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Comparison Study between effect of Methanolic Extract of *Moringa oleifera* and Exogenous Ghrelin on Lipid Profile in Atherogenic Rats.

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ABSTRACT

We compared between the hypolipdemic effect of methanolic extract of *Moringa oleifera* leaves and exogenous ghrelin in atherogenic rats. This study was conducted on (n = 50) 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 ten groups (G1-G10) including 5 animals each. Groups G1 fed a standard diet, G2 fed an atherogenic diet, G3 administered 250 mg/kg of *M. oleifera* and atherogenic diet, G4 administered atherogenic diet and 250 mg/kg of *M. oleifera*, G5 administered 500 mg/kg of *M. oleifera*and atherogenic diet, G6 administered atherogenic diet and 500 mg/kg of *M. oleifera*, G7 administered 100 µg/kg of ghrelin and atherogenic diet, G8 administered atherogenic diet and 100 µg/kg of ghrelin, G9 administered 300 µg/kg of ghrelin and atherogenic dietand G10 administered atherogenic diet and 300 µg/kg body weight of ghrelin. Dyslipidaemia induced by atherogenic diet for 60 days. Two concentrations of Methanolic leaf extract of *M. oleifera* and exogenous ghrelin administered before and after 2 hours of atherogenic diet. The serum concentration of total cholesterol, triglyceride (TG), LDL-C, VLDL-C, HDL-C, and body weight and organs weight were estimated in control, non-treated and treated animals groups. The results revealed significant increase (P≤0.05) in serum Cholesterol, TG, LDL-C, VLDL-C levels and significant decrease (P≤ 0.05) in serum HDL-C levels in atherogenic groups in comparing to control group. The results also revealed significant decrease (P≤0.05) in serum Cholesterol, TG, LDL-C, VLDL-C levels and significant increase (P≤0.05) serum HDL-C levels in atherogenic groups administered methanolic extract of M. oleifera leaves and ghrelin hormone compared to atherogenic group. The results also revealed significant increase (P≤ 0.05) in body weight and Kidney, liver and heart weight in atherogenic groups in comparing with control group. The results also showed significant decrease (P≤ 0.05) in body weight and weight of kidney, liver and heart in atherogenic groups administered methanolic extract of *M. oleifera* leaves and ghrelin hormone compared to atherogenic group.no differences between the effects of methanolic extract of *M. oleifera* leaves and exogenous ghrelin hormone on different study parameters estimated.

Keywords: *Moringa oleifera*, Ghrelin, Atherogenic diet, obesity.

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INTRODUCTION

Atherosclerosis is the main cause of cardiovascular diseases and the lead to death. Atherosclerosis is a complex, chronic disease characterized by lipid accumulation and inflammation within the tunica intima of vessel wall [1].

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 [2,3]. Common England names include Moringa and drumstick tree [4]. 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 [5]. Studies by Limon-Pacheco and Gonsebatt, Amaglo et al. and Mahajan and Mehta [6,7,8] have documented the antioxidant, pharmacological, and anti-inflammatory characteristic of *M. oleifera* respectively. Moreover, Awodele et al [9] estimate the toxicity of the aqueous extract of *M. oleifera*. Gupta et al [10] worked on the estimation of antioxidant and activity antidiabetic of *M. oleifera* in animal induced diabetes mellitus. Choudhary et al., [11] estimate 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 [12]. It is represent a natural bind to the growth hormone secretagogue receptor (GHSR), which is largely distributed in the body, involving the cardiovascular system [13]. In addition to its obvious releasing activity of growth hormone (GH) [12], ghrelin increase the appetite [14]. Ghrelin has different cardiovascular activities independent of GH releasing activity, such as decreasing ischemic reperfusion injury, stimulating angiogenesis, relieving heart failure and increasing vasodilation [15].

Aim of study: the current study was designed to show the hypolipidemic effect of both *Moringa oleifera* leave extract and ghrelinas comparative study between themin rats treatedwith 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) at the faculty of science/ kufa university.

Animals

Male rats (n = 50) 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 1/2/2015. 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°).

Serum cholesterol estimation

This was done by a method based on (CHOD-POD) enzymatic colorimetric test, executed with specific rat's kit for test, supplied by (cypress diagnostic, Langdrop – Belgium. Cat. No. HB006).

Serum triglyceride estimation

This was done by a method based on (GPO-POD) enzymatic colorimetric test, executed with specific rat's kit for test, supplied by (cypress diagnostic, Langdrop – Belgium. Cat. No. HB021).

Serum HDL-cholesterol estimation

This was done by a method based on Phosphotungstic test, executed with specific rat's kit for test, supplied by (cypress diagnostic, Langdrop – Belgium. Cat. No. HB007).

