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Effect of Methanolic Leaf Extract of *Moringa oleiferaon* some Biochemical Markers in obesity induced rats.

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ABSTRACT

This study was conducted on (n = 40) Albino Wistar male rats (200-250) g, ages were range of (10-17) weeks, in laboratory of animal physiology of science collage/ university of Kufa. The animals were distributed into eight groups (G1-G8) including 5 animals each. Groups G1 standard diet for two months, G2fed a standard diet for three months,G3 fed an atherogenic dietfor two months, G4 fed an atherogenic diet for three months, G5 administered atherogenic diet and 250 mg/kg of *M. oleifera* for two months, G6 administered atherogenic diet and 250 mg/kg of *M. oleifera* for three months, G7 administered atherogenic diet and 500 mg/kg of *M. oleifera*for two months, G8 administered atherogenic diet and 500 mg/kg of *M. oleifera*for three months. obesity induced by atherogenic diet for two and three months. The serum concentration of ghrelin, obestatin, and VEGF were estimated in control, non-treated and treated animals groups. Theresults revealed significant increase (P≤0.05) in serum obestatin, and VEGF levels and significant decrease (P≤ 0.05) in serum ghrelin in obese groups in comparing to control group.The results also revealed significant decrease (P≤0.05) in serum obestatin, and VEGF levels and significant increase (P≤0.05) serum ghrelin levels in atherogenic diet groups administered methanolic extract of *M. oleifera* leaves compared to obese group. The present study concluded the good effect of *M. oleifera* by both concentrations on altered biochemical markers induced by atherogenic diet.
Keywords:*Moringa oleifera*, Ghrelin,obestatin, VEGF, Atherogenic diet, obesity.

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INTRODUCTION

The obesity is progressively increasing around the world and has become an epidemic. Obesity is associated with the early development of diseases such as cardiovascular disease and type 2 diabetes. The ghrelin and obestatin play significant roles in regulating body mass.

Moringa oleifera is the most commonly cultivated species of the family Moringaceae, which comprise 13 species of shrubs and trees distributed in sub Himalayan ranges of Sri Lanka, India, South-western and North-eastern Africa, Arabia, Madagascar, and parts of West Africa particularly Nigeria [1,2] . Common England names include Moringa and drumstick tree [3]. The Moringa tree is a plant that has many functions. It has been cultivated in tropical areas of the world for the following reasons: high protein, mineral, carbohydrate content, and vitamins of plants; high content of oil (42%) in the seed, is edible; high nutrition value for both humans and livestock; and with medicinal uses, the seeds coagulant can be used for treatment of wastewater [4]. Studies by Limon-Pacheco and Gonsebatt, Amaglo et al. and Mahajan and Mehta [5,6,7] have documented the antioxidant, pharmacological, and anti-inflammatory characteristic of *M. oleifera* respectively. Moreover [8] estimated the toxicity of the aqueous extract of *M. oleifera*. Gupta et al., [9] worked on the estimation of antioxidant and activity antidiabetic of *M. oleifera* in animal induced diabetes mellitus. Choudhary et al., [10] estimated the antiulcer activity of *M. oleifera* root bark extract in rats. Ghrelin is a small peptide hormone, which is essentially secreted from the oxyntic cells of stomach [11]. It is represent a natural bind to the growth hormone secretagogue receptor (GHSR), which is largely distributed in the body, involving the cardiovascular system [12]. In addition to its obvious releasing activity of growth hormone (GH) [11], ghrelin increase the appetite [13]. Ghrelin has different cardiovascular activities independent of GH releasing activity, such as decreasing ischemic reperfusion injury, stimulating angiogenesis, relieving heart failure and increasing vasodilation [14].

Obestatin peptide hormone consists of 23 amino acid produced by preproghrelin cleavage, that is mainly generated in cells of the gastric mucosa, pancreas, myenteric plexus, and in testis leydig cells [15]. It has been documented to bind to and stimulate the orphan receptor, G protein-coupled receptor-39 (GPR39) [16]. Biological action of obestatin is part of a complex network of gut–brain, the satiety or hunger interactions [17]. Obestatin activity is opposite to ghrelin, where it act as satiety hormone [18].

Vascular Endothelial Growth Factor (VEGF) a heparin binding growth factor with 45 kD induce group of biological influences on endothelium both in vivo and in vitro involving maintaining, proliferation and migration, enhance vascular permeability, and NO production [19]. Raised levels of plasma VEGF has been reported in hyperlipidemia and atherosclerosis in many studies [20,21,22]. Aim of study: the current study was designed to show the effects of *Moringa oleifera* leaf extract on some biochemical markers in rats induced by atherogenic diet.

MATERIALS AND METHODS

Plant collection and Diagnosis

Moringa oleifera leaves were obtained from Canada and classified by special taxonomist (Ass. Prof. Dr. Ahmed obeys motar) in faculty of science/ kufa university.

Animals

Male rats (n = 60) in number weighting from (200-250) g and aged between (10-17) weeks were obtained from high institutes of fertility and / Al-Nahrain university and the study begun from 1/12/2014 to 1/3/2015. Animals were housed in the animal house/ university of kufa/ faculty of science under control condition light 12 and 12 dark hour and temperature (2021-24C°).

