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Study the Effect of Isolated Osteocalcin from Human and Rats on Lipids Profile in Normal and Alloxan-Induced Diabetic Male Rats

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Abstract: This study concerned with an attempts to isolate and purify osteocalcin from healthy human blood plasma and healthy rats serum using different biochemical techniques. This included precipitation by cold acetone and gel filtration chromatography. It was found that only the second peak (peak B) from human and rats had concentration of osteocalcin, and showed that fold of purification of isolated osteocalcin 57 and 43 for human and rats respectively. The research also included study the effect of isolated osteocalcin from human plasma and rats serum on lipids profile in normal and alloxan induced diabetic males rats. The results showed after one week of treatment by the isolated osteocalcin from human plasma and rats serum at the dose (1ng/Kg of body weight/d) caused a significant decrease in the concentration of all from total lipids, total cholesterol, triglycerides, VLDL-C, LDL-C, atherogenic index, and a significant increase in the concentration of HDL-C, and antiatherogenic index in normal and alloxan induced diabetic rats.

Keywords: Osteocalcin, Isolation, Lipids profile, Diabetes, Rats

Introduction

Osteocalcin is a bone matrix protein, Osteocalcin, also called bone Gla-protein or the vitamin k-dependent protein of bone and synthesized predominantly by osteoblasts and in lower way by odontoblasts, is incorporated into the extracellular matrix of bone (Rehder et al., 2015). In particular, osteocalcin or is a small abundant non-collagenous calcium binding protein, indigenous to the organic matrix of bone dentin and possibly other mineralized tissue, which circulates in the blood It is accepted as a marker of osteoblast activity(Shao et al., 2015 ; Zoch et al., 2016). In 2007 research from Columbia University Medical Center demonstrates that bone cells release a hormone called osteocalcin, which controls the regulation of blood sugar (glucose) and fat deposition through synergistic mechanisms, so osteocalcin directs the pancreas' beta cells, which produce the body's supply of insulin, to produce more insulin, at the same time, osteocalcin directs fat cells to release a hormone called adiponectin, which improves insulin sensitivity, this discovery showed for the first time that one hormone has a synergistic function in regulating insulin secretion and insulin sensitivity, and that this coordinating signal comes from the skeleton, additionally, osteocalcin enhances the production of insulin-producing beta cells, which is considered one of the best, to treat diabetes(Zoch et al., 2016; http://www.columbia.edu/cu. (2007)). The scientists reported that osteocalcin-deficient mice were glucose intolerant, insulin resistant, obesity prone, and characterized by decreased secretion of insulin and adiponectin (Lee et al., 2007).

Aim of the Research

Because there is few previous studies in Iraq about effect of osteocalcin on normal and diabetes experimental animals. So, there was suggestion to study the effect of isolated osteocalcin from human plasma and rats serum on the concentration of lipids in normal and diabetes experimental animals.

Method

Samples

A human fresh plasma (45 mL) was obtained from one healthy male person age (35 years), with assistance of Blood Bank in Mosul city, and male albino rats serum (25 mL) was separated by centrifugation from blood of male albino rats (13±1 week old) from animal house, college of veterinary medicine, university of Mosul.

The methods used to purification of osteocalcin

Organic solvent precipitation

Proteinous materials were precipitated by using acetone (Robyt and White 1987). Gradual addition (60:40 v/v) cold acetone to plasma with slow stirring at 4°C for 60 min. The mixture was left in a refrigerator for 24 h and the precipitated protein was isolated by centrifugation for 30 minutes (12000 g) at a refrigerated centrifuge. Protein and osteocalcin concentration were estimated after dissolving the precipitate in a lowest volume of distilled water. Then the solution of proteinous precipitate was kept in a tight sample tube for further step.

Gel Filtration Chromatography

Packing of the column: It has a dimension of (2 × 100 cm) which contained a gel sephadex G-50. Proteinous precipitates solutions which were prepared in section (1) were applied to this column.

Lyophilization Technique

Peaks B (from the column) were dried by freeze drying, this technique was performed in the department of chemistry, college of science, university of Mosul.

Osteocalcin Assay

Osteocalcin concentration was determined by enzyme linked immunosorbent assay (ELISA) technique (Nagasue et al., 2003) using Epitope Diagnostics, Inc kit (USA). This analysis was performed in the immunity laboratory in Al-Salam hospital in Mosul city by using (BIO-TEK INSTRUMENTS, INC), USA.

Determination of Protein Concentration

Protein was determined by the method of modified lowry (Schacterle and Pollack 1973). Bovine serum albumin was used as a standard protein.