Serum LDL-cholesterol estimation

LDL-cholesterol, (mg/dl) was calculated according to the flowing formula:

LDL Chol= Total Chol. –(Triglyceride /5) – HDL Chol.

Serum VLDL-cholesterol estimation

VLDL-cholesterol, (mg/dl) was calculated according to the flowing formula:

VLDL Chol= (Triglyceride /5).

METHODS

Atherogenic diet

The constituents of atherogenic diet according to (Saso et al., 1992) illustrated in table 1.

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 months before and after 2 hours of atherogenic diet administration.

Ghrelin administration

Ghrelin were injected inter operationally for two months at two doses 100 µg/kg and 300 µg/kg before and after 2 hours of atherogenic diet intake by animals.

Body and organs weight measurement

Animal rat's weights and organs weight were measured at the end of experiment by using balance.

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

Animal Grouping

The study included (10) groups as follows:

G1 fed a standard diet for 60 days

G2 fed an atherogenic diet for 60 days

G3 administered 250 mg/kg of *M. oleifera* and atherogenic diet for 60 days

G4 administered atherogenic diet and 250 mg/kg of *M. oleifera* for 60 days

G5 administered 500 mg/kg of *M. oleifera* and atherogenic diet for 60 days

G6 administered atherogenic diet and 500 mg/kg of *M. oleifera* for 60 days

G7 administered 100 µg/kg of ghrelin and atherogenic diet for 60 days

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G8 administered atherogenic diet and 100 µg/kg of ghrelin for 60 days
G9 administered 300 µg/kg of ghrelin and atherogenic diet for 60 days
G10 administered atherogenic diet and 300 µg/kg body weight of ghrelin for 60 days

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 EXCEL program of Microsoft office 2013 be done figures. Significant difference (P≤ 0.05).

RESULTS

Effect of atherogenic diet and different concentrations of methanolic extract of *Moringa oleifera* on lipid profile

The results of table 2 indicate a significant increase (P≤0.05) in serum Cholesterol, TG, LDL and VLDL levels in atherogenic group (G2) (190.6 ± 2.22, 260 ± 2.63, 105 ± 2.05 and 52 ± 0.53) respectively in comparing with control group (G1) (142.2 ± 2.63, 216.6 ± 2.56, 30.68 ± 1.89, 43.32 ± 0.51) respectively, LSD value was (10.785, 12.067, 12.595 and 2.413) respectively, and a significant decrease (P≤ 0.05) in serum HDL level in G2 (33.6 ± 0.51) in comparing with G1 (68.2 ± 1.32) LSD value of HDL was (7.157). Also the results show that the group which administered atherogenic diet and 500 mg/kg of methanolic extract of *Moringa oleifera* (G6) as treatment elicited higher significant decrease (P≤ 0.05) in serum Cholesterol, TG, LDL and VLDL levels (145.6 ±1.50, 227 ± 1.36, 32.48 ± 1.54 and 45.52 ± 0.33) respectively and higher significant increase (P≤ 0.05) in serum HDL level (67.6 ± 0.93) when compared with other treatment (G4) and protective groups (G3 andG5). The results also show significant differences at (P≤ 0.05) between groups, LSD value of Cholesterol, TG, LDL, HDL and VLDL (10.785, 12.067, 7.157, 12.595 and 2.413) respectively.

Table 2 Effect of atherogenic diet and oral administration of methanolic extract of *M. oleifera* leaves on lipid profile

Parameters / Groups	Mean ± SE				
	Cholesterol mmol/L	TG mmol/L	HDL mmol/L	LDL mmol/L	VLDL mmol/L
G1	142.2 ± 2.63	216.6 ± 2.56	68.2 ± 1.32	30.68 ± 1.89	43.32 ± 0.51
G2	190.6 ± 2.22	260 ± 2.63	33.6 ± 0.51	105 ± 2.05	52 ± 0.53
G3	177.2 ± 0.86	250.2 ±1.66	44.4 ± 1.03	82.76 ± 1.8	50.04 ± 0.33
G4	166.8 ± 1.46	241.8 ± 2.20	57.4 ± 2.09	61.04 ± 3.04	48.36 ± 0.44
G5	155.2 ±1.85	235.8 ± 1.39	62.2 ± 0.86	45.84 ± 2.31	47.16 ± 0.28
G6	145.6 ±1.50	227 ± 1.36	67.6 ± 0.93	32.48 ± 1.54	45.52 ± 0.33
LSD	10.785	12.067	7.157	12.595	2.413