Ghrelin Rats Elisa kit measurement

The assessment of ghrelin rats Elisa kits provided by (elabscience – china) Sandwich immunoassay technique (enzyme linked immunesorbent assay – automated microtiter plate), Elisa reader (Biokit ELX 800 reader, ELX50 washer/USA).

Obestatin rats Elisa kit measurement

The assessment of obestatin rats Elisa kits provided by (us biological life science – china) sandwich immunoassay technique (enzyme – linked immunosorbant assay - automated microtiter plated). Elisa reader (Biokit ELX 800 reader, ELX50 washer/USA).

Vascular endothelial growth factor Elisa kit measurement

The assessment of Vascular endothelial growth factor rats Elisa kits provided by (us biological life science – china) sandwich immunoassay technique (enzyme – linked immunosorbant assay - automated microtiter plated). Elisa reader (Biokit ELX 800 reader, ELX50 washer/USA).

METHODS

Atherogenic diet

The constituents of atherogenic diet according to [23] illustrated in table 1.

Table 1 constituents of atherogenic diet

Composition	Weight Kg	Standard diet (%)	Atherogenic diet (%)
Maize	8.97	18	17.678
Soya bean meal	3.74	8	7.37
Brewery dry grain (BDG)	17.5	35	34.49
Wheat bran(WB)	5	10	9.85
Rice bran (RB)	12.5	25	24.635
Oyster shell (OS)	1	2	1.97
Bone meal (BM)	0.5	1	0.985
Common salt (CS)	0.13	0.5	0.256
Methionine	0.5	0.1	0.985
Fish meal	0.5	0.1	0.985
Groundnut oil	0.3	-	0.59
cholesterol	0.1	-	0.197

Preparation of *Moringa oleifera*leaf extract

M. oleifera Leaves were dried by using oven under (45C°) for several days and then crushed to a powder by using an electrical blender, the methanolic extract was prepared by adding 200 ml of methanol alcohol to 20 g of powder and by using Sexholate for 24 hour, the extract was dried and obtained for experiments. The extract was prepared by two doses 250 and 500 mg/kg of methanolic extract. The procedure of plant doses administered to male animal rats was orally for two and three months after 2 hours of atherogenic diet administration.

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Animal Grouping

The study included (12) groups as follows:
Group 1: included 5 males were fed a standard diet for two months (C2).
Group 2: included 5 males were fed a standard diet for three months (C3).
Group 3: included 5 males were fed an atherogenic diet for two months (OB2).
Group 4: included 5 males were fed an atherogenic diet for three months (OB3).
Group 5: included 5 males administered 250 mg/kg of *M. oleifera* after 2 h of atherogenic diet administration for two months (250OBM2).
Group 6: included 5 males administered 250 mg/kg of *M. oleifera* after 2 h of atherogenic diet administration for three months (250OBM3).
Group 7: included 5 males administered 500 mg/kg of *M. oleifera* after 2 h of atherogenic diet administration for two months (500OBM2).

Group 8: included 5 males administered 500 mg/kg of *M. oleifera* after 2 h of atherogenic diet administration for three months (500OBM3).

Blood samples

The blood was drawn by heart puncture by using disposable syringe (5 ml in volume) and then left in room temperature for clotting, and then centrifuged at 3000 r pm for 15 minutes, then serum was isolated and stored at deep freeze in Al- Sadar teaching city in Al-Najaf Al-Ashraf province until using for estimation the lipid profile.

Statistical analysis

The data of current study was statically analysis by (mean ± standard error). Statistical analysis by spss package (v.17).The descriptive analysis between main groups of animals (mean ± SE) and performed using multivariate ANOVA and LSD for comparison among groups in the testing parameters. By EXCELL program of Microsoft office 2013 be done figures. Significant difference (P≤ 0.05) [24].

RESULTS

Effect of atherogenic diet and concentration of methanolic extract of *M. oleifera* on Ghrelin, Obestatin and VEGF level

The results of table 2 indicate a significant increase (P≤ 0.05) in body weight, serum obestatin and vascular endothelial growth factor (VEGF) levels in obese group (300 ± 2.23, 105.26 ± .865, and 126.08 ± 9.043) respectively in comparing with control group (251.8 ± 1.11, 63.34 ± 1.334 and 66.72 ± 0.711) respectively, LSD value was (8.200, 6.7842, and 22.9398) respectively, and a significant decrease (P≤ 0.05) in serum ghrelin level in obese group (0.24 ± 0.04) in comparing with control group (3.18 ± 0.365) LSD value of ghrelin was (1.0194). Also the results show that the obese group which administered concentration of 500 mg/kg of methanolic extract of *Moringa oleifera* recorded higher significant increase (P≤ 0.05) in serum ghrelin level (2.5 ± 0.10) and higher significant decrease (P≤ 0.05) in body weight, serum Obestatin, and VEGF levels (266.4 ±1.86, 65.780 ±1.764, and 60.92 ± 1.99) respectively when compared with other group(OBM250) in relative to obese group (0.24 ± 0.04, 300 ± 2.23, 105.26 ± .865,and 126.08 ± 9.043) respectively. The results also show significant differences at (P≤ 0.05) between groups, LSD value of body weight Ghrelin, Obestatin and VEGF (8.200,1.0194, 6.7842 and 22.9398) respectively.

