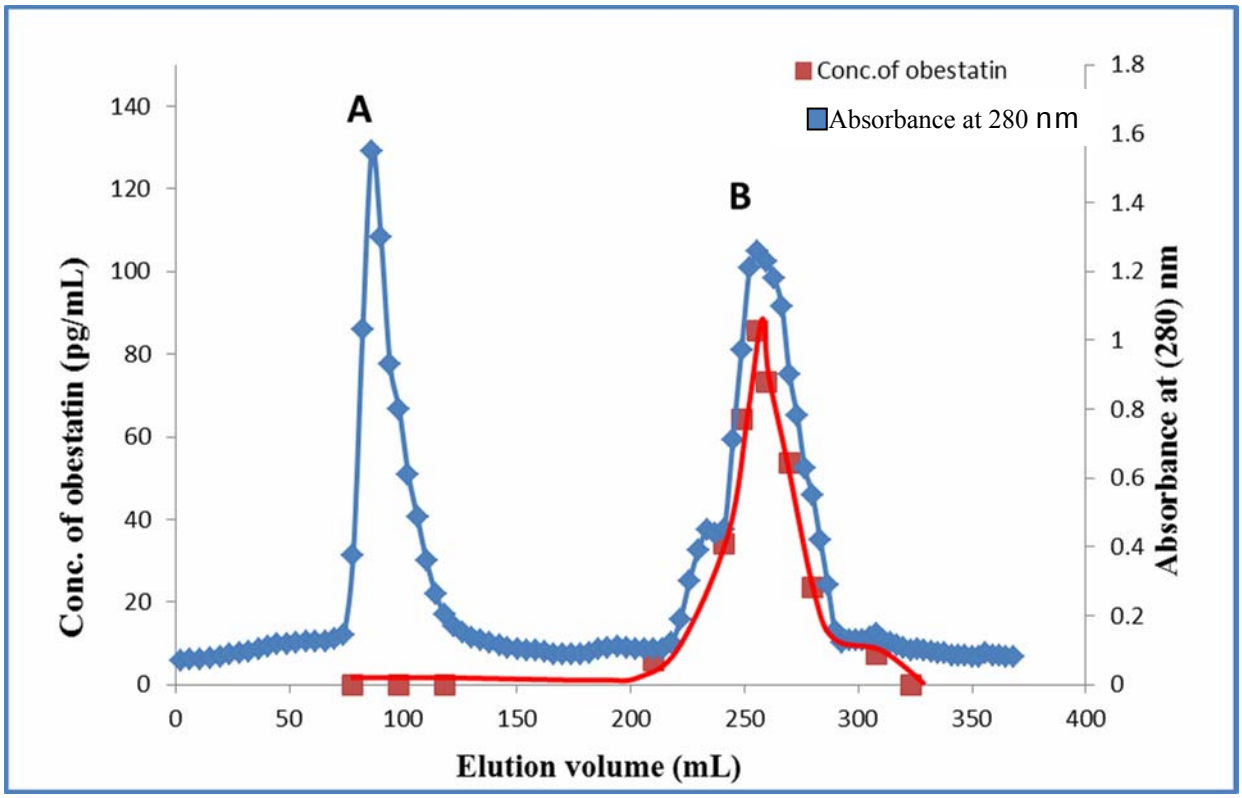

Fig. 1: Elution profile of prteinous precipitate solution obtained from acetone precipitation on Sephadex G-50. The dimension of the column is (2×100cm) at the flow rate is (88 ml/h)

Second column: This technique was applied to separate the proteinous materials, obtained from the first column gel filtration (peak B). The results of elution as shown in (Fig. 2), indicated that there were two peaks (C and D). The elution volume of peak (C and D) were (39.8 ml), (112.5 ml) respectively. The peak D has obtained with high concentration of obestatin hormone. So, the peak (C) was neglected.

The results of all purification steps for obestatin hormone were listed in Table (1).