Effect of isolated osteocalcin from human plasma and rats serum in normal and alloxan–induced diabetic rats

Animals used

Forty six healthy male albino rats with age (13±1 week old), and weight (300±35g) obtained from animal house, college of veterinary medicine, university of Mosul were used in the experiments. They were housed under standard environmental conditions, pelleted food and water were available ad Libitum.

Induction of diabetes

The animals were fasted for (24 h) and made diabetes by injecting with alloxan tetrahydrate (180 mg/ Kg of body weight, i.p) dissolved in sterile normal saline (Miura et al., 1995). The diabetic state was monitored by

hyperglycemia (Colorimetric assay kit, Randox, United Kindom) along the next ten days. Rats with blood glucose concentration more than (250 mg/dL) were considered diabetic and used for study.

Experimental Design

The dose of isolated osteocalcin was (1ng/kg of body weight) (Dou *et al*., 2014; Sabek *et al*., 2015).

The male rats were divided into six group and all groups were injected for one week as shown below:

1. The first group: non fasted control male rats group was injected intraperitoneally with physiological saline solution.
2. The second group: non fasted male rats group before and after induced them diabtes by injecting with alloxan tetrahydrate (180 mg/ Kg of body weight, i.p).
3. The third group: non fasted healthy male rats group was injected intraperitoneally with (1 ng/kg of body weight) isolated osteocalcin from human plasma.
4. The fourth group: non fasted healthy male rats group was injected intraperitoneally with (1 ng/kg of body weight) isolated osteocalcin from rats serum.
5. The fifth group: non fasted and alloxan induced diabetic male rats group was injected intraperitoneally with (1 ng/kg of body weight) isolated osteocalcin from human plasma.
6. The sixth group: non fasted and alloxan induced diabetic male rats group was injected intraperitoneally with (1 ng/kg of body weight) isolated osteocalcin from rats serum.

Collection of blood

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

Total lipids: was found by colorimetric method manually (Chabrol and Chardonnet 1937).
-Total cholesterol: was determined by enzymatic colorimetric method (Allain *et al*., 1974), using BIOLABO kit (France).
-Triglycerides: was determined by enzymatic colorimetric method (Fossati and Principle 1982), using BIOLABO kit (France).
-Very low density lipoprotein-cholesterol (VLDL-C): was calculated by using the following equation: VLDL Conc. (mmol/L) = TG Conc./2.2 (Yeboah *et al*., 2017).
-Low density lipoprotein-cholesterol (LDL-C): was calculated by using the following equation:
LDL Conc. (mmol/L) = Total cholestrol Conc.-HDL Conc.-(TGconc./2.2) (Burtis and Ashwood 1982).
-High density lipoprotein-cholesterol (HDL-C): was determined by precipitation method (Burtis and Ashwood 1982), using BIOLABO kit (France).
-Atherogenic Index(AI): was calculated by using the following equation:
Atherogenic Index (AI) = Log(TG/HDL-C) (Nagasue *et al*., 2003).
-Antitherogenic Index(AAI): was calculated by using the following equation:
100 / (TC -HDL-C) (Saravanan et al., 2011).× Antiatherogenic index (AAI) = HDL-C

Data Analysis

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

1. Standard statistical method used to determined the mean and standard error.
2. Indepent – sample T-test 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

Purification of osteocalcin from human plasma and rats serum

Organic solvent precipitation

The precipitate solution which obtained by cold acetone from plasma human was with high concentration of osteocalcin (23.77 ng/mL) compared with plasma (16.55 ng/mL), while the filtrate was not obtained any concentration of osteocalcin hormone. So, the filtrate was neglected. The precipitate solution which obtained by cold acetone from rats serum was with high concentration of osteocalcin (2.1 ng/mL) compared with plasma (1.33 ng/mL), while the filtrate was not obtained any concentration of osteocalcin hormone. So, the filtrate was neglected.

Gel filtration chromatography

This technique was applied to separate the proteinous materials, which were obtained by acetone precipitation method from human plasma and rats serum. The results of elution of isolated osteocalcin from human and rats shown in figure (1) and (2) respectively and indicated that there were two peaks (A & B). The elution volume of peak A & B were (89.47 mL) , (223 mL) respectively for isolated osteocalcin from human, and elution volume of peak A & B were (93.0 mL) , (230 mL) respectively for isolated osteocalcin from rats. Only peak (B) from human plasma and rats serum was obtained with high concentration of osteocalcin.