Table 2 effect of atherogenic diet and concentration of methanolic extract of M. oleifera on Ghrelin, Obestatin and VEGF level

Parameters Groups	Mean ± SE			
	Body weight g	Ghrelinpmol/L	Obestatinpmol/L	VEGFpmol/L
C	251.8 ± 1.11	3.18 ± 0.365	63.34 ± 1.334	66.72 ± 0.711
OB	300 ± 2.23	0.24 ± 0.04	105.26 ± 0.865	126.08 ± 9.043
OBM250	271.4 ± 0.98	2 ± 0.07	70.21 ± 0.615	68.91 ± 1.329
OBM500	266.4 ±1.86	2.5 ± 0.10	65.780 ±1.764	60.92 ± 1.99
LSD	8.200	1.0194	6.7842	22.9398

C = control group, OB = obese group, OBM250 =atherogenic diet+250 mg/kg of methanolic extract of *M. oleifera*,OBM500 = atherogenic diet + 500 mg/kg of methanolic extract of *M. oleifera*

Effect of duration (two and three months) of atherogenic diet and methanolic extract of *M. oleifera* biochemical markers

Ghrelin level

The figure 1 show significant decrease (P≤ 0.05) in ghrelin level in obese groups for both durations two and three months (0.44 and 0.2) in comparing with control groups (3.18 and 3.2) LSD value was (0.9684). Also show that the duration of two months in group which administered *M. oleifera* extract recorded higher

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significant increase (P≤ 0.05) in ghrelin level (1.7) in comparing with obese groups (0.44 and 0.2). Also the results reveal significant differences at (P≤ 0.05) between groups, LSD value (0.9684).

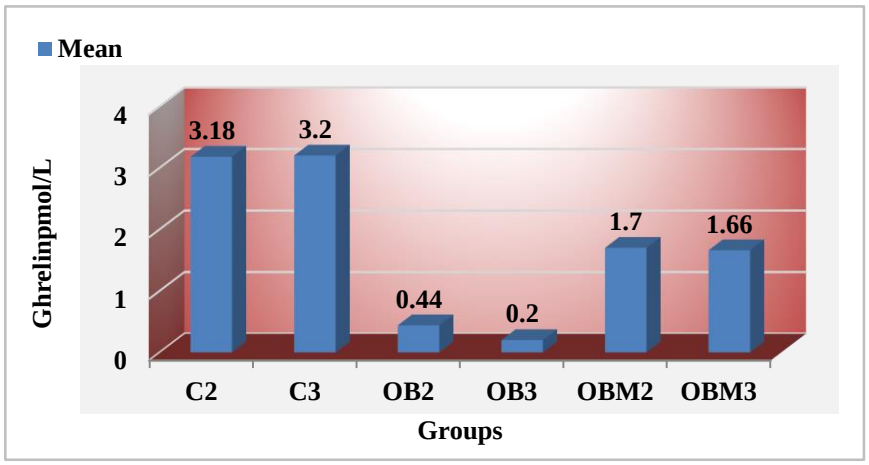

Figure 1: Effect of duration (two and three months) of atherogenic diet and methanolic extract of M. oleifera on ghrelin level

C2 = control group (two months), C3 = control group (three months), OB2 = obese group (two months), OB3 = obese group (three months), OBM2 = atherogenic diet + methanolic extract of *M. oleifera*(two months), OBM3 = atherogenic diet + methanolic extract of *M. oleifera* (three months).

Obestatin level

The figure 2 show significant increase (P≤ 0.05) in obestatin level in obese groups for both durations two and three months (95.72 and 104.54) in comparing with control groups (63.34 and 63.3), LSD value was (7.0842). Also show that the duration of three months in group which administered *M. oleifera* extract recorded higher significant decrease (P≤ 0.05) in obestatin level (67.5) in comparing with obese groups (95.72 and 104.54). Also the results reveal significant differences at (P≤ 0.05) between groups, LSD value (7.0842).

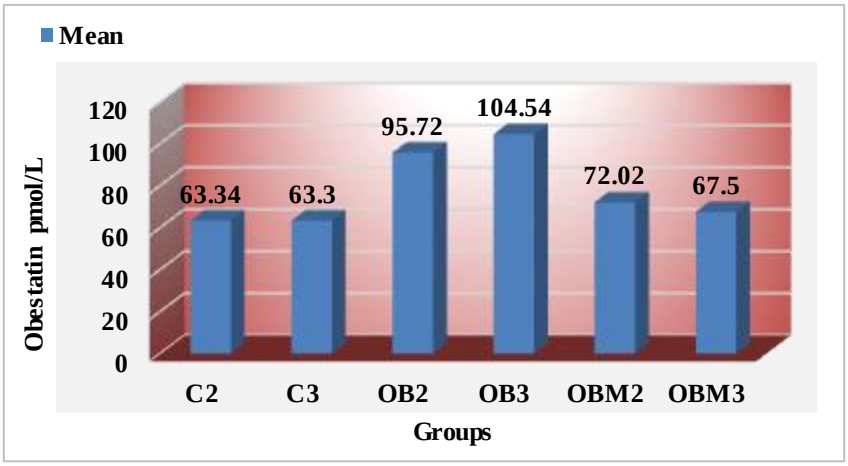

Figure 2: Effect of duration (two and three months) of atherogenic diet and methanolic extract of *M. oleifera* on obestatin level

C2 = control group (two months), C3 = control group (three months), OB2 = obese group (two months), OB3 = obese group (three months), OBM2 = atherogenic diet + methanolic extract of M. oleifera(two months), OBM3 = atherogenic diet + methanolic extract of M. oleifera (three months).