N = 5 for each group
Significant difference ≤ 0.05

G1 = control group, G2 = atherogenic group, G3 = 250 mg/kg of methanolic extract of *M. oleifera* + atherogenic diet, G4 = atherogenic diet + = 250 mg/kg of methanolic extract of *M. oleifera*, G5 = 500 mg/kg of methanolic extract of *M. oleifera* + atherogenic diet, G6 = atherogenic diet + 500 mg/kg of methanolic extract of *M. Oleifera*

Body weight and body organs weights

The results of table 3 indicate a significant increase (P≤ 0.05) in body weight and Kidney, liver and heart weight in G2 (300 ± 2.23, 1.69 ± 0.11, 8.02 ± 0.18 and 2.92 ± 0.05) respectively in comparing with G1 (251.8 ± 1.11, 0.7 ± 0.04, 5.88 ± 0.05 and 1.5 ± 0.08) respectively, LSD value was (8.200, 0.413, 0.735 and 0.378) respectively. Also the results show that the treatment of atherogenic group with the concentration 500

mg/kg of methanolic extract of *Moringa oleifera* elicited higher significant decrease (P≤ 0.05) in body weight and weight of kidney, liver and heart (266.4 ±1.86, 1.0 ± 0.054, 6.12± 0.18and 1.92 ± 0.06) respectively when compared with other treatment (G4) and protective groups (G3 and G5) in relative to atherogenic group (300 ± 2.23, 1.69 ± 0.11, 8.02 ± 0.18 and 2.92 ± 0.05). The results also show significant differences at (P≤ 0.05) between groups, LSD value of weight of body, kidney liver and heart (8.200, 0.413, 0.735 and 0.378) respectively.

Table 3 effect of atherogenic diet and concentrations of methanolic extract of *M. oleifera* leaves on body weight and body organs weights

Parameters Groups	Mean ± SE			
	Body weight g	Kidney g	Liver g	Heart g
G1	251.8 ± 1.11	0.7 ± 0.04	5.88 ± 0.05	1.5 ± 0.08
G2	300 ± 2.23	1.69 ± 0.11	8.02 ± 0.18	2.92 ± 0.05
G3	280.8 ± 0.49	1.42 ± 0.03	6.66 ± 0.10	2.72 ± 0.03
G4	276.2 ± 0.97	1.27 ± 0.08	6.14 ± 0.10	2.46 ± 0.05
G5	271.4 ± 0.98	1.10 ± 0.04	5.96 ± 0.05	2.22 ± 0.07
G6	266.4 ±1.86	1.0 ± 0.054	6.12± 0.18	1.92 ± 0.06
LSD	8.200	0.413	0.735	0.378

N = 5 for each group
Significant difference ≤ 0.05

G1 = control group, G2 = atherogenic group, G3 = 250 mg/kg of methanolic extract of *M. oleifera* + atherogenic diet, G4 = atherogenic diet + = 250 mg/kg of methanolic extract of *M. oleifera*, G5 = 500mg/kg of methanolic extract of *M. oleifera* + atherogenic diet, G6 = atherogenic diet + 500 mg/kg of methanolic extract of *M. oleifera*

Effect of atherogenic diet and intramuscular injection of concentrations of ghrelin hormone on lipid profile

The results of table 4 indicate a significant increase (P≤ 0.05) in serum Cholesterol, TG, LDL and VLDL levels in atherogenic group (190.6 ± 2.22, 260 ± 2.63, 105 ± 2.05 and 52 ± 0.53) respectively in comparing with control group (142.2 ± 2.63, 216.6 ± 2.56, 30.68 ± 1.89, 43.32 ± 0.51) respectively, LSD value was (11.198, 11.518, 13.055 and 2.303) respectively, and a significant decrease (P≤ 0.05) in serum HDL level in G2 (33.6 ± 0.51) in comparing with G1 (68.2 ± 1.32) LSD value of HDL was (7.314). Also the results show that the group which administered atherogenic diet and concentration 300 µg/kg of ghrelin (G10) as treatment mean elicited higher significant decrease (P≤ 0.05) in serum Cholesterol, TG, LDL and VLDL levels (150.2 ± 1.85, 238.8 ± 1.35, 40.44 ± 3.19and 47.76 ± 0.27) respectively and higher significant increase (P≤ 0.05) in serum HDL level (65 ± 1.51) when compared with other treatment (G8) and protective groups (G7 and G9)). The results also show significant differences at (P≤ 0.05) between groups, LSD value of Cholesterol, TG, LDL, HDL and VLDL (11.198, 11.518, 7.314, 13.055 and 2.303) respectively.