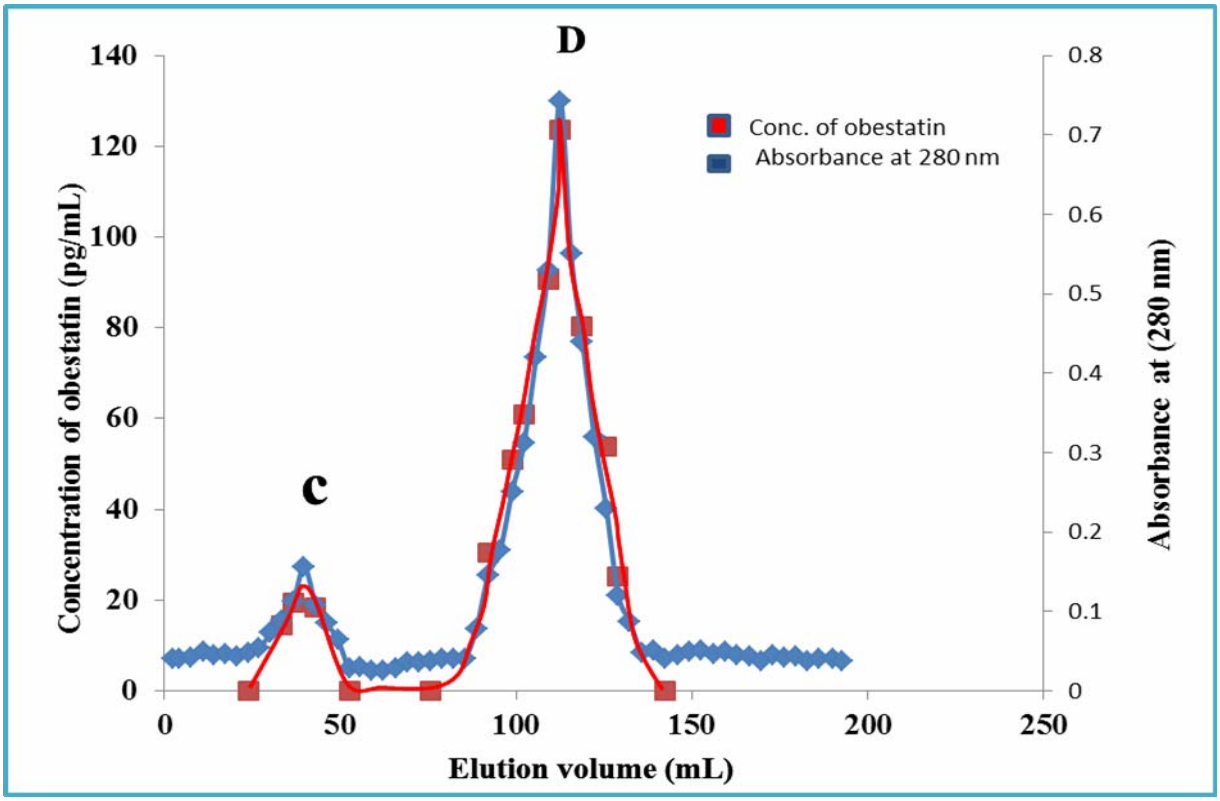

Fig. 2: Elution profile of peak (B), using Sephadex G-50 on Sephadex G-25. The dimension of the column is (1.21×110cm) at a flow rate of (68 ml/h)

Table 1: Partial purification of obestatin hormone from human plasma

Purification steps	Total volume	Total protein (mg/mL)	Total conc. of obestatin (pg/mL)	Total specific conc. of obestatin (pg / mg)	Fold of purification	Recovery %
Plasma	50	398.00	15072.00	37.86	1	100
Porteinous precipitate solution	38	236.74	14148.92	59.76	1.57	93.87
Gel filtration /Sephadex G-50 (peak B) after lyophilizer	26	41.93	12503.66	298.14	7.87	82.95
Gel filtration /Sephadex G-25 (peak D) after lyophilizer	25	7.25	11262.25	1553.41	41.02	74.72

To check the purity of isolated obestatin hormone (peak D)from plasma, SDS-PAGE and RP-HPLC were used as shown below:

1- SDS-poly acrylamide gel electrophoresis

Obestatin hormone (peak D isolated using sephadex G-25) exhibited only one single bond, using SDS-electrophoresis as shown in (Fig. 3). This result was in a highly agreement with previous results published by other investigators (Subasinghage *et al*., 2010; Alen *et al.*, 2012; Raucci *et al.*, 2013). Who found that obestatin hormone was a single peptide consist of (23 amino acids).