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VEGF level

The figure 3 shows significant increase (P≤ 0.05) in VEGF level in obese groups for both durations two and three months (111.21 and 127.67) in comparing with control groups (66.72 and 66.7), LSD value was (19.6646). Also show that the duration of three months in group which administered *M. oleifera* extract recorded higher significant decrease (P≤ 0.05) in VEGF level (60.55) in comparing with obese groups (111.21 and 127.27).Also the results reveal significant differences at (P≤ 0.05) between groups, LSD value (19.6646).

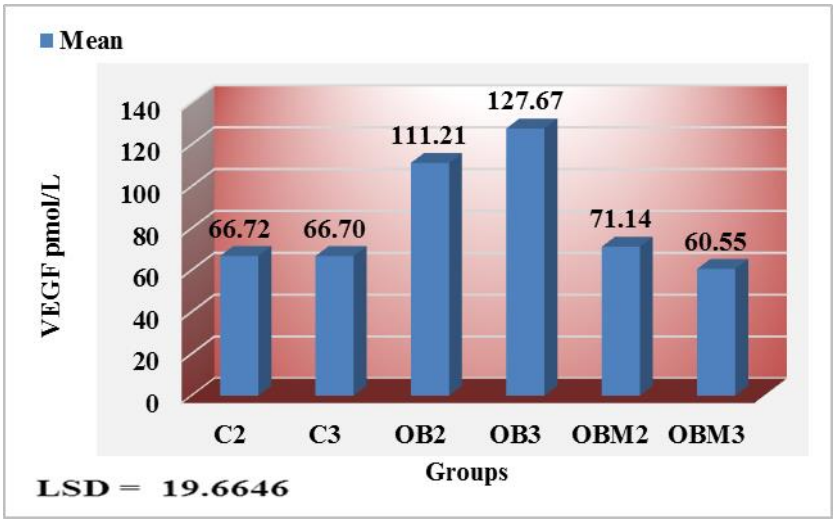

Figure 3: Effect of duration (two and three months) of atherogenic diet and methanolic extract of M. oleifera on VEGF level

C2 = control group (two months), C3 = control group (three months), OB2 = obese group (two months), OB3 = obese group (three months), OBM2 = atherogenic diet + methanolic extract of M. oleifera(two months), OBM3 = atherogenic diet + methanolic extract of M. oleifera (three months).

Effect of interaction between the concentrations and duration of methanolic extract of *M. oleifera* biochemical markers

Ghrelin level

Figure 4 show that the concentration of 500 mg/kg of *M. oleifera* extract for three months give higher significant increase (P≤ 0.05) in serum ghrelin level (2.92) in comparing with obese groups for two and three months (0.44) and (0.2), LSD value (0.435).

Obestatin level

Figure 5 show that the concentration of 500 mg/kg of *M. oleifera* extract for three months give higher significant decrease (P≤ 0.05) in serum obestatin level (67.5) in comparing with obese groups for two and three months (95.7) and (104.5), LSD value (6.578).

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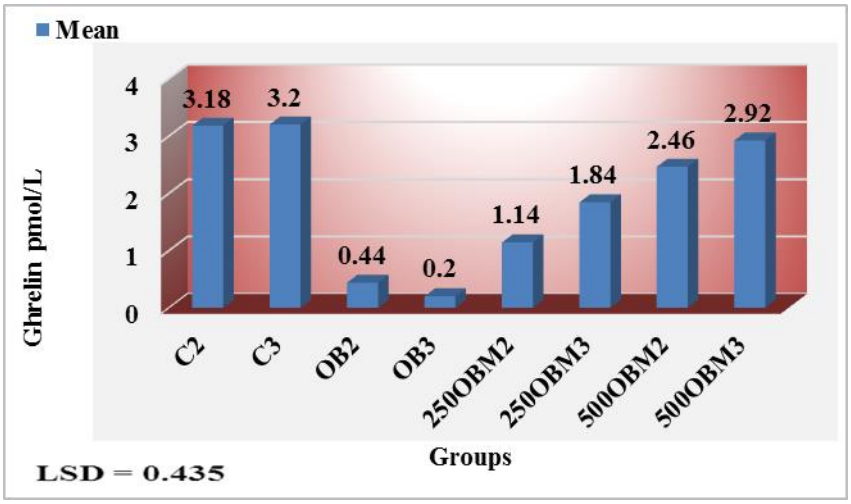

Figure 4: Effect of interaction between the concentrations and duration of methanolic extract of *M. oleifera* on ghrelin level

C2 = control group (two months), C3 = control group (three months), OB2 = obese group (two months), OB3 = obese group (three months), 250OBM2 = atherogenic diet + 250 mg/kg of methanolic extract of M. oleifera(two months), 250OBM3 = atherogenic diet + 250 mg/kg of methanolic extract of M. oleifera(three months), 500OBM2 = atherogenic diet + 500 mg/kg of methanolic extract of M. oleifera(two months), 500OBM3 = atherogenic diet + 500 mg/kg of methanolic extract of M. oleifera(three months).