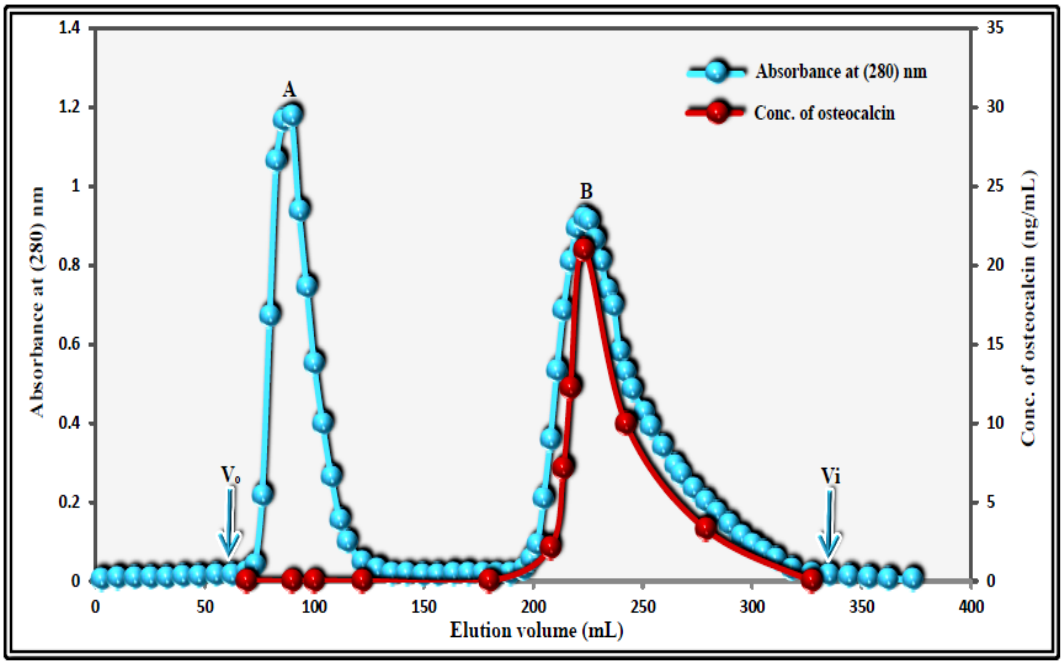

Figure 1.Elution profile of proteinous precipitate solution obtained from acetone precipitation of human plasma on Sephadex G-50.The dimensions of the column are (2×100 cm).

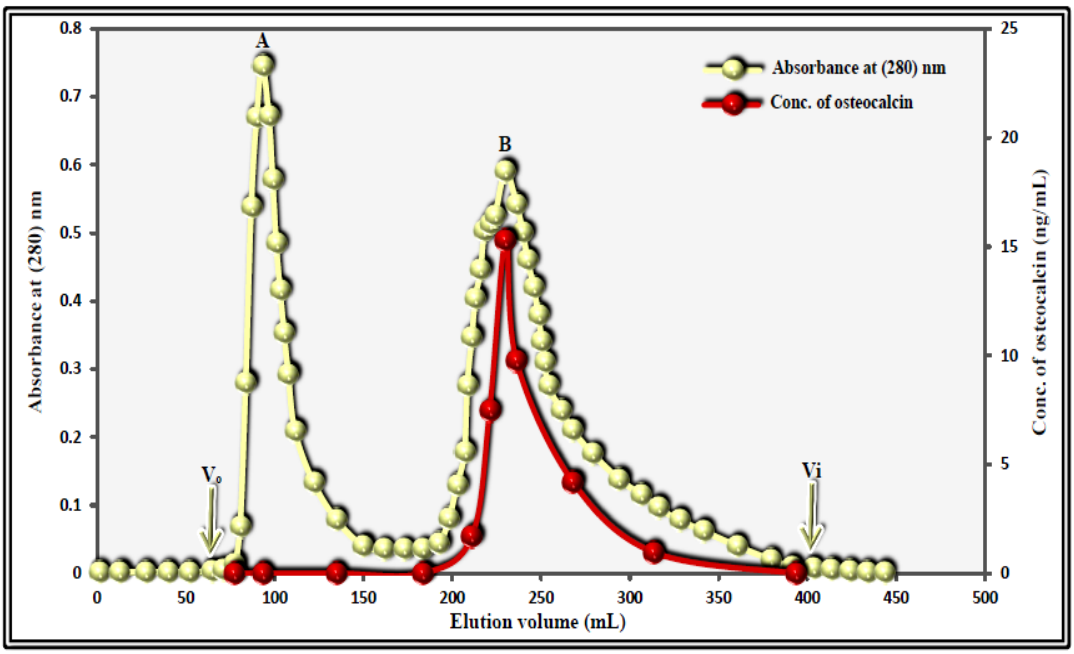

Figure 2.Elution profile of proteinous precipitate solution obtained from acetone precipitation of rats serum on Sephadex G-50.The dimensions of the column are (2×100 cm)

The results of all purification steps for osteocalcin from human plasma and rats serum were listed in table (1) and table (2) respectively:

Table 1.Partial purification of osteocalcin from human plasma

Purification steps	Volume (mL)	Total protein (mg)	Total conc. Of osteocalcin (ng)	Total specific conc. Of osteocalcin (ng/mg)	Recovery %	Fold of purification
Plasma	45	319.5	744.75	2.33	100	1
Proteinous precipitate solution by cold acetone	30	143.4	713.1	4.97	95.75	2
Gel filtration/Sephadex G-50 (peak B)	10	4.7	620	131.91	83.24	57

The results in Table (1) showed that the concentration of osteocalcin was increased from (16.55 ng/mL) in plasma to (23.77 ng/mL) for proteinous precipitate solution concentration of osteocalcin and to (62 ng/mL) for peak B concentration of osteocalcin used in gel filtration by using (Sephadex G-50).While the concentration of protein was decreased.

Table 2.Partial purification of osteocalcin from rats serum

Purification steps	Volume (mL)	Total protein (mg)	Total conc. Of osteocalcin (ng)	Total specific conc. Of osteocalcin (ng/mg)	Recovery %	Fold of purification
Serum	25	158.5	33.25	0.21	100	1
Proteinous precipitate solution by cold acetone	14	60.34	29.4	0.49	88.42	2
Gel filtration/Sephadex G-50 (peak B)	5	2.65	23.85	9	71.73	43

The results in Table (2) showed that the concentration of osteocalcin was increased from (1.33 ng/mL) in serum to (2.1 ng/mL) for proteinous precipitate solution concentration of serum rats and to (4.77 ng/mL) for peak B concentration of osteocalcin used in gel filtration by using (Sephadex G-50).While the concentration of protein was decreased.

Effect of isolated osteocalcin on some clinical parameters in serum of normal and alloxan-induced diabetic rats:

When injected intraperitoneally the isolated osteocalcin from human plasma and rats serum at the dose (1ng/Kg of body weight/d) in healthy and alloxan induced diabetic male rats for one week, the results show effect of osteocalcin on lipids profile as shown bellow:

1. Total lipids, Total cholesterol, Triglycerides, Very low density lipoprotein cholesterol, Low density lipoprotein cholesterol & High density lipoprotein cholesterol

The results revealed that the percentage of reduction in the third group and the fourth group in the concentration of all from total lipids were (-13.62%) and (-7.99%) respectively, total cholesterol were (-40.89%) and (-31.06%) respectively, triglycerides were (-19.88%) and (-11.37%) respectively, very low density lipoprotein cholesterol were (-19.51%) and (-10.66%) respectively, low density lipoprotein cholesterol were (-75.16%) and (-56.50%) respectively, while the percentage of reduction in the fifth group and the sixth group in the concentration of all from total lipids were (-34.13%) and (-27.24%) respectively, total cholesterol were (-44.62%) and (-38.39%) respectively, triglycerides were (-45.51%) and (-38.76%) respectively, very low density lipoprotein cholesterol were (-45.20%) and (-38.77%) respectively, low density lipoprotein cholesterol were (-59.01%) and (-49.90%) respectively after one week of treatment with osteocalcin as shown in the tables (3), (4), (5), (6), and (7).

Table 3. Effect of intraperitoneally administration of isolated osteocalcin for one week on total lipids concentration in normal and alloxane-induced diabetic male rats

Group No.	Treatment	TL conc. (mg/dL) Mean ± S.E		Change %
		Pre-treatment (Zero time)	Post-treatment (After one week)	
1	Control group+ 0.9%NaCl	572.84 ± 5.18A	b,c,d,e 586.33 ± 8.85A	2.35
2	Diabetic rats+0.9%NaCl	577.01 ± 1.71A	a,c,d,e,f 798.54 ± 8.81B	38.39
3	Normal rats + (1ng/Kg/d) of isolated human osteocalcin	590.82 ± 7.84A	a,b,d,f 510.33 ± 1.33B	-13.62
4	Normal rats + (1ng/Kg/ d) of isolated rats osteocalcin	587.16 ± 6.09A	a,b,c,e,f 540.24 ± 3.03A	-7.99
5	Diabetic rats + (1ng/Kg/d) of human osteocalcin	790.57 ± 8.12A	a,b,d,f 520.73 ± 1.20B	-34.13
6	Diabetic rats + (1ng/Kg/d) of rats osteocalcin	797.32 ± 5.98A	b,c,d,e 580.11 ± 1.14B	-27.24

Different letters horizontally A,B indicate different significant at (P≤0.05) in the lipid parameter concentration between pre-treatment and post-treatment, while differents letters vertically (a), (b), (c), (d), (e) and (f) indicate that the mean is different significantly at (P≤0.05) in the lipid parameter concentration between each group after one weak (post-treatment).