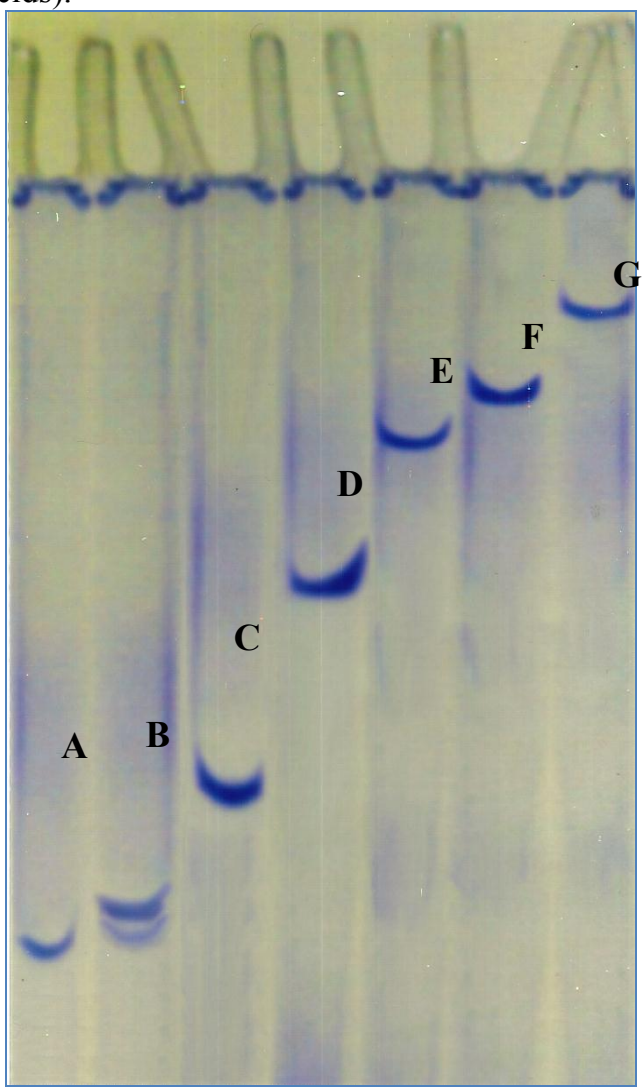

Fig. 3: SDS –polyacrylamide gel electrophoresis of partially purified obestatin hormone from plasma and known molecular weight proteins.
Band (A) represents (obestatin hormone) peak D from gel filtration (sephadex G-25), band (B) insulin + β-ME, band (C) insulin without β-ME, band (D) for papein, band (E) for pepsin, band (F) for egg albumin, band (G) for α-amylase. (β-ME; is β-mercapto ethanol).

2- RP-HPLC

To determine the purity of obestatin hormone (peak D) the standard solution of obestatin was introduced into the RP-HPLC system to know it's retention time under the following conditions:

Flow rate: 0.5 ml/min, Temp. Ambient, Wave length: 205.4, Pressure: 12 Mpa

Mobile phase: (60:40 v/v) acetonitrile in 0.1% triflouroacetic acid.

The results in (Fig. 4) and Table (2) indicated that there were two peaks, the first and the second (main) peaks were appeared at (6.277) min., (6.713) min respectively.

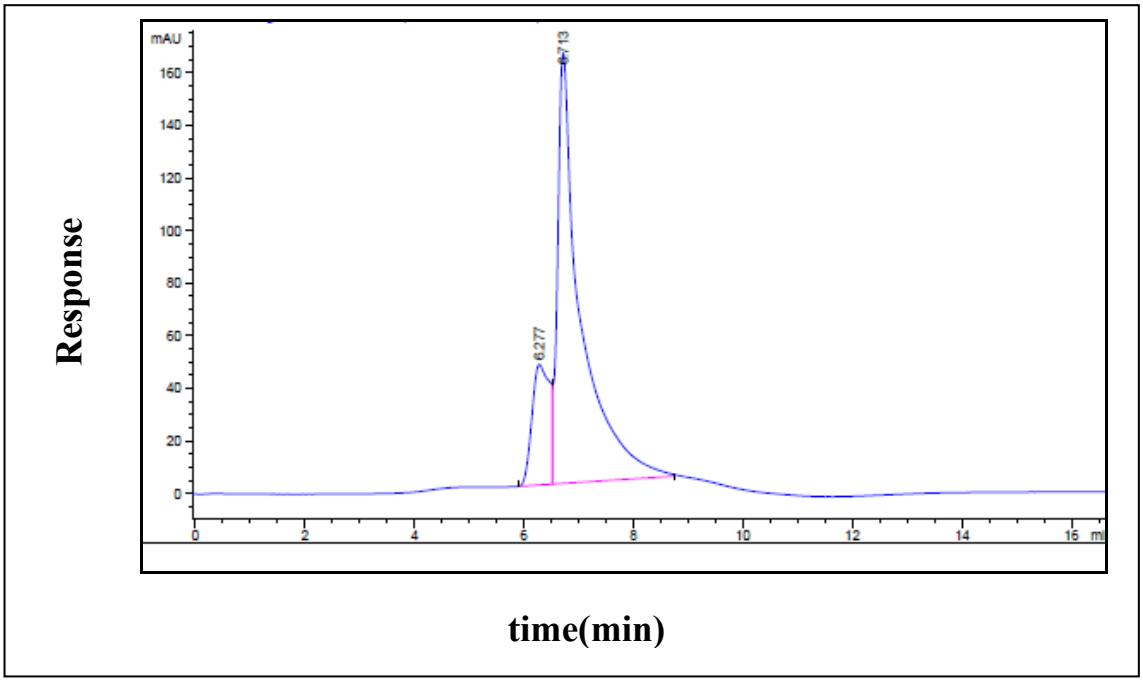

Fig. 4: Chromatogram of obestatin standard solution

Table 2: Obtained data from RP-HPLC of obestatin standard solution and isolated hormone (sample)