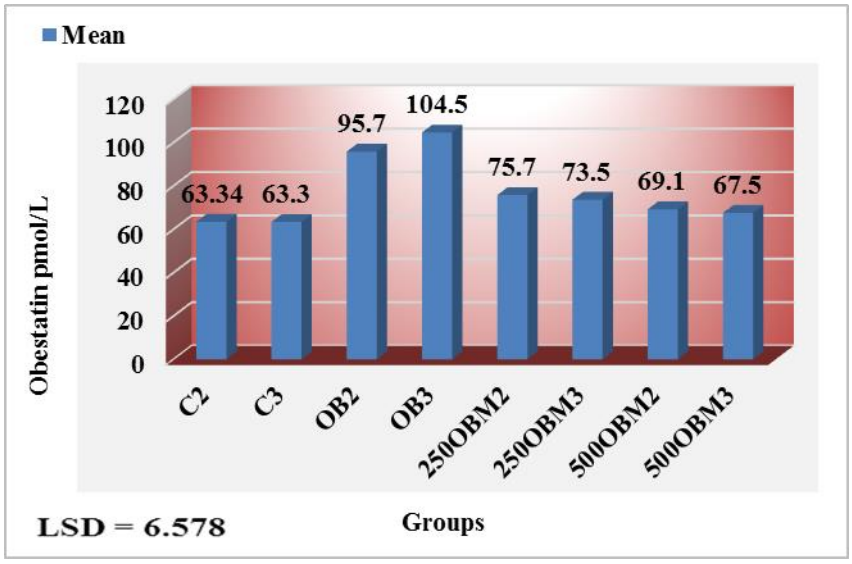

Figure 5: Effect of interaction between the concentrations and duration of methanolic extract of *M. oleifera* on obestatin level

C2 = control group (two months), C3 = control group (three months), OB2 = obese group (two months), OB3 = obese group (three months), 250OBM2 = atherogenic diet + 250 mg/kg of methanolic extract of M. oleifera(two months), 250OBM3 = atherogenic diet + 250 mg/kg of methanolic extract of M. oleifera(three months), 500OBM2 = atherogenic diet + 500 mg/kg of methanolic extract of M. oleifera(two months), 500OBM3 = atherogenic diet + 500 mg/kg of methanolic extract of M. oleifera(three months).

VEGF level

Figure 6 show that the concentration of 500 mg/kg of *M. oleifera* extract for three months give higher significant decrease (P≤ 0.05) in serum VEGF level (60.2) in comparing with obese groups for two and three months (111.2) and (127.7), LSD value (16.92).

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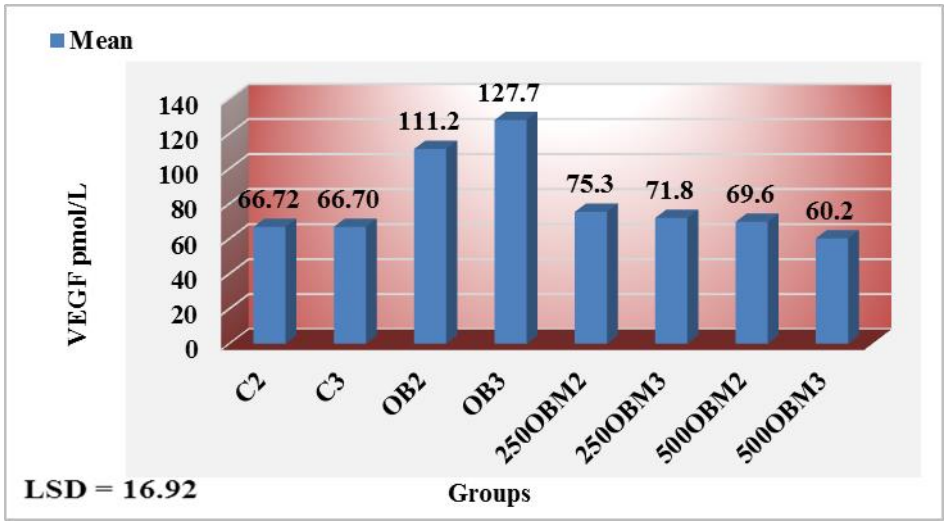

Figure 6 Effect of interaction between the concentrations and duration of methanolic extract of *M. oleifera* on VEGF level

C2 = control group (two months), C3 = control group (three months), OB2 = obese group (two months), OB3 = obese group (three months), 250MOB2 = 250 mg/kg of methanolic extract of M. oleifera + atherogenic diet (two months), 250OBM2 = atherogenic diet + 250 mg/kg of methanolic extract of M. oleifera(two months), 250OBM3 = atherogenic diet + 250 mg/kg of methanolic extract of M. oleifera(three months), 500OBM2 = atherogenic diet + 500 mg/kg of methanolic extract of M. oleifera(two months), 500OBM3 = atherogenic diet + 500 mg/kg of methanolic extract of M. oleifera(three months).

