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Effect of Moringa oleifera Extract on blood Levels of Lipocalin-2 and Omentin Parameters in High Fat Diet-induced Obesity in Rats.

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ABSTRACT

The present study was carried out to investigate the efficacy of Moringa Oleifera (MO). Forty Wistar rats with body weight (150-200gm) and were randomly divided into four (4) groups and treated for 12 weeks: group I- rats treated with normal diet (Control), group II: Obese group received HFD for 12 weeks, group III `- Obese group rats treated with HFD for 12 weeks then received with MO extract orally in a dose of 600 mg kg^{-1} b.wt, and group IV: Obese group treated with HFD for 12 weeks then received with simvastatin at a daily dose for 12 weeks of 5 mg kg^{-1} b.wt .Blood samples were collected for studying biochemical changes (Lipocalin-2 and omentin) associated with the use of MO extract on HFD -induced diabetic rats. The present study indicated that administration of high fat diet in rats led to an significant increase in body mass index (BMI) in t that is in group II , compared to obese group. On the other side, in group III &group IV treatment with MOE and Simvastatin resulted in significant decrease in BMI compared to HFD group. There was significant increase in the levels of serum Lipocalin-2 (172.6 ± 1.73ng/µl)whereas significant decrease omentin-1 (20 ± 0.57 pg/ml) in the HFD animals when compared to normal fed rats(63.94 ± 2.19 ng/µl), and(38.15 ± 1.071 pg/ml).On the other hand the levels of Lipocalin-2 decreased and omentin-1 (20 ± 0.57 pg/ml increased in the obese group following MO(118.5 ± 1.21 ng/µl), (30.62 ± 0.94pg/ml) and Simvastatin treatment(116 ± 1.83ng/µl), (31.70 ± 0.871 pg/ml) respectively. The results indicate that MO has anti-obesity effects in an experimental mouse model of high-fat-diet-induced obesity.

1. Introduction

Obesity is defined as abnormal or excessive fat accumulation due to multifactorial conditions, involving psychological, biochemical, metabolic, anatomic and social alterations, it's a chronic, and serious public health problem because of its prevalence, cutting across all age groups, sex and race. Worldwide about 1.9 million adults are overweight and 600 million of them are clinically obese[1-3).Although several available anti-obesity drugs, including orlistat, rimonabant, and sibutramine, have been approved as a therapeutic strategy to combat obesity[4), the development of new therapies from plants that are able to control obesity is of great interest[5).

Moringa oleifera Lam. (M. oleifera) belongs to an'on-generic' family Moringaceae. It is commonly called drum Stick tree, which is a widespread growing plant in tropical and subtropical areas. Phytochemical analyses have shown that *MO* has been recognized as containing a great number of bioactive compounds, which are rich in potassium, calcium, phosphorous, iron, vitamins A, B,E, C and D, essential amino acids, β-carotenoids, polyphenols, phenolic acids, flavonoids, alkaloids, glucosinolates, isothiocyanates, tannins and saponins [6,7]. It is exhibited antioxidant, antibiotic, hypotensive, anti-ulcer, anti-inflammatory and anti-cancer properties[8].

Simvastatin belongs to statin which works by inhibiting 3-Hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase inhibitors the master enzyme of the cholesterol synthesis pathway, act by inhibiting in a competitive manner the conversion of HMG-CoA to mevalonic acid. Its increase the expression of LDL receptors in the liver, increasing LDL catabolism and lowering total cholesterol causing a reduction of LDL and TG and an increase of HDL[9- 11].

Adipose tissue is an active endocrine organ that secretes various bioactive mediators, the so-called adipokines. These factors signal to several organs including liver, skeletal muscle, brain, and the immune system, modulating lipid and glucose metabolism as well as inflammation[12-14].

Lipocalin-2 (LCN2), also known as neutrophil gelatinase-associated lipocalin (NGAL), are bioactive peptides that belong to a novel adipokines, a 25-kDa secretory glycoprotein was first identified in activated neutrophils[15].