Table 4 effect of atherogenic diet and intramuscular injection of ghrelin hormone on lipid profile

Parameters Groups	Mean ± SE				
	Cholesterol mmol/L	TG mmol/L	HDL mmol/L	LDL mmol/L	VLDL mmol/L
G1	142.2 ± 2.63	216.6 ± 2.56	68.2 ± 1.32	30.68 ± 1.89	43.32 ± 0.51
G2	190.6 ± 2.22	260 ± 2.63	33.6 ± 0.51	105 ± 2.05	52 ± 0.53
G7	182.2 ± 1.24	254.6 ± 1.36	47 ± 1.41	84.28 ± 0.68	50.92 ± 0.27
G8	160.4 ± 1.88	245.4 ± 2.20	57.4 ± 1.02	53.92 ± 2.68	49.08 ± 0.43
G9	171.8 ± 1.28	248.2 ± 1.15	59.6 ± 1.43	62.56 ± 2.09	49.64 ± 0.23
G10	150.2 ± 1.85	238.8 ± 1.35	65 ± 1.51	40.44 ± 3.19	47.76 ± 0.27
LSD	11.198	11.518	7.314	13.055	2.303

N = 5 for each group
Significant differences ≤ 0.05

G1 = control group, G2 = atherogenic group, G7 =100µg/kg of ghrelin + atherogenic diet, G8 = atherogenic diet + = 100µg/kg of ghrelin, G9 = 300µg/kg of ghrelin + atherogenic diet, G10 = atherogenic diet + 300µg/kg of ghrelin

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Body weight and body organs weights

The results of table 5 indicate a significant increase (P≤ 0.05) in body weight and kidney, liver, and heart weight in G2 (300 ± 2.23, 1.69 ± 0.11, 8.02 ± 0.18 and 2.92 ± 0.05) respectively in comparing with G1 (251.8 ± 1.11, 0.7 ± 0.04, 5.88 ± 0.05 and 1.5 ± 0.08) respectively, LSD value was (8.56, 0.3554, 0.5276 and 0.5244) respectively. Also the results show that the group which administered atherogenic diet and concentration 300 mg/kg of ghrelin as treatment elicited higher significant decrease (P≤ 0.05) in body weight and weight of kidney, liver and heart (271.2 ± 1.77, 1.08 ± 0.05, 6.1 ± 0.04 and 2.28 ± 0.10) respectively when compared with other treatment and protective groups (GAD100, GAD300 and ADM100) in relative to atherogenic group (300 ± 2.23, 1.69 ± 0.11, 8.02 ± 0.18 and 2.92 ± 0.05). The results also show significant differences at (P≤ 0.05) between groups, LSD value of weight of body, kidney liver and heart (8.56, 0.3554, 0.5276 and 0.5244) respectively.

Table 5 Effect of atherogenic diet and intramuscular injection of ghrelin hormone on body weight and body organs weights

Parameters / Groups	Mean ± SE			
	Body weight g	**Kidney g**	**Liver g**	Heart g
G1	**251.8 ± 1.114**	**0.7 ± 0.04**	**5.88 ± 0.06**	1.5 ± 0.09
G2	**300 ±2.236**	**1.694 ± 0.12**	**8.02 ± 0.18**	2.92 ± 0.06
G7	**288 ±1.38**	**1.64 ± 0.04**	**7.02 ± 0.05**	3.14 ± 0.07
G8	**281 ± 0.89**	**1.44 ± 0.02**	**6.68 ± 0.07**	2.82 ± 0.09
G9	**278.2 ± 0.92**	**1.28 ± 0.04**	**6.38 ± 0.06**	2.54 ± 0.12
G10	**271.2 ± 1.77**	**1.08 ± 0.05**	**6.1 ± 0.04**	2.28 ± 0.10
LSD	**8.56**	**0.3554**	**0.5276**	0.5244

N = 5 for each group
Significant difference ≤ 0.05

G1 = control group, G2 = atherogenic group, G7 =100µg/kg of ghrelin + atherogenic diet, G8 = atherogenic diet + = 100µg/kg of ghrelin, G9 = 300µg/kg of ghrelin + atherogenic diet, G10atherogenic diet + 300µg/kg of ghrelin

Effect of atherogenic diet and concentrations of methanolic extract of *M. oleifera* leaves on Comparison the effect of *M. oleifera* with ghrelin on lipid profile

The results in figures 1, 2, 3, 4and 5 are indicated no significant differences (P≤ 0.05) between the effects of extract of *M. oleifera* and hormone on content of cholesterol, TG, HDL, LDL, and VLDL either as protective mean (165, 243, 57, 59.4, and 48.6) , (167.8, 247.6, 53.4, 61.4, and 49.52) respectively, or as treatment mean (145, 232.2, 64.2, 34.36, and 46.44), (151.4, 238.8, 61, 42.64, and 47.76.