DISSCUSION

Ghrelin level

The results in table 2 show significant increase in body weight in atherogenic diet feeding rats. These results are in accordance with [25]. The food rich with fat was the best mean studied by researchers in eliciting the obesity in animal model due to its high similarity of mimicking the usual route of obesity episodes in human [26]. Also the results revealed significant decreasein serum ghrelin. These result in accordance with [27]. These results indicate the down regulation of ghrelin in obesity and high fat feeding [28]. This ghrelin down regulation in obesity is related to insulin resistance [29]. Previous study by Tschop et al., [30] they attribute the cause of such down regulation to an increasing in leptin or insulin, because fasting levels of plasma ghrelin are inversely correlated with fasting levels of plasma insulin and the levels of leptin, they also interpret the decreased plasma ghrelin as an body attempt to overcome the positive energy balance associated with obesity by this physiological adaptation [31]. The suggestions that the decreased ghrelin levels in obesity related to elevated levels of serum insulin still conflicting although there is index that the ghrelin is significantly decreased during obesity associated only with insulin resistance and high plasma insulin levels and disappear in obesity with sensitivity to insulin action [32].

Another study by Toshinai et al., [33] reported that the ghrelin mRNA expression in fundus of db/db mice stomach (an obese model characterized by null mutation in the gene of leptin receptor) is decreased and become less regulated when compared to normal weight mice. They attributed the reason of that to physiological adaptation to long term positive energy balance.

The results in same table revealed significant increase in levels of serum ghrelin when the obese group treated with methanolic *M. oleifera* leaf extract. There are no previous researches in this field. The probable cause of elevated serum ghrelin levels by *M. oleifera* administration is decreased body weight in this group, where the previous studies indicated the role of *M. oleifera* in reduction the weight of obese individuals [34], and Serum ghrelin level is negatively correlated with the body mass index [29]. Also the elevation in the levels of ghrelin hormone after *Moringa oleifera*adminstation may be due to presence of active compound such as flavonoids.

Obestatin level

The results in table 2 revealed a significant increase in serum obestatin level in obese group when compared to control group. These results are in accordance with [35], But disagree with [36,37]. The studies disparate between them with regard to the level of obestatin in obese individuals, the differences between studies in this field may be attributed to different fates of cleavage of preproghrelin after translation into two related peptides (ghrelin and obestatin) which may be undergo to different regulation during obesity or due to different coordination by common regulatory factors responsible of levels of these hormones in response to nutritional status [38]. The differences in the obese status may also responsible of these disparate obestatin values. The study of Hainer et al., [37] was on massive obese individuals, whereas the study of Guo et al., [36] on Chinese subjects who are suffered from ethnic-specific values of waist circumference and BMI [38]. The same interpret for such disparate applies to studies performed in this field on laboratory animals with different strains.

Many studies correlate the decreased ghrelin levels in obesity with insulin resistance and hyper insulinemia in these individuals [39] while there is no evidence ascribe the elevation in obestatin levels in obesity to this reason.

The ratio of ghrelin/obestatin was reduced in obesity and this reduction was significantly correlated to indices of insulin, they interpret this finding as disparate effect of insulin on ghrelin and obestatin in various studies.

The results in same table indicated a significant reduction in the levels of obestatin in high fat feeding groups administered *M. oleifera* extract. This reduction may be due to decreased body weight in these groups and returned the disturbed ghrelin and obestatin concentrations caused by obesity to normal or nearly normal levels.

Vascular Endothelial Growth Factor (VEGF) level

The results in table 2 revealed a significant increase in VEGF level in rich fat diet group when compared to control group. This results in accordance with [40,41] who revealed that the level of serum VEGF was positively linked with body mass index and visceral fat mass in overweight and obese individuals. But disagree with [42].

Obesity is correlated with capillary bed expansion in a region of fat stores and this expansion appears to be important in obesity development [43]. Previous study by Rupnick et al., [44] demonstrated that the obese mouse model treated with antiangiogenic agents cause regression in depots of fat suggesting that the regulation of vasculature of adipose tissue is an important contributor in obesity. Angiogenesis is strongly linked with adipogenesis [45] and is represents an essential component in development and expansion of adipose tissue [46]. The expansion of adipose tissue creates hypoxia, the regulatory factor of vascular endothelial growth factor which is secreted by several endothelial cells in adipose tissue [47] . The important role of VEGF in vascular development indicated by the fact that loss of single allele of VEGF-A cause abnormal blood vessel development and embryonic death [48].

These observations provide a suggestion that the obesity may be correlated with increased angiogenic factors levels, and may be directly related to the pathogenesis of obesity or, as a result of increased adiposity; it is closer to the right.

Obese individuals with many types of cancers have worse prognosis, reduced interval without disease after first treatment and metastatic disease increment which suggest that angiogenesis may be increased in obesity [49]. In same table the results showed significant decrease in VEGF level in group treated with both atherogenic diet and *M. oleifera* extract, which may be related to reduced body mass in treated group.

REFERENCES

[1] Pinder RM, Brogden RN, Sawyer PR, Speight TM and Avery GS (1975). “Fenfluramine: A Review of Its Phar- macological Properties and Therapeutic Efficacy in Obesity,” Drugs, 10(4): 241-323.

[2] Yun JW, (2010). “Possible Anti-Obesity Therapeutics from Na- ture—A Review,” Phytochemistry, 71(14-15): 1625-1641.

[3] Kishino E, Ito T, Fujita K. andKiuchi Y (2006). "A Mixture of the Salaciareticulata (KotalaHimbutu) Aqueous Extract and Cyclodextrin Reduces the Accumulation of Visceral Fat Mass in Mice and Rats with High-Fat Diet-Induced Obesity," J Nut. 136(2): 433-439.