Omentin, also named intelectin-1, is a novel adipokine and a Ca^{2+}-dependent galactofuranose-binding lectin, consisting of a 313-amino acid peptide , preferentially produced by visceral stromal adipose tissue., but not by mature adipocytes , its expression and production are modified in several pathological situations, such as obesity and insulin resistance[16, 17].

The aim of the present investigation was to evaluate the influence of Moringa oleifera ethanol extract on serum Lipocalin-2in rats fed on high fat diet.

2. Material and Methods

Moringa oleifera leaf extract was used in this study after collected from Shorja , Baghdad City, Iraq, Then the leaves were shade-dried by spreading over a sheet of paper under a ceiling fan for five days. The dried leaves were ground an electric mixer into fine powder and stored in clean sterile glass container. 50 mg of M. oleifera leaf powder/rat/day was administered with normal diet and high fat diet according to experimental design[18].

High-Fat Diet Formula: HFD that consists of 58% fat, 25% protein and 17% carbohydrate, lard (13%), cholesterol (1%), vitamin, and minerals (0.6%) as a percentage of total kcal ad libitum, respectively was administered [19].Food intake was calculated every day and bodyweight was measured once in every two days.

2.1 Experimental protocol

Forty healthy male Wistar rats ranging in weight from (150-200gm) were used in the study, housed in Faculty of Veterinary Medicine, Tikrit, University. Rats were kept in suitable circumstances, such as, temperature, humidity.

The animals were divided into four main groups (10 rats for each) as follows:

Group I: (NC) consisting of(10) rats treated with normal diet (Control).

Group II: Obese rats treated with HFD for 12 weeks and left untreated for the other 12 weeks,

Group III: Obese rats treated with HFD for 12 weeks then received with Moringa oleifera ethanolic extract orally in a dose of 600 mg kg b.wt[12].

Group IV: Obese rats treated with HFD for 12 weeks then received with Simvastatin at a daily dose for 12 weeks of 5 mg kg b.wt. (Mbikay,2012)[6].

The rats were weighed at the beginning and at the end of the experiment. At the end of study period, animals were weighed, fasted overnight, blood samples from each group were collected at weekly intervals. The obtained blood sample from each rat(retro-orbital venous plexus).The samples were centrifuged for 15 min. The serum was carefully harvested in dry clean Wasserman tubes using a Pasteur pipette and kept frozen until examination at -20 ^{o}C. Then determine concentration of Lipocalin-2 and omentin in serum by ELISA kit. The animals of different groups were sacrificed under with 60 mg/kg sodium pentobarbital intra peritoneal injection in other to ensure rapid and painless death of rats.

2.2 Statistical analysis

All data were analyzed with one-way ANOVA (SPSS 19.0, USA) followed by Tukey's multiple comparisons test. $P \leq 0.05$were considered as significant.

3. Results & Discussion

High-fat-diet feeding is critical for obesity development because excess calorie intake can promote the development of a positive energy balance followed by increased visceral fat deposition leading to abdominal obesity.

The data revealed that there is significant increase in the level of lipocalin-2 and BMI in obese group (7.21±2.16kg/m^2) , and (172.6 ± 1.731 pg/ml) respectively (P <0.05) with respect to the lean control group(4.35±1.05kg/m^2) , (63.94 ± 2.19 ng/µl)respectively. On the other hand, there is significant decrease in lipocalin-2 (P <0.05) of obese groups treated with Moringa oleifera leaf (5.3±1.74 kg/m^2) ,and (30.62 ± 0.94pg/ml) respectively , or simvastatin (4.69±1.55b kg/m^2), (116 ± 1.83 pg/ml) versus the HFD group (7.21±2.16a kg/m^2), (20 ± 0.57 pg/ml) respectively as illustrated in Table (1).