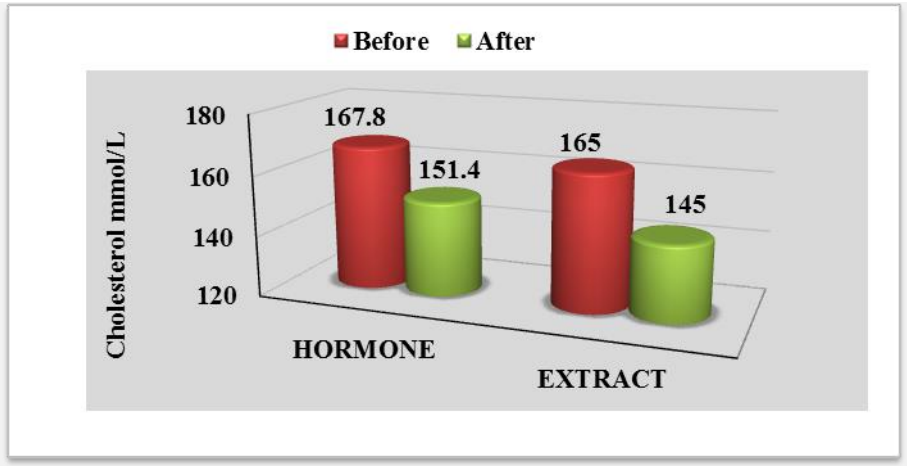

Figure 1: Comparison between the effect of *M. oleifera* and ghrelin on cholesterol level (Significant difference ≤0.05)

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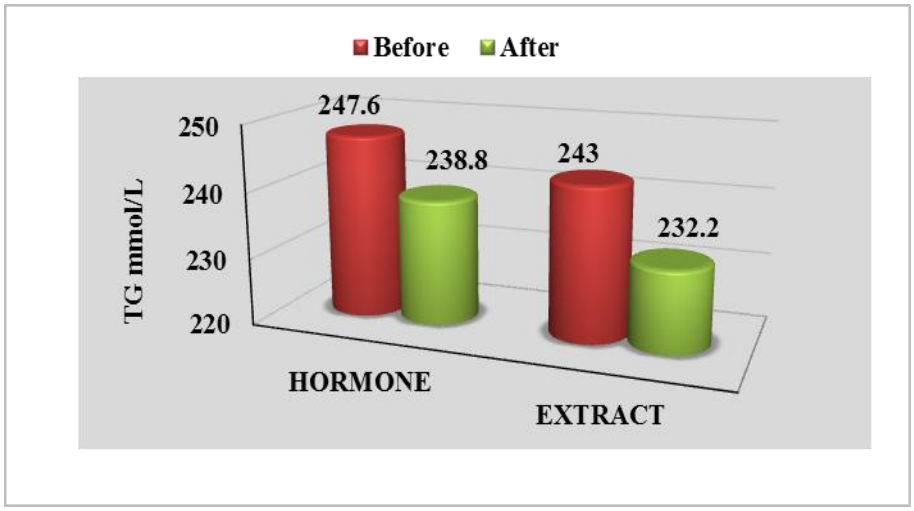

Figure 2: Comparison between the effect of *M. oleifera* and ghrelin on TG level (Significant difference ≤0.05)

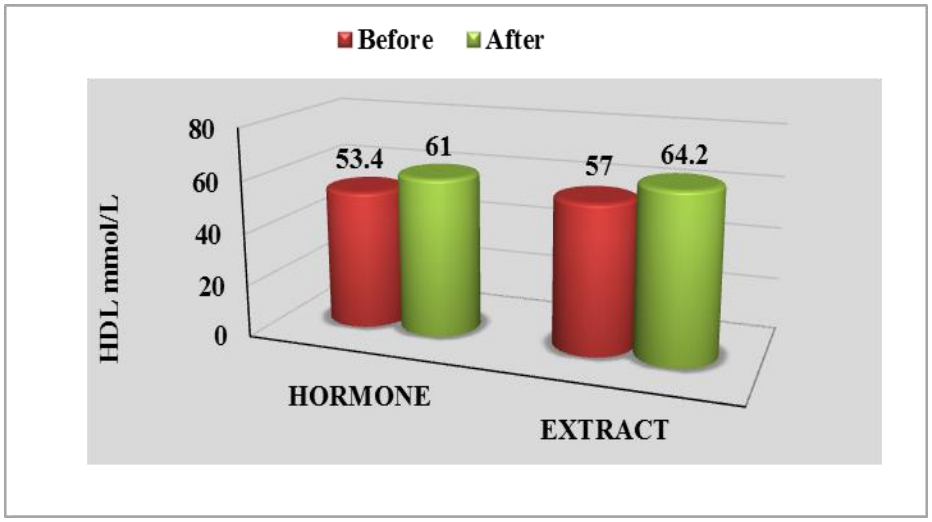

Figure 3: Comparison between the effect of *M. oleifera* and ghrelin on HDL level (Significant difference ≤0.05)