Name of the solution	peak	Ret. time (min)	width	Area	%area
Standard	1	6.277	0.3222	1029.688	17.6598
	2	6.713	0.3944	4800.995	82.3402
Sample	1	6.448	0.1904	233.31	5.9568
	2	6.776	0.4201	3472.2	88.6521
	3	9.196	0.6466	211.156	5.3912

On the other hand, the results in (Fig. 5) and Table (2) showed that three peaks were appeared after introducing a sample of obestatin (peak D) into RP-HPLC system under the same conditions of standard. The first, second (main peak) and the third peaks were appeared at (6.448), (6.776) and (9.196) min respectively.

After comparing the chromatogram of standard with that of the sample, it was found that there was a good identity between the retention time of standard with sample as shown in (Fig. 4, 5) and Table (2). In order to make sure that the isolated proteinous compound (obestatin) was identical with the standard one, 5,10 µl of the standard was added to the sample (peak D) and passed through RP-HPLC under the same conditions as shown

formerly. The results in (Fig. 6 and 7) and Table (3) demonstrated that the area under the peak of the sample was a gradualy increased with the addition of standard solution to the sample (peak D). Furthermore, the stabilization of retention time after each addition. After comparing the chromatograms in (Figs. 5, 6 and 7), it was found that there was an identity between the obestatin standard and the isolated obestatin hormone (peak D). so this indicated that peak D (obestatin) represent the same properties of standard obestatin.

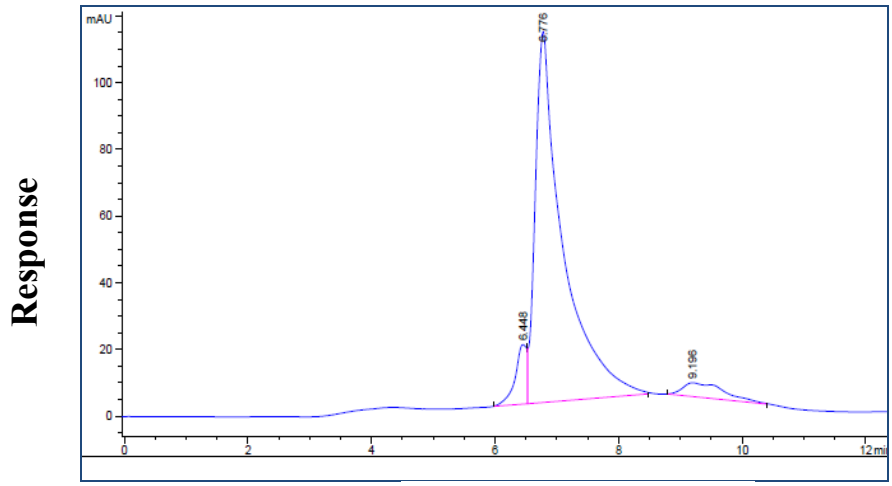

Fig. 5: Chromatogram of sample solution (peak D) from gel filtration (Sephadex G-25)