$$\textbf{Change\%} = \frac{\textbf{Post-treatment Conc.} - \textbf{Pre-treatment Conc.}}{\textbf{Pre-treatment Conc.}} \times 100$$

The cause may be due to that osteocalcin increases energy consumption in muscles and adipose tissues (Lacombe et al., 2013), also (Pi and Quarles 2012) showed when injected osteocalcin, it enhace insulin sensitivity via its direct effect on its GPRC6A receptor activating on skeletal muscles, liver, and adipose cells, and through its role in enhance adiponectin secretion from adipose cells, so adiponectin regulates energy metabolism through increases fatty acid oxidation in liver and skeletal muscle, inhibits gluconeogenesis in liver, and enhances glucose uptake in skeletal muscles, as well as osteocalcin decreases leptin concentration when leptin rises at fat mass increasing (Grau et al., 2010).

Whereas the results showed that the percentage of elevation of HDL-C concentration in the third group and the fourth group were (17.33%) and (11.76%) respectively, while the percentage of elevation in the fifth group and the sixth group were (63.76%) and (56.45%) respectively after one week of treatment with osteocalcin as shown in the table (8), these elevation could be attributed to that osteocalcin stimulates insulin secretion, so the activity of lipoprotein lipase will increase and lead to increase HDL-C, or the cause may be due to that osteocalcin promotes adiponectin secretion, so osteocalcin correlated positively with HDL-C (Zoch et al., 2016, Albadah et al., 2015).

Table 4. Effect of intraperitoneally administration of isolated osteocalcin for one week on total cholesterol concentration in normal and alloxane-induced diabetic male rats

Group No.	Treatment	TC conc. (mmol/L) Mean ± S.E Pre-treatment (Zero time)	Post-treatment (After one week)	Change%
1	Control group+ 0.9%NaCl	4.94 ± 0.10A	b,c 5.17 ± 0.12A	4.65
2	Diabetic rats+0.9%NaCl	5.52 ± 0.14A	a,c,d,e,f 7.01 ± 1.06B	26.99
3	Normal rats + (1ng/Kg/d) of isolated human osteocalcin	5.38 ± 0.07A	a,b 3.18 ± 0.30B	-40.89
4	Normal rats + (1ng/Kg/ d) of isolated rats osteocalcin	5.44 ± 0.05A	b 3.75 ± 0.21A	-31.06
5	Diabetic rats + (1ng/Kg/d) of human osteocalcin	7.26 ± 0.73A	b 4.02 ± 0.13B	-44.62
6	Diabetic rats + (1ng/Kg/d) of rats osteocalcin	7.11 ± 0.52A	b 4.38 ± 0.10B	-38.39

Table 5. Effect of intraperitoneally administration of isolated osteocalcin for one week on triglycerides concentration in normal and alloxane-induced diabetic male rats

	Treatment	TG conc. (mmol/L) Mean ± S.E Pretreatment (Zero time)	Post-treatment (After one week)	Change%
1	Control group+ 0.9%NaCl	1.73 ± 0.07A	b 1.66 ± 0.06A	-4.04
2	Diabetic rats+0.9%NaCl	1.96 ± 0.03A	a,c,d,e,f 3.27 ± 0.23B	66.83
3	Normal rats + (1ng/Kg/d) of isolated human osteocalcin	1.81 ± 0.01A	b,f 1.45 ± 0.14B	-19.88
4	Normal rats + (1ng/Kg/ d) of isolated rats osteocalcin	1.67 ± 0.05A	b,f 1.48 ± 0.23B	-11.37
5	Diabetic rats + (1ng/Kg/d) of human osteocalcin	3.23 ± 0.41A	b 1.76 ± 0.07B	-45.51
6	Diabetic rats + (1ng/Kg/d) of rats osteocalcin	3.25 ± 0.48A	b,c,d 1.99 ± 0.10B	-38.76

Table 6. Effect of intraperitoneally administration of isolated osteocalcin for one week on VLDL-C concentration in normal and alloxane-induced diabetic male rats

Group No.	Treatment	VLDL-C conc. (mmol/L) Mean ± S.E Pretreatment (Zero time)	Post-treatment (After one week)	Change %
1	Control group+ 0.9%NaCl	0.78 ± 0.03A	b 0.75 ± 0.02A	-3.84
2	Diabetic rats+0.9%NaCl	0.89 ± 0.01A	a,c,d,e,f 1.48 ± 0.10B	66.29
3	Normal rats + (1ng/Kg/d) of isolated human osteocalcin	0.82 ± 0.005A	b,f 0.66 ± 0.06B	-19.51
4	Normal rats + (1ng/Kg/ d) of isolated rats osteocalcin	0.75 ± 0.02A	b,f 0.67 ± 0.11B	-10.66
5	Diabetic rats + (1ng/Kg/d) of human osteocalcin	1.46 ± 0.18A	b 0.80 ± 0.03B	-45.20
6	Diabetic rats + (1ng/Kg/d) of rats osteocalcin	1.47 ± 0.21A	b,c,d 0.90 ± 0.04B	-38.77