[4] El-Olemy MM, Al-Muhtadi FJ, Afifi AA (1994). Experimental Phytochemistry.A laboratory Manual. Riyadh, Saudi Arabia: King Saud University Press.

[5] Limon-Pacheco J, and Gonsebatt ME (2009). The role of antioxidants and antioxidant-related enzymes in protective responses to environmentally induced oxidative stress. Mutat. Res. 674, 137–147.

[6] Amaglo NK, Bennett RN, Lo-Curto RB, Rosa EAS, Lo-Turco V, Giuffrid A, Lo CurtoA.Crea F, and Timpo GM (2010). Profiling selected phytochemicalsand nutrients in different tissues of the multipurpose tree Moringa oleifera L. grown in Ghana. Food Chem. 122, 1047–1054.

[7] Mahajan S, and Mehta A (2009). Curative effect of hydroalcoholic extract of leaves of Moringa oleifera lam. Against adjuvant induced established arthritis in rats. Niger. J. Nat. Prod. Med. 13, 13–22.

[8] Awodele O, Oreagba IA, Odoma S, Silva JAT, Osunkalu VO (2012). Toxicological evaluation of the aqueous extract of Moringa oleifera Lam (Moringaceae).J Ethnopharmacol. 139: 330-336.

[9] Choudhary MK, Bodakhe SK, Gupta SK (2013). Assessment of the antiulcer potential of Moringa oleifera root bark extract in rats. J Acupunct Meridian Stud.

[10] Gupta et al (2012). Evaluation of antidiabeticand antioxidant activity of Moringa oleifera in experimental diabetes.J Diabetes.

[11] Kojima M, Hosoda H, Date Y. (1999). Ghrelin is a growthhormone- releasing acylated peptide from stomach. Nature.402:656–60.

[12] Gnanapavan S, Kola B, Bustin SA, Morris DG, McGee P, Fairclough P et al (2002). The tissue distribution of the mRNA of ghrelin and subtypes of its receptor, GHS-R, in humans.J ClinEndocrinolMetab. 87: 2988–91.

[13] Shintani M, Ogawa Y, Ebihara K, Aizawa-Abe M, Miyanaga F, Takaya K et al (2001). Ghrelin, an endogenous growth hormone secretagogue, is a novel orexigenic peptide that antagonizes leptin action through the activation of hypothalamic neuropeptide Y/Y1 receptor pathway. Diabetes. 50: 227–32.

[14] Xu L, Depoortere I, Tomasetto C, Zandecki M, Tang M, Timmermans JP &Peeters TL (2005). Evidence for the presence of motilin, ghrelin, and the motilin and ghrelin receptor in neurons of the myenteric plexus.RegulPept 124(1–3): 119–125.

[15] Dun SL, Brailoiu GC, Brailoiu E, Yang J, Chang JK and Dun NJ (2006). Distribution and biological activity of obestatin in the rat. J. Endocrinol. 191: 481-489.

[16] Zhang JV, Ren PG, Avsian-Kretchmer O, Luo CW, Rauch R, Klein C &Hsueh AJ (2005). Obestatin, a peptide encoded by the ghrelin gene, opposes ghrelin's effects on food intake. Science 310(5750): 996–999.

[17] Pan W, Tu H, and Kastin AJ (2006). Differential BBB interactions of three ingestive peptides: obestatin, ghrelin, and adiponectin. Peptides, 27: 911–6.

[18] Kaiya H, Miyazato M, and Kangawa K (2011). Recent advances in the phylogenetic study of ghrelin. Peptides, 32: 2155–2174.

[19] Zachary I, (2003). VEGF signalling: Integration and multitasking in endothelial cell biology. BiochemSoc Trans. 31Pt, 6: 1171-1177.

[20] Couffinhal T, Kearney M, Witzenbichler B, Chen D, Murohara T, Losordo DW, Symes J and Isner JM (1997). Vascular endothelial growth factor/vascular permeability factor VEGF/VPF. in normal and atherosclerotic human arteries. Am J Pathol. 1505: 1673-1685.

[21] Trape J, Morales C, Molina R, Filella X, Marcos JM, Salinas R, and Franquesa J (2006).Vascular endothelial growth factor serum concentrations in hypercholesterolemic patients. Scand J. Clin. Lab. Invest., 663: 261-267.

[22] Giurgea AG, Margeta C, Maca T, Rezaie-Majd A, Bucek RA, M Manavi, Afarideh R, Minar E, and Baghestanian M (2006). Simvastatin reduces serum level of vascular endothelial growth factor in hypercholesterolemic patients. J CardiovascPharmacol 471: 30-36.

[23] Saso Y, kitamura K, Yasoshima A, iwasaki HO, Yasoshima K, Doi K and Morita T (1992). Rapid induction of atherosclerosis in rabbits. Histol.Histopathol. 7: 315–330.

[24] AL-RawikM (2000). "Introduction to Statistics" , 2nd.ed , College of Agriculture and Forestry. Mosul University. [17] Saso Y, kitamura K, Yasoshima A, iwasaki HO, Yasoshima K, Doi K and Morita T (1992). Rapid induction of atherosclerosis in rabbits. Histol.Histopathol. 7: 315–330.