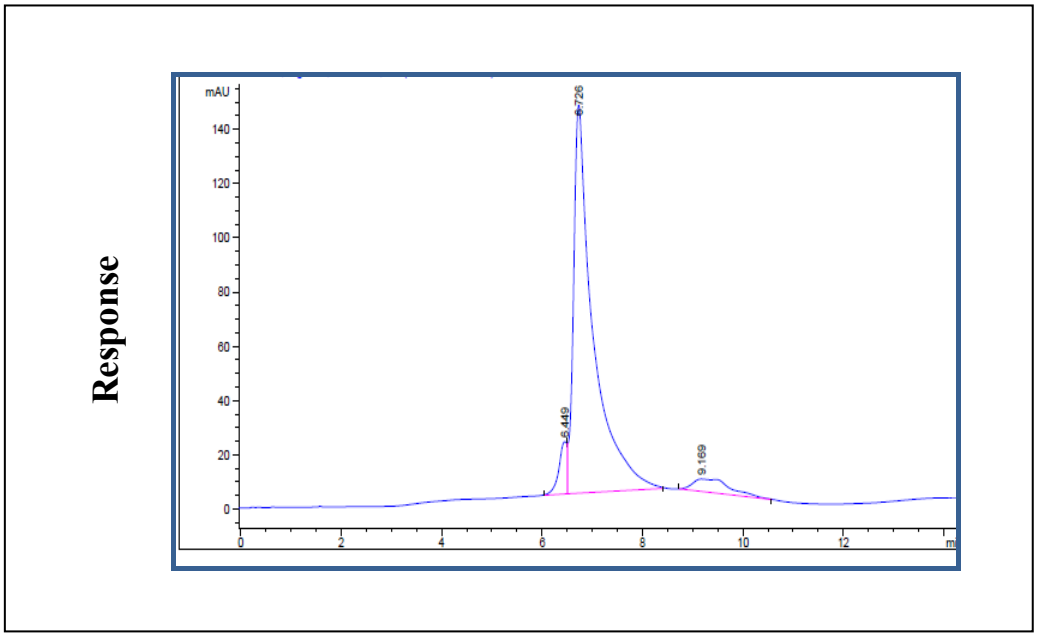

Fig. 6: Chromatogram of sample solution (peak D) from gel filtration (Sephadex G-25) after addition of 5µl of obestatin standard solution.

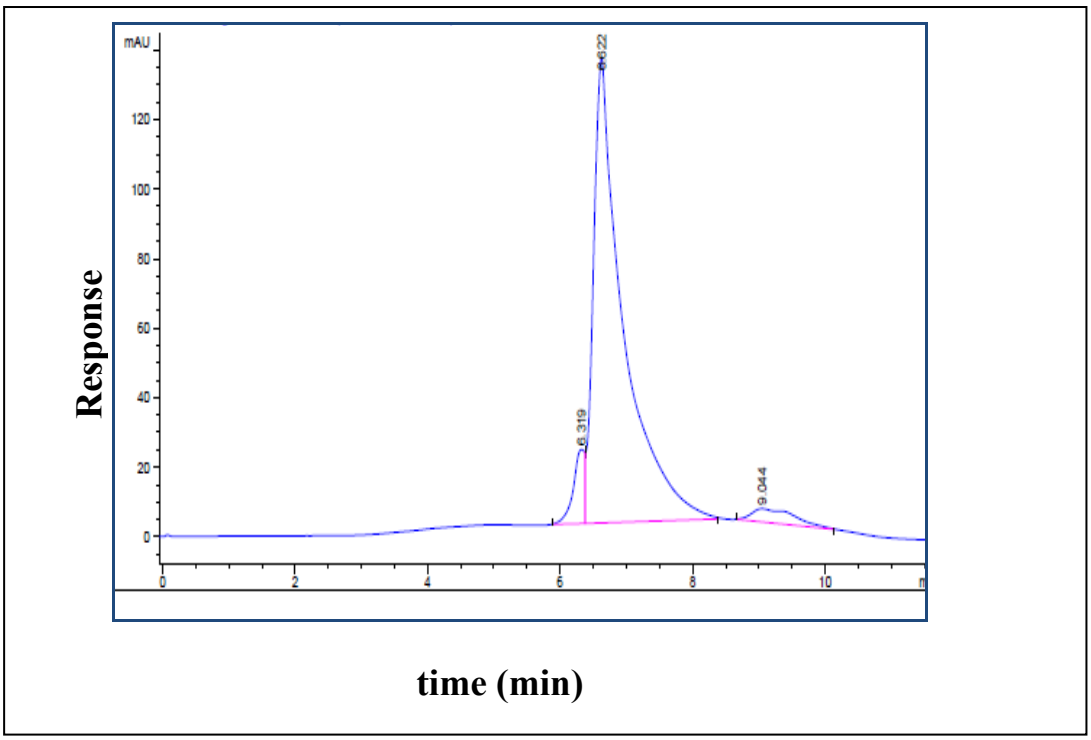

Fig. 7 : Chromatogram of sample solution (peak D) from gel filtration (Sephadex G-25) after addition of 10µl of obestatin standard solution.

Table 3: Data from RP-HPLC of sample after addition of 5, 10 µL of obestatin standard solution

Name of the solution	peak	Ret. time (min)	width	Area	%area
Sample+5 µL standard obestatin	1	6.449	0.1718	221.42609	5.0708
	2	6.726	0.3624	3882.41406	88.91
	3	9.169	0.7078	262.83627	6.0191
Sample+10 µL standard obestatin	1	6.319	0.1763	253.0191	5.4658
	2	6.622	0.4192	4190.5083	90.5251
	3	9.044	0.6111	185.58301	4.0090

Determination of the molecular weight of obestatin hormone

I- Determination of the molecular weight of obestatin hormone by gel filtration chromatography using sephadex G-25:

The approximate molecular weight of peak (D) as a source of obestatin was determined from the elution volume on a sephadex G-25 column. The calibration curve obtained by using known molecular weight (Table 4) proteins is shown in (Fig. 8). The molecular weight of peak (D) is approximately equal to (2573 ± 100) Dalton as shown in (Fig. 8).