Table 7. Effect of intraperitoneally administration of isolated osteocalcin for one week on LDL-C concentration in normal and alloxane-induced diabetic male rats

Group No.	Treatment	LDL-C conc. (mmol/L) Mean ± S.E		Change%
		Pretreatment (Zero time)	Post-treatment (After one week)	
1	Control group+ 0.9%NaCl	2.62 ± 0.06A	b,c,d 2.92 ± 0.03A	11.45
2	Diabetic rats+0.9%NaCl	3.11 ± 0.05A	a,c,d,e,f 4.89 ± 1.03B	57.23
3	Normal rats + (1ng/Kg/d) of isolated human osteocalcin	3.06 ± 0.15A	a,b,f 0.76 ± 0.23A	-75.16
4	Normal rats + (1ng/Kg/ d) of isolated rats osteocalcin	3.15 ± 0.17A	a,b 1.37 ± 0.17A	-56.50
5	Diabetic rats + (1ng/Kg/d) of human osteocalcin	5.10 ± 0.53A	b 2.09 ± 0.12B	-59.01
6	Diabetic rats + (1ng/Kg/d) of rats osteocalcin	5.01 ± 0.63A	b,c 2.51 ± 0.08B	-49.90

Table 8. Effect of intraperitoneally administration of isolated osteocalcin for one week on HDL-C concentration in normal and alloxane-induced diabetic male rats

Group No.	Treatment	HDL-C conc. (mmol/L) Mean ± S.E		Change %
		Pretreatment (Zero time)	Post-treatment (After one week)	
1	Control group+ 0.9%NaCl	1.54 ± 0.13A	b,f 1.50 ± 0.19A	-2.59
2	Diabetic rats+0.9%NaCl	1.52 ± 0.10A	a,c,d,e 0.63 ± 0.01B	-58.55
3	Normal rats + (1ng/Kg/d) of isolated human osteocalcin	1.50 ± 0.21A	b,e,f 1.76 ± 0.02B	17.33
4	Normal rats + (1ng/Kg/ d) of isolated rats osteocalcin	1.53 ± 0.23A	b,e,f 1.71 ± 0.04B	11.76
5	Diabetic rats + (1ng/Kg/d) of human osteocalcin	0.69 ± 0.01A	b,c,d 1.13 ± 0.17B	63.76
6	Diabetic rats + (1ng/Kg/d) of rats osteocalcin	0.62 ± 0.02A	a,c,d 0.97 ± 0.14B	56.45

Atherogenic index

The results demonstrated that the percentage of reduction of atherogenic index (AI) in the third group and the fourth group were (-214.28%) and (-200%) respectively, while the percentage of reduction in the fifth group and the sixth group were (-69.69%) and (-54.92%) respectively after one week of treatment with osteocalcin as shown in the table (9).The cause may be due to that osteocalcin correlates by negative correlation with triglycerides and by positive correlation with HDL-C (Kang, 2016), therefore osteocalcin may be a marker for assessing the risk of atherosclerosis and cardiovascular in type II diabetes (Alfadda *et al.*, 2013), and these results were in agreement with those obtained by other investigators (Dou *et al.*, 2014) who demonstrated that osteocalcin had protective effect from atherosclerosis and cardiovascular.

Table 9. Effect of intraperitoneally administration of isolated osteocalcin for one week on atherogenic index (AI) in normal and alloxane-induced diabetic male rats

Group No.	Treatment	Atherogenic index (AI) Mean ± S.E Pretreatment (Zero time)	Post-treatment (After one week)	Change %
1	Control group+ 0.9%NaCl	0.05 ± 0.05A	b,f 0.06 ± 0.19A	20
2	Diabetic rats+0.9%NaCl	0.11 ± 0.06A	a,c,d,e,f 0.71 ± 0.43B	545.45
3	Normal rats + (1ng/Kg/d) of isolated human osteocalcin	0.07 ± 0.21A	b,e,f -0.08± 0.07A	-214.28
4	Normal rats + (1ng/Kg/ d) of isolated rats osteocalcin	0.06 ± 0.21A	b,e,f -0.06 ± 0.14A	-200
5	Diabetic rats + (1ng/Kg/d) of human osteocalcin	0.66 ± 0.58A	b,c,d 0.20 ± 0.07B	-69.69
6	Diabetic rats + (1ng/Kg/d) of rats osteocalcin	0.71 ± 0.69A	a,b,c,d 0.32 ± 0.08B	-54.92

Antitherogenic index

The results showed that the percentage of elevation of antitherogenic index (AAI) in the third group and the fourth group were (241.09%) and (112.87%) respectively, while the percentage of elevation in the fifth group and the sixth group were (277.80%) and (192.11%) respectively after one week of treatment with osteocalcin as shown in the table (10). These elevation could be attributed to that osteocalcin correlates by positive correlation with HDL-C and by negative correlation with total cholesterol (Kang, 2016), and these results were in agreement with those obtained by other investigators (O'Connorand Durack 2017) who demonstrated the correlation of osteocalcin concentration with clinical parameters concentration of atherosclerosis in type II diabetes.