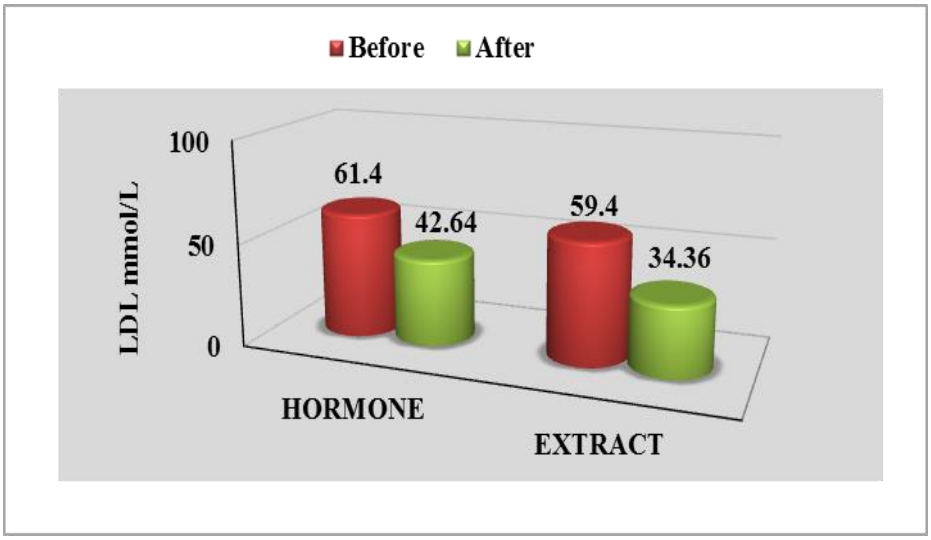

Figure 4: Comparison between the effect of *M. oleifera* and ghrelin on LDL level (Significant difference ≤0.05)

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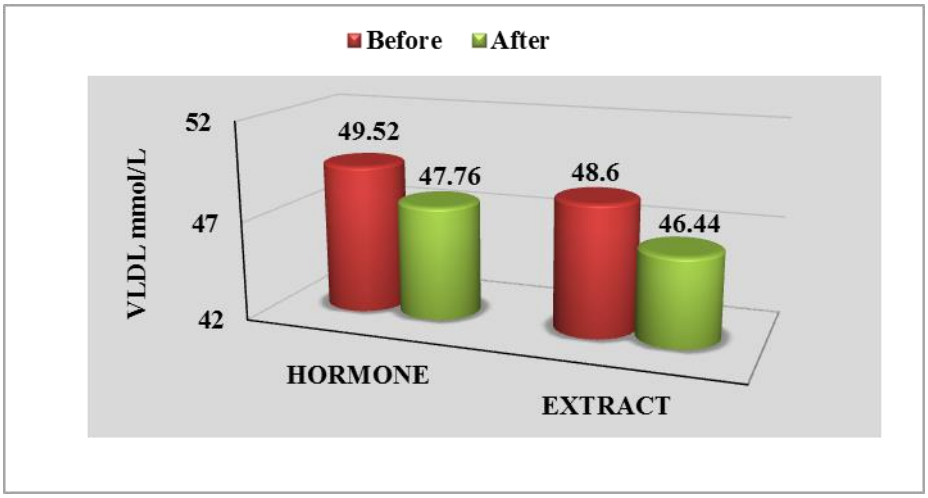

Figure 5 Comparison between the effect of *M. oleifera* and ghrelin on VLDL level (Significant difference ≤0.05)

Comparison the effect of *M. oleifera* with ghrelin on body weight

The results in figure 6 are indicated no significant differences (P≤ 0.05) between the effects of extract of *M. oleifera* and hormone on the body weight either as protective mean (276.2)and (281.4) respectively, or as treatment mean (273.6) and (280.2).