Table 4: Elution volumes of known molecular weight materials on sephadex G-25

Materials	Molecular weight (Dalton)	Elution volume (mL)
Blue dextran	2000000	53
Bovine serum albumin	67000	56
Egg albumin	45000	61
Prolactin hormone	24000	71
Papain	23000	75
Insulin hormone	5734	92
Oxytocin hormone	1051	126.8
Tryptophan	204	186.6
Peak D	2573	112.5

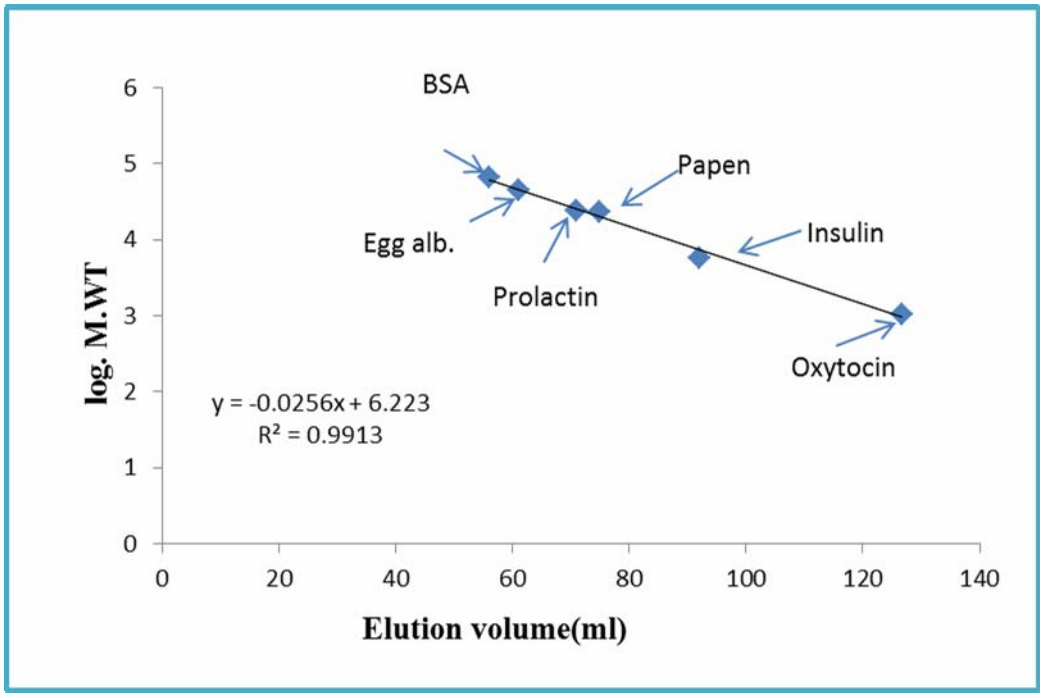

Fig. 8: Relation of molecular weight of known proteins and elution volumes on a Sephadex G-25

II- Determination of molecular weight by SDS-PAGE

The molecular weight of peak (D) as a source of obestatin was determined, using known molecular weight compounds, and it was found to be in the range of (2660) Dalton as shown in Table (5) and (Fig. 9).

This finding agrees well with the previous results, where it was reported that the molecular weight of obestatin purified from stomach of rats and human plasma was (2500) Dalton (Zhang *et al*., 2005; Lacquaniti *et al*., 2011).

Table 5 :Distance of known molecular weight materials on SDS-PAGE

Materials	Molecular Weight (Dalton)	Distant (cm)
α-amylase	58000	1.1
Egg albumin	45000	1.8
Pepsin	36000	2.2
Papen	23000	3.4
Insulin (without β-M.E)	5734	5.2
(Insulin +β-M.E) chain B	3430	6.2
(Insulin +β-M.E) chain A	2384	6.4
Peak D	2660	6.5