Table 10. Effect of intraperitoneally administration of isolated osteocalcin for one week on antitherogenic index in normal and alloxane-induced diabetic male rats

Group No.	Treatment	Antitherogenic index (AAI) Mean ± S.E Pretreatment (Zero time)	Post-treatment (After one week)	Change%
1	Control group+ 0.9%NaCl	45.41 ± 4.26A	c,d 40.99 ± 5.90A	-9.73
2	Diabetic rats+0.9%NaCl	38.14 ± 1.22A	c,d 10.74 ± 4.62B	-71.84
3	Normal rats + (1ng/Kg/d) of isolated human osteocalcin	39.15 ± 6.89A	a,b,d,e,f 133.54 ± 22.66A	241.09
4	Normal rats + (1ng/Kg/ d) of isolated rats osteocalcin	39.85 ± 7.42A	a,b,c,e,f 84.83 ± 5.04A	112.87
5	Diabetic rats + (1ng/Kg/d) of human osteocalcin	10.77 ± 1.27A	c,d 40.69 ± 9.66B	277.80
6	Diabetic rats + (1ng/Kg/d) of rats osteocalcin	9.76 ± 0.72A	c,d 28.51 ± 4.45B	192.11

Conclusion

From this research we concluded that the isolated osteocalcin from human plasma and rats serum had a major role in the metabolism of glucose and lipids profile in normal and alloxan induced diabetic rats, also it was concluded that isolated osteocalcin from human plasma was more effect than isolated osteocalcin from rats

serum on the concentration of lipids in normal and alloxan induced diabetic males rats, especially the effect of isolated osteocalcin from human plasma was higher than isolated osteocalcin from rats serum on the concentration of lipids in alloxan induced diabetic males rats compared with normal male rats.

References

Albadah, M.S.; Dekhil, H.; Shaik, S.A.; Alsaif, M.A ; Shogair, M; Nawaz, S. and Alfadda, A.A. (2015). " Effect of weight loss on serum osteocalcin and its association with serum adipokines". Internat. J. Endocrinol. 10: 1-10.

Alfadda, A.A.; Masood, A.; Shaik, S.A.; Dekhil, H. and Goran, M. (2013). "Association between osteocalcin, metabolic syndrome, and cardiovascular risk factors: Role of total and undercarboxylated osteocalcin in patients with type 2 diabetes". Internat. J. Endocrinol. 10: 1-6.

Allain, C.C.; Poon, L.S.; Chan, C.S.; Richmond, W. and Fu, P.C. (1974). "Determination of serum total cholesterol by enzymatic colorimetric method". J. Clin. Chem. 20(4): 470-475.

Burtis, C. A. and Ashwood, E.R. (1982). " Teits Fundamental of Clinical Chemistry". 2nd ed., W.B. Saunders Company, U.S.A. Philadelphia. :302, 337, 539, 901.

Chabrol and Chardonnet. (1937). Cited by AL-Mollah, L. A. M. (1990). "Studies of some metabolic effects of noradrendine insulin inpresence of Ca++. M.Sc. Thesis, University of Mosul.

Dou, J.; Li, H.; Ma, X.; Zhang, M.; Fang, Q.; Nie, M.; Bao, Y. and Jia, W. (2014). "Osteocalcin attenuates high fat diet-induced impairment of endothelium-dependent relaxation through Akt/eNOS-dependent pathway". J. Cardiovascular Diabetol. 13(74): 1-12.

Fossati, P. and Principle, L. (1982). "Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide". J. Clin. Chem. 28(10): 2077-2080.

Grau, G.A.; Ara, I., Dorado, C.; Rodriguez, V.G.; Gomez, P.J.; Cabrero, J.C.; Sanchez, S.J.A.; Santana, A. and Calbet, J.A. (2010). "Osteocalcin as a negative regulator of serum leptin concentration in humans: insight from triathlon competitions". Eur. J. Appl. Physiol. 110(3): 635-643.

Kang, J.H. (2016). " Association of Serum Osteocalcin with Insulin Resistance and Coronary Atherosclerosis". J. Bone Metab. 23: 183-190.