Table 1: The Body Mass Index, Lipocalin-2 and Omentin-1 values

Parameters	Normal diet	HFD	Ob+M. oleifera	Ob+Sim
Body Mass Index (kg/m^2)	4.35±1.05	7.21±2.16a	5.3±1.74	4.69±1.55b
Lipocalin-2 (ng/µl)	63.94 ± 2.19	172.6 ± 1.73	118.5 ± 1.21	116 ± 1.83
Omentin-1 (pg/ml)	38.15 ± 1.071	20 ± 0.57a	30.62 ± 0.94^b	31.70 ± 0.871^b

a, b mean significant differences at the $P \leq 0.05$

The present study showed that the Wistar rats fed HFD had significant greater body weight than normal fed rats , This increase might be due to the consumption of a diet rich in energy in the form of saturated fats and its deposition in various body fat pads and decreased energy expenditure. These observations are similar to previous

reports that demonstrated higher BMI in rats fed HFD for 8 weeks as compared to the control group [21]. Also this study indicated that simvastatin, and dietary MOE was capable of reducing body weight in an HFD-induced of obesity which suggests that supplementation are capable of preventing body weight gain, concomitantly helping in maintaining the current body weight, may be due to the inhibition of dietary lipid utilization. This result is consistent with previous investigations Adedapo [22], and Dongmeza and co-workers[21],indicated that a higher inclusion level of moringa extract or its fractions such as saponins and tannins have been associated with the reduced energy required for protein and lipid biosynthesis leading to lower growth performance and nutrient utilization. Therefore, moringa has the ability to reduce body lipid and consequently energy retention.

Lipocalin-2 is secreted by adipocytes and immune cells such as neutrophils and macrophages which induced by granulocyte macrophage colony-stimulating factor (GM-CSF) via many pro- and anti-inflammatory cytokines and factors such as lipopolysaccharide (LPS), tumor necrosis factor-α (TNF-α), interleukin(IL)-1β, IL-6 or IL-17 in a variety of cell types[23-25]. Lipocalin-2 implicated in several cardio-metabolic disease states including obesity, inflammation and insulin resistance[26].

The results in table revealed significant increase in levels of serum LCN2 when the obese group treated with Simvastatin and methanolic M. oleifera leaf extract. There are no previous researches in this field. The probable cause is the presence of active compound such as flavonoids.

In contrast, there is significant decrease in serum omentin-1 levels ($P<0.05$) in obese group (20 ± 0.57a pg/ml)when compared with the lean control group(38.15 ± 1.071pg/ml). While, significant elevation ($P<0.05$) in serum omentin-1 level was detected in obese groups treated with the ethanolic extract of Moringa oleifera or Simvastatin (30.62 ± 0.94 pg/ml), (31.70 ± 0.871pg/ml) respectively when compared to the HFD group.

Omentin-1 exhibits powerful anti-inflammatory properties and plays a significant role in modulating insulin sensitivity by paracrine and endocrine factor where it enhances the insulin sensitivity and glucose metabolism on the local level of omental adipose tissue, as it increases the insulin transduction by activating the Akt protein kinase B in both visceral and subcutaneous adiposity and down regulating tumour necrosis factor (TNF)-α-induced expression of endothelial adhesion molecules and TNF-α induced cyclooxygenase-2 expression. This indicates that omentin-1, like adiponectin, may act as a protective adipokine[27-30].

Our data recorded significant depletion in the serum level of Omentin-1 in the HFD group treated with ethanolic extract of Moringa oleifera. Our findings could be attributed to that that insulin and glucose significantly decrease the omentin mRNA expression and omentin protein production in vitro omental adipose tissue therefore hyperinsulinemia leads to decrease the circulating omentin-1 level significantly in normal subjects and this lead insulin and glucose play a role in the regulation of omentin-1 synthesis either directly or indirectly[31,32].

Treatment of obese groups with the ethanolic extract of Moringa oleifera resulted in a significant elevation in Omentin-1 in comparison with the obese group. Moringa oleifera possesses anti-inflammatory capacity and it can inhibit the level of TNF-α. This may be due to presence of the anti-inflammatory compounds in Moringa oleifera namely 4-[(2′-O-acetyl-α-l-rhamnosyloxy) benzyl] isothiocyanate , 4-[(3′-O-acetyl-α-l-rhamnosyloxy)benzyl] isothiocyanate and S-methyl-N-{4-[(α-l-rhamnosyloxy) benzyl]} thiocarbamate. Thus, Moringa oleifera extract

may elecit an improvement in the omentin serum level in obese rats via inhibition the TNF-α level[33].