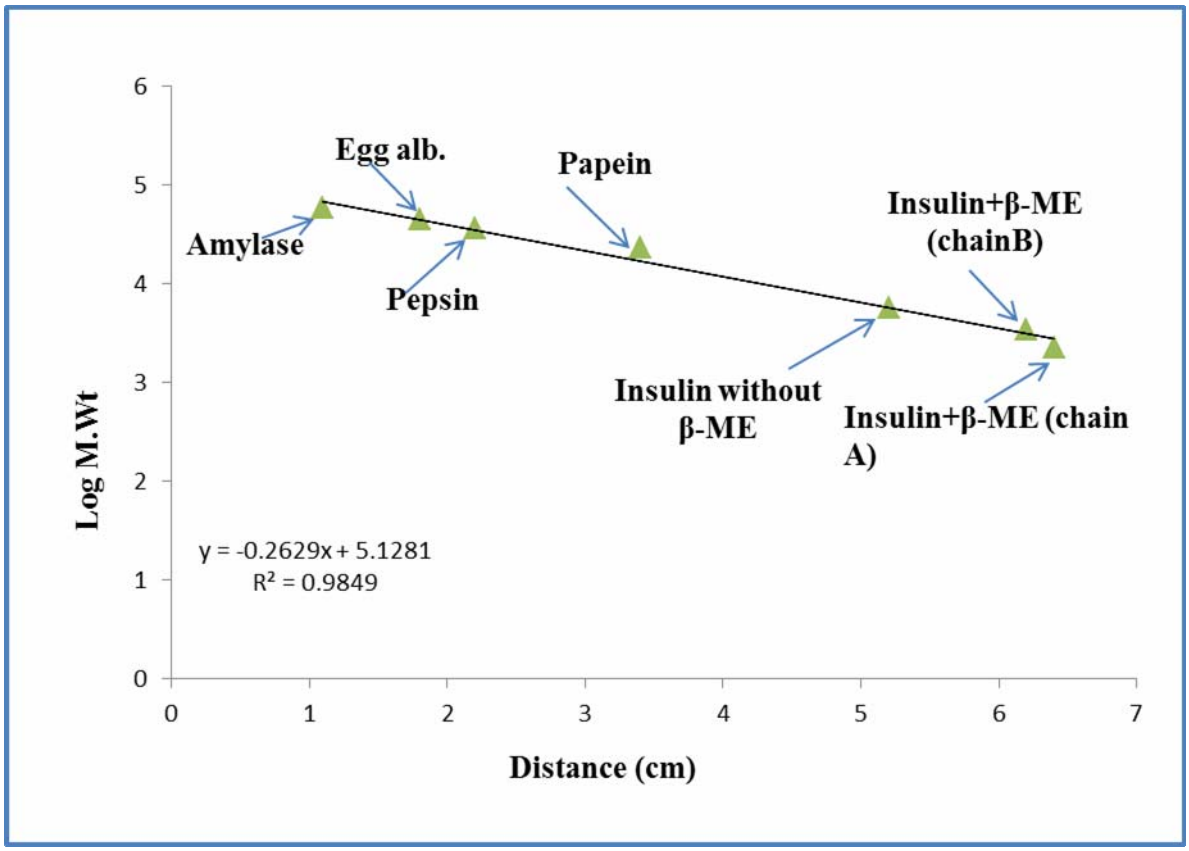

Fig. 9: Calibration curve for molecular weight estimation by SDS-Polyacrylamide gel electrophoresis using known molecular weight proteins

The second part of this research focused on the studding the effect of obestatin (peak D) on normal and diabetic rats.

Effect of isolated obestatin hormone on some biochemical parameters in serum of normal and alloxan-induced diabetic rats.

The results in Table (6) showed that the intraperitoneally injection of (1 µmol / kg / day) obestatin for a period of one week, the normal and alloxan induced diabetes rats caused a

significant decrease in serum glucose level compared to the control groups which are related to these groups (group 1 and 2). These results were in agreement with those obtained by other investigators (Granata *et al*., 2012). These reductions could be attributed to the important role of obestatin in stimulation the insulin biosynthesis, glucose uptake in either absence or presence of insulin, promoting glucose transporter type 4(GLUT4) translocation, whereas GLUT4 plays the role of tissue glucose uptake and regulates the body glucose homeostasis (Garanta *et al*., 2012; Gandhi *et al*., 2013). Further more, obestatin promoting survival and preventing apoptosis in β-cells of pancreas and up regulating of glucokinase expression (Granata *et al*., 2008). Furthermore, the treatment with obestatin might cause an increase of β-cells mass like ghrelin (Granata *et al*., 2012). Also the reduction of glucose level might cause the induction of adiponectin, whereas adiponectin decreases gluconeogensis, while the increase in glucose uptake and level lead to increase insulin sensitivity (Huerta, 2006).

Moreover, the results also showed that there was a significant decrease of serum total lipids compared to the control group which is related to these groups. This might be due to the role of obestatin in inhibiting food intake (Nagaraj *et al*., 2009; Brunelti *et al*., 2010), and the role of obestatin in inhibiting gastric emptying and gastrointestinal motility (Zhang *et al*., 2005; Fujimiya *et al*., 2011).