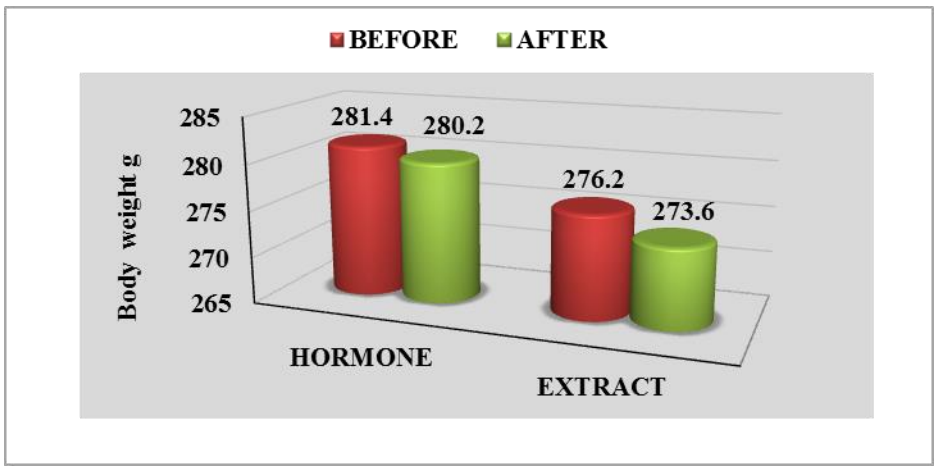

Figure 6: Comparison between the effect of *M. oleifera* and ghrelin on body weight (Significant difference ≤0.05)

DISSCUSION

The results in table 2 revealed significant elevation in serum levels of total cholesterol, triglyceride, low density lipoprotein, very low density lipoprotein, accompanied with significant decrease in serum high density lipoprotein level in animals fed on atherogenic diet in comparing to control group. These results are in accordance with Adekunle et al., [16] who measured the total cholesterol and triglyceride concentrations in rats fed on high fat diet and their results showed hypercholesterolemia and triglyceridemia. These High levels of serum total cholesterol, TG, LDL-C, and VLDL-C have been attributed to increased amount of fat in the diet [17]. Lipid hypothesis states, hypercholesterolemia are associated with higher serum concentration of LDL and lower serum concentration of HDL as well as high level of TG with cardiovascular disease where it promote arthroma development in arteries [18].

Use of methanolic extract of *M. oleifera* leaves and administration of ghrelin hormone cause significant decrease in serum total CHO, TG, LDL-C, and VLDL-C levels and significant increase in serum HDL-C level as indicated in the results of table 2 and 4 respectively, these results agree with Denen et al ., [19] who

suggested the potent activity of *M. oleifera* and its importance in preventing the atherosclerosis . One safe way which provides protection against atherosclerosis with no or less side effects is by using the medicinal plants with hypolipidimic activity. The hypolipidemic activity of many medicinal plants is may be achieved by one or more than one of the following mechanisms: decreased energy intake, decreased lipid absorption, decreased pre-adipocyte proliferation and differentiation, increased energy expenditure, and increased lipolysis and decreased lipogenesis [3].

This study aim to reveal the role of *M. oleifera* leaves extract as anti-obesity treatment and show its hypolipidemic activity in comparing to exogenous ghrelin.

Dongmeza and co- workers revealed that the ingestion of higher level of *M. oleifera* extract or its fractions of saponins and tannins have been correlated with decreased energy important for protein and lipid biosynthesis which cause lower growth performance and utilization of nutrient. By this clarifying the *M. oleifera* has the ability to decrease body lipids and cause energy retention [20].

Homeostasis of cholesterol is maintained by two processes: the first one that biosynthesis of cholesterol in which HMG-Co- A reductase stimulate the rate limiting step and cholesterol absorption of both, the cholesterol removed from the liver via biliary secretion and dietary cholesterol. The ratio of HMG-Co-A/ mevalonate has anegative relationship to the activity HMG-Co-A reductase [21].

The leaves of *M. oleifera* extract contain bioactive components: phenolic compounds, tannins, alkaloids, glycosides, and flavonoids (Kassa and Mesay, 2014) [22]. These bioactive components also responsible of the hypolepdemic activity of medicinal plants as many of previous studies indicated, such that the hypolipidemic activity of alkaloids which may achieved through the up-regulation of the activities of lipolytic enzymes or by promoting the excretion of fecal bile acid [23]. The leaves of *M. oleifera* contain large amounts of bioactive phytoconstituents β-sitosterol as reported by previous studies [24]. β-sitosterol is a plant sterol has similar structure to that of cholesterol with exception of replacement of an ethyl group at C24 of its side chain. β-sitosterol have the ability to reduce the cholesterol by taking the place of dietary and biliary cholesterol in micelles produced in the lumen of intestine, thus it decrease the cholesterol absorption in the body [25]. And the β-sitosterol will absorbed instead of cholesterol and remove it outside the body with faeces in form of neutral steroids.

M. oleifera leaves are a good source of neutral antioxidant, where it contains many types of antioxidant compounds such flavonoids, phenolic compounds, ascorbic acid, and carotenoids [26] thus the *M. oleifera* leaves extract increase the level of HDL-C due to preventing the oxidation of LDL-C. HDL-C has anti-atherogenic properties.