Simvastatin can reduce the expression of these cytokines and adipokines ,whereas enhancing anti-inflammatory adipokine expression and secretion by adipocytes by up regulating PPARγ expression in adipocytes[34]. Wang et al [35]. reported that statins able to decrease inflammation in pericarotidal AT from high-fat diet- (HFD-) treated mice and down regulation of pro-inflammatory adipokines /cytokines.

Conclusion

OM suppressed the increases in body weight observed with a high-fat-diet-induced model of obesity. It also significantly reduced the serum levels Lipocalin-2 and increased omentin-1.

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تأثير مستخلص المورينجا أوليفيرا على مستويات ليبوكالين 2 و 2 وأومنتين في جرذان التجارب المسمنة بغذاء عالي الدهون

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الملخص

أجريت الدراسة الحالية للتحقق من فعالية المورينجا اوليفيرا . تم تقسيم أربعين جرذا بوزن جسم (150-200 غرام) بشكل عشوائي إلى أربع (4) مجموعات وعولجت لمدة 12 أسبوع: المجموعة الأولى – الجرذان التي عولجت بنظام غذائي عادي (مجموعة السيطرة) ، المجموعة الثانية: مجموعة الجرذان التي تلقت غذاء عالي الدهن لمدة 12 أسبوع ، المجموعة الثالثة مجموعة الجرذان التي عولجت بـغذاء عالي الدهن لمدة 12 أسبوع ثم تلقت المستخلص الإيثانولي للمورينجا اوليفيرا عن طريق الفم بجرعة 600 ملغم لكل كغم من وزن الجسم ، والمجموعة الرابعة: مجموعة الجرذان التي عولجت بـغذاء عالي الدهن لمدة 12 أسبوع ثم تلقت سيمفاستاتين في جرعة يومية 5 مغم لكل كغم من وزن الجسم لمدة 12 أسبوع جمعت عينات الدم لدراسة التغيرات البيوكيميائية (ليبوكالين 2 وأومنتين) المرتبطة باستخدام مستخلص (MO) على الجرذان المصابة بالسمنة . وأشارت الدراسة الحالية إلى أن اتباع نظام غذائي عالي الدهون في الجرذان أدى إلى زيادة معنوية في مؤشر كتلة الجسم في المجموعة الثانية ، مقارنة مع مجموعة السيطرة . من ناحية أخرى ، في المجموعة الثالثة والمجموعة الرابعة ، أدى العلاج باستخدام (MOE و Simvastatin) إلى انخفاض معنوي في مؤشر كتلة الجسم مقارنة بمجموعة المعالجة. كانت هناك زيادة معنوية في مستويات ليبوكالين -2 في المصل (172.6 ± 1.73 نانوغرام / ميكرولتر) بينما انخفاض معنوي في أومنتين -1 (20 ± 0.57 بيكوغرام / مل) في المجموعة غذاء عالي الدهن بالمقارنة مع الجرذان التي تتغذى بشكل طبيعي (63.94 ± 2.19 نانوغرام / ميكرولتر) ميكرولتر) و (38.15 ± 1.071 بيكوغرام / مل) ومن ناحية أخرى انخفضت مستويات ليبوكالين 2 وزاد أومنتين 1 (20 ± 0.57 بيكوغرام / مل في المجموعة البدينة التي تلي 1.21 ± 118.5) MO نانوغرام / ميكرولتر) ، (30.62 ± 0.94 جالون / مل) وسيمفاستاتين (116 ± 1.83 نانوغرام / ميكرولتر) ، (31.70 ± 0.871 بيكوغرام / مل) على التوالي. وتشير النتائج إلى أن MO له تأثيرات مضادة للسمنة في النموذج التجريبي للجرذان المسمنة بغذاء عالي الدهون التي يسببها النظام الغذائي