[25] NajlaGS ,Nazamid S, Amin I, Alfi K, Mohamad FM, and Azizah AH (2015). An Investigation into the Antiobesity Effects of Morindacitrifolia L. Leaf Extract in High Fat Diet Induced Obese Rats Using a 1H NMR Metabolomics Approach. J Diabetes Res. 2015: 304142.
[26] Buettner R, Scholmerich J, and Bollheimer LC (2007). "High-fat diets: modeling the metabolic disorders of human obesity in rodents," Obesity. 15(4): 798–808.
[27] Lindqvist A, Cour CD, Stegmark A, Hakansonb R, Erlanson-Albertsson C (2005). Overeating of palatable food is associated with blunted leptin and ghrelin responses. Regulatory Peptides 130: 123 – 132.
[28] Nakagawa E, Nagaya N, Okumura H, Enomoto M, Oya H, Ono F, et al (2002). Hyperglycaemia suppresses the secretion of ghrelin, a novel growth-hormone-releasing peptide: responses to the intravenous and oral administration of glucose. ClinSci (Lond). 103(3):325– 8.
[29] Cummings DE, Weigle DS, Frayo RS, Breen PA, Ma MK, Dellinger EP, and Purnell JQ (2002). Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery. The New Eng J Med 346: 1623-1630.
[30] Tschop M, Smiley DL, HeimanML(2000). Ghrelin induces adiposity in rodents. Nature, 407(6806):908–13.
[31] St-Pierre DH, Karelis AD, Coderre L, Malita F, Fontaine J, Mignault D, Brochu M, Bastard JP, Cianflone K, Doucet E, Imbeault P &Rabasa-Lhoret R (2007). Association of acylated and nonacylated ghrelin with insulin sensitivity in overweight and obese postmenopausal women.J ClinEndocrinolMetab. 92(1): 264–269.
[32] Murdolo G, Lucidi P, Di Loreto C, et al (2003). Insulin is required for prandial ghrelin suppression in humans. Diabetes. 52(12):2923-7.
[33] Toshinai K, Mondal MS, Nakazato M, Date Y, Murakami N, Kojima M, et al (2001). Upregulation of Ghrelin expression in the stomach upon fasting, insulin-induced hypoglycemia, and leptin administration.BiochemBiophys Res Commun 281(5):1220–5.
[34] Denen A, Ejike DE, et al (2014). Hypolipidaemic effect of ethanol leaf extract of Moringa oleifera Lam. in experimentally induced hypercholesterolemicwistar rats.Int J Food SciNutr 3(4): 355-360.
[35] Zamrazilova H, Hainer V, et al (2008). Plasma Obestatin Levels in Normal Weight, Obese and Anorectic Women. Physiol Res. 57 (1): 49-55 .
[36] Guo ZF, Zheng X, Qin YW, Hu JQ, Chen SP and Zhang Z (2007). Circulating preprandial ghrelin to obestatin ratio is increased in human obesity. J ClinEndocrinol and Metab. 92: 1875–1880.
[37] Hainer DG, Schindler K, et al (2007). Serum retinol-binding protein-4 is reduced after weight loss in morbidly obese subjects. J ClinEndocrinolMetab. 92 1168–1171.
[38] Chen C and Lu FC (2004). The guidelines for prevention and control of overweight and obesity in Chinese adults.Biomed and Enviro Sci. 17: 1–36.
[39] Kojima M, Kangawa K. (2005). Ghrelin: structure and function. Physiol Rev. 85:495–522.
[40] Miyazawa-Hoshimoto S, et al (2003). Elevated serum vascular endothelial growth factor is associated with visceral fat accumulation in human obese subjects. Diabetologia. 46: 1483–1488.
[41] Gomez-Ambrosi J, Catalan V, Rodriguez A, Ramirez B, Silva C, Gil MJ, Salvador J, Fruhbeck G (2009). Involvement of serum vascular endothelial growth factor family members in the development of obesity in mice and humans. J NutrBiochemepub ahead of print, PMID: 19615882.
[42] KhazaeiM andTahergorabi Z (2013). "Systemic ghrelin administration alters serum biomarkers of angiogenesis in diet-induced obese mice,"Int J Pep, vol. 2013, Article ID 249565, 5 pages.
[43] Hausman GJ, Richardson RL (2004).Adipose tissue angiogenesis. J Anim Sci. 82: 925–934.
[44] Rupnick MA, Panigrahy D, Zhang CY, Dallabrida SM, Lowell BB, Langer R, Folkman MJ (2002). Adipose tissue mass can be regulated through the vasculature. ProcNatlAcadSci USA. 99: 10730–10735
[45] Christiaens V and. Lijnen HR (2010). "Angiogenesis and development of adipose tissue," Molecular and Cellular Endocrinol. 318(1-2) , pp. 2–9.
[46] Liu L and Meydani M (2003), "Angiogenesis inhibitors may regulate adiposity," Nutrition Reviews, 61(11), pp. 384–387.
[47] Mick GJ, Wang X, McCormick K (2002). White adipocyte vascular endothelial growth factor: regulation by insulin. Endocrinology. 143: 948–953.
[48] Ferrara N, Chen H, et al (1998). Vascular endothelial growth factor is essential for corpus luteum angiogenesis. Nat Med 4:336–340.
[49] Freedland SJ, Aronson WJ, et al (2004). Impact of obesity on biochemical control after radical prostatectomy for clinically localized prostate cancer: a report by the Shared Equal Access Regional Cancer Hospital database study group. J ClinOncol. 22: 446−453.