Kirkwood, B. R. (1988). Essentials of Medical Statistics. 1st edn., Black well Scientific Publication, Oxford; 43-56.

Lacombe, J.; Karsenty, G. and Ferron, M. (2013). "In vivo analysis of the contribution of bone resorption to the control of glucose metabolism in mice". J. Molecular. Metab. 8: 1-7.

Lee, N.K.; Sowa, H.; Hinoi, e.; Ferron, M.; Ahn, J.D.; Confavreux, C.; Dacquin, R.; Mee, P.J.; Mckee, M.D.; Jung, D.Y.; Zhang, Z.; Kim, J.K.; Mauvais,J. F; Ducy, P. and Karsenty, G. (2007). "Endocrine regulation of energy metabolism by the skeleton". J. Cell. 130: 456-469.

Miura, T.; Usami, M.; Tsuura, Y.; Ishida, H. and Seino, Y. (1995). "Hypoglycemic and hyolipidemic effect of chitosan in normal and neonantal streptotocin induced diabetic mice". J. Biol. Pharm. Bull. 18(1): 1623-1625.

Nagasue, K.; Inaba, M.; Okuno, S.; Kitatani, K.; Imanishi, Y.; Ishimura, E.; Miki, T., Kim, M. and Nishizawa, Y. (2003). "Serum N-terminal midfragment vs. intact osteocalcin immunoradiometric assay as markers for bone turnover and bone loss in hemodialysis patients". J. Biomed. Pharmacother. 57(2):98-104.

Nansseu, J.R.N.; Moor, V.J.A.; Nouaga, M.E.D.; Awona, B.Z.; Tchanana, G. and Ketcha, A. (2016). " Atherogenic index of plasma and risk of cardiovascular disease among Cameroonian postmenopausal women". J. Lipids in Health and Disease. 15:49: 1-5.

O'Connor, E.M. and Durack, E. (2017). " Osteocalcin: The extra-skeletal role of a vitamin K-dependent protein in glucose metabolism". J. Nutr. & Intermed. Metab. 7: 8-13.

Pi, M. and Quarles, L.D. (2012). "Multiligand specificity and wide tissue expression of GPRC6A reveals new endocrine networks". J. Endocrinol. 153(5): 2062-2069.

Rehder, D.S.; Gundberg, C.M.; Booth, S.L. and Borges, C.R. (2015). " Gamma-carboxylation and fragmentation of osteocalcin in human serum defined by mass spectrometry". J. Mol. & Cell. Proteomics. 14(6): 1546-1555.

Research shows skeleton to be endocrine organ. http://www.columbia.edu/cu. (2007).

Robyt, F.J. and White, J. B. (1987). "Biochemical Techniques Theory and Practice". Books Cole Publishing Co., U.S.A., 141, 235-236, 246, 263, 269

Sabek, O.M.; Nishimoto, S.K.; Fraga, D.; Tejpal, N.; Ricordi, C. and Gaber, A.O. (2015). "Osteocalcin effect on human β-cells mass and function". J. Endocrinol. 156(9): 3137-3146.

Saravanan, G; Kalaiselvi, .B. and Reddy, G.P.(2011). "Hypolipidemic effect of zignidd (polyherbal formulation) in streptozotocin-induced diabetic rats". INT. J. Drug Formulation and Research. 2(6): 151-160.20.

Schacterle, G.R. and Pollack , R. L. (1973). "A simplified method for the quantitatiue assay of small amounts of protein biological material". J. Anal. Biochem. 51: 654-655.

Shao, J.; Wang, Z.; Yang, T.; Ying, H.; Zhang, Y. and Liu, S. " (2015). "Bone regulates glucose metabolism as an endocrine organ through osteocalcin". Internat. J. Endocrinol. 10: 1-9.

Tomoda, M.; Shimizu, N.; Gonda, R.; Kanari, M.; Yamada, H. and Hikino, H. (1990). "Anti-Complementary and hypoglycemic activities of the glycans from the seeds of malva verticillata". J. Planta. Med. 56:168-170.

Yeboah, J.O.; Owiredu, W.K.B.A.; Norgbe, G.K.; Lokpo, S.Y.; Gyamfi,J.; Allotey, E.A.; Aduko, R.A.; Noagbe, M. and Attah, F.A. (2017). " The Prevalence of metabolic syndrome and its components among people with type 2 diabetes in the Ho Municipality, Ghana: A cross-sectional study". Internat. J. Chronic Dis. 10: 1-8.

Zoch, M.L.; Clemens, T.L. and Riddle, R.C. (2016). " New insights into the biology of osteocalcin". J. Bone and diabetes. 82: 42–49.

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