The results in Table (6) revealed that there were a significant decrease in total serum cholesterol, triglycerides, LDL-C and VLDL-C levels compared to control groups which were related to those groups. These results were in agreement with those obtained by other investigators (Nagaraj *et al*., 2009; Aragno *et al*., 2012). The reduction might be due to that obestatin increase the phosphorylation of AMP-activated protein kinase (AMPK) (Bourron *et al*., 2010) and then inhibit HMG-CoA reductase which contributes in biosynthesis of cholesterol (Agnew *et al*., 2011). Also, the phosphorylation of AMPK will inhibit acetyl CoA carboxylase which contributes in biosynthesis of fatty acid and triglycerides (Fogarty *et al*., 2010).

On the other hand, the results also showed that there was a significant increase inserum HDL-C levels compared to control groups which were related to these groups. The cause might due to that obestatin stimulates insulin secretion, so the activity of lipoprotein lipase will be increased and leads to increase HDL-C (Granata *et al*., 2008), or the cause might be due to that obestatin induced phosphorylation of AMPK and this will reduce inflammatory markers such as TNF-α and IL-6 (Arango *et al*., 2012) and lead to induce adiponectin which correlates positively with HDL-C (Hsu *et al*., 2012; Allwsh and Mohammad, 2013).

Furthermore, the results in Table (6) show that there was a significant decrease in serum MDA level compared to control groups which were related to these groups. The reason might be due to the ability of obestatin to restore oxidative balance and leads to decrease oxidative stress and ROS. So, obestatin exerts protective effect against oxidative stress (Arango *et al*., 2012), or the cause might be attributed to the antioxidant properties of adiponectin (Raucci *et al*., 2013), which correlated positively with obestatin (Allwsh and Mohammad, 2013).

Table 6: Effect of intraperitoneally injection of isolated obestatin hormone for a period of one week on some biochemical parameters on normal and alloxan-induced diabetic males rats

Group No.	biochemical parameters / Treatment	Glu mmol/L	T.L mg/dL	T.C mmol/ L	T.G mmol/ L	LDL-C mmol/ L	VLDL-C mmol/ L	HDL mmol/ L	MDA µmol/ L	GSH µ
1	Control + N.S	(b) 4.86 ± 0.1	(b) 767.02 ± 3.61	(b) 3.46 ± 0.22	(b) 1.29 ± 0.06	(b) 2.01 ± 0.32	(b) 0.56 ± 0.02	(b) 1.22 ± 0.07	(b) 1.4 ± 0.14	(b 8.16 ±
2	Diabetic rats (untreated with obestatin)+N.S	(d) 22.24 ± 0.22	(c) 880.16 ± 4.48	(c) 4.92 ± 0.23	(c) 2.35 ± 0.29	(c) 3.07 ± 0.29	(c) 1.06 ± 0.09	(a) 0.78 ± 0.06	(c) 3.9 ± 0.25	(a 4.32 ±
3	Normal rats + (1µmol/ kg/ d) of isolated obestatin hormone	(a) 3.24 ± 0.41	(a) 611.46 ± 2.4	(a) 2.51 ± 0.06	(a) 0.89 ± 0.07	(a) 0.65 ± 0.09	(a) 0.40 ± 0.01	(c) 1.55 ± 0.05	(a) 0.82 ± 0.06	(b 9.10 ±
4	Diabetic rats + (1µmol/ kg/ d) of isolated obestatin	(c) 7.29 ± 0.26	(b) 743.42 ± 8.28	(b) 3.56 ± 0.07	(ab) 1.01 ± 0.11	(b) 1.48 ± 0.12	(ab) 0.45 ± 0.02	(c) 1.61 ± 0.12	(b) 1.74 ± 0.13	(b 6.89 ±

Different letters vertically (a),(b),(c),(d), indicate that the mean are different significantly at $P \leq 0.05$ between each group after one week of treatment, the values are mean ± S.E of six rats each group, N.S, normal saline.

The results (Table 6) also showed that there were a non significant increase inserum GSH level compared to control group which related to this group, while caused a significant increase of GSH level in alloxan induced diabetic mals rats. The results were in agreement with those obtained by other investigators (Arango *et al*., 2012), who found the ratio of GSSG/GSH was partially decreased when the diabetic rats treated with obestatin and showed that obestatin exerted protective effects against oxidative stress.

Furthermore, the results in Table (6) showed that there were no significant differences in GPT, GOT, ALP and ACP levels after intraperitoneally injection of (1 μmol / kg / d) for one week in normal and alloxan induced diabetic males rats.

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