Another suggested mechanism in lowering the lipid by *M. oleifera* extract is through binding it to bile acid in intestine and thus prevent its reabsorption. When the pool of bile acid become eliminated, the liver enzyme, cholesterol 7-α- hydroxylase, is up-regulated increasing the cholesterol conversion to bile acids, this result in enhanced requirement to cholesterol in hepatocytes producing increased effect of activity and transcription of enzyme of cholesterol biosynthesis, 3- hydroxyl-3- methyl-gutaryl Co enzyme A reductase, and increase the receptor numbers of hepatic LDL, thus lead to increased removing of LDL-C from the blood , and decreased levels of serum LDL-C [27].

The results of this study also shown observed reduction in the level of triglyceride in rats administered *M. oleifera* leaves extract, the hypotriglyceridemia effect of *M. oleifera* may be attribute to reduced hepatic TG synthesis [28], increased catabolism of LDL [29], and inhibition of acetyl-CoA carboxylase [30].

The results in table 4 show revealed a significant decrease in the levels of serum CHO, TG, LDL-C, and VLDL-C and significant increase in serum HDL-C level in animals administered ghrelin hormone.

Ghrelin hormone play important role in growth hormone secretion and appetite regulation, the thing that made the scientists plane to use it in preventing and treating obesity [31]. The way by which the ghrelin regulate the appetite is by binding to growth hormone secretagogue receptor (GHS-R) which is receptor coupled to G protein (GPCR) and has important role in the stimulation of growth hormone secretagogue

receptor 1 a (GHS-R1a). This elevates the intracellular Ca+2 concentrations via inositol 1, 4, 5-triphosphate (TP3) signal transduction. High levels of calcium lead to releasing the growth hormone from pituitary [32].

Ghrelin also perform the appetite regulation effect by acting on the hypothalamus to stimulate the Neropeptide Y (NPY) releasing which is the most potent recognized orexigen and agouti-related protein (AgRP) expressing neuron , and suppress the release of pro-opiomelanocortin (POMC) neuron [33]. Thus the role of ghrelin in treatment and prevention obesity is indirect and largely mediated by its effect on GH secretion.

A previous study done by Álvarez-Castroet al., [34] revealed that the GH secretion in obese individuals increase slightly by growth hormone releasing hormone (GHRH), but the significant increase in its secretion occur by administration of either ghrelin alone or in combination with GHRH. GH may have direct effects on lipid and lipoprotein metabolism. Previous study by Attanasio et al [35] compared through which between the levels of plasma lipid and lipoprotein in growth hormone deficient adults with age matched healthy one, the results revealed increased concentrations of total cholesterol (TC), TG, LDL-C, VLDL-C and low level of serum HDL-C.

Fasting ghrelin levels in obese individuals have been found to be lower in comparing to normal individuals, and increase following weight loss induced by diet [31]. Low levels of ghrelin in obese individuals seem to be correlated with high fat which may results in central resistance to ghrelin.

The results in table 3 indicated a significant increase in body and organs weight in group of rats with atherogenic diet induced obesity when compared to control group, these results in accordance with [36]. Increased weight of body and studied organs can ascribed to feeding on hypercoloric diet which represent the etiology of obesity and its best and multiply its pathological characteristics [37]. 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 [38].

Previous study done by Souravh et al.,[39] revealed no significant differences in the daily intake of food of animals although there was a significant difference in body weight between normal diet and high fat diet groups. Their observation gives guide that an increase in body weight is independent of consumed food amount by animals.

The results in table 3 indicated a significant decrease in body and organs weight in rats given extract of *M. oleifera* leaves, This results in accordance with [40] . The reduced body weight by extract of *M. oleifera* leaves may be ascribed to inhibition of cholesterol deposition in body tissues or inhibition of HMG-CoA reductase activity [41]. Previous study by Ijeoma et al., [42] demonstrated that the extract of *M. oleifera* in addition to hypolipidemic activity, it has effect on appetite and daily feed consumption where caused reduction in both of them when compared to rats fed on high fat diet only.

The body and organs weight also significantly decreased by exogenous ghrelin hormone administered into rats, as indicated in the results of table 5. These results in accordance with [43]. Previous study done by Tomomi et al., [44] also reported that the plasma concentrations of ghrelin are negatively correlated with body mass index in individuals with normal weight, obese individuals, patients with anorexia nervosa and type II diabetes mellitus.

In conclusion the results revealed approximately equal effects of *M. oleifera* leaves extract and ghrelin hormone and without significant differences between them in its hypolipidemic effect and reducing body weight.

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