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Hypolipidemic potential of Moringa extract comparing with simvastatin in rats

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ABSTRACT

Background: Overweight and obesity are hazardous for public health and complex disease caused by the interaction of complex combination of genetic, nutritional, lifestyle, and environmental factors favoring a long-term positive energy balance and resulting in increased body fat accumulation. **Aim of the work:** To investigation the effect of Moringa oleifera extract as hypolipidemic agents combined with the commercial drug, simvastatin in obese rats. **Material and methods:** 35 male Wistar rats were assigned to five groups fed various diets as follows: a standard diet group, HFD group, HFD with Moringa oleifera extract (300 mg/kg body weight), HFD with Simvastatin (40 mg/kg body weight) and HFD group with Simvastatin with Moringa oleifera extract. After 10 weeks after the period of treatment, the body weight and lipid profile were measured from serum. **Result:** The administration of Moringa oleifera extract plus simvastatin to HFD rats maximize the efficacy of Simvastatin caused a significant reduction body weight through the period of experiment showed decrease total cholesterol, Triglyceride, LDL and increase HDL in serum. **Conclusion:** Our findings provided empirical evidence for the traditional use of Moringa oleifera extract as an alternative treatment for obesity and maximize the efficacy of Simvastatin when used in combination with each other.

Introduction:

One of the biggest epidemic problems in the world nowadays is obesity, which is thought to pose a severe threat to human health. Obesity contributes to atherogenic dyslipidemia and, over time, is implicated in the pathophysiology of additional metabolic syndrome components such as insulin resistance, hypertension, and abdominal obesity, depending on the

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consumption of high-cholesterol fast food [1].

The prevalence of metabolic disorders, especially obesity, has sharply increased globally [2].Obesity is predisposed to by the Western diet (i.e., a high-calorie, high-saturated-fat diet) [3].

Obesity is described as abnormal or excessive deposit of fat that presents a risk to health [4]. Contrary to popular belief that obesity is just a risk factor for diseases, the World Obesity Federation has proclaimed obesity to be a chronic, relapsing progressive disease [5].

Observational data from multiple research suggests that a subset of obese people may be protected from obesity-related cardiometabolic disorders or have a considerably lower risk than the positive relationship between BMI and cardiometabolic risk suggests. Despite substantial body fat accumulation, this subphenotype has been labelled MHO and is characterised by the absence of cardiometabolic problems such as insulin resistance, poor glucose tolerance, dyslipidemia, and hypertension [6].

Currently prevalent worldwide, hyperlipidemia is a metabolic and endocrine condition that includes faulty lipid metabolism and difficulty transporting fat [7]. The problem of triglycerides (TG) and cholesterol is the predominant symptoms of hyperlipidemia [8-9]. A high-cholesterol diet and sedentary lifestyles have contributed to the rise in the number of individuals with hyperlipidemia in recent years, both of which have adverse impacts on people's quality of life [10]. Currently, The medications niacin, cholic acid binding resin, and simvastatin are used for treating hyperlipidemia, among others. Yet due to their multiple hazardous adverse effects, these medicines are not recommended for long-term or high-dose use [11]. As a result, developing novel natural nutrients for the management of hyperlipidemia that have asymptomatic side effects is preferable as Zingiber officinale , Moringa oleifera and Phoenix Dactylifera that having the ability to restore normalized hepatic tissue, antioxidant state, and serum biochemical parameters in obese male rats [12].

Medicinal plants have long been used to treat a variety of diseases as well as to help people lose weight and avoid the difficulties that come with obesity [13]. There are promising reports of different antiobesogenic phytochemicals [14-15-16-17].

The ethnopharmacological and ethnobotanical methodologies used to assess the therapeutic value of plants provide a solid foundation for selecting plants to be further evaluated in the development of innovative phytotherapeutic medications [18].

Moringa oleifera Lam. (drumstick tree) belongs to a family called Moringaceae of the angiosperm plants [19]. Antioxidants are found in moringa oil extract (vitamin C, flavonoids, and phenolics) [20]. The plant's leaves, blooms, immature pods, seeds, bark, and roots have all been utilised as food and medicine for a variety of conditions [21-22]. The hypolipidemic and antiobesity effects of M. oleifera extracts have been discovered in animal investigations utilizing aqueous, alcohol, or hydroalcohol extracts [23]. Flavonoids, phenolics, and carotenoids are abundant in the plant's leaves, all of which have biological actions that are advantageous to metabolic health [24].
Antibiotic, hypotensive, anti-ulcer, anti-inflammatory, and anti-cancer activities were found in various M.

oleifera preparations [17]. For ages, The M. oleifera tree's edible leaves have been utilised as a diabetic meal [24]. M. oleifera leaf aqueous extract showed significant anti-oxidant and anti-diabetic properties [25]. Furthermore, in rats with induced obesity, a M. oleifera leaf extract of methanol reduced dyslipidemia and gain in weight [26]. In obese mice, an ethanolic extract of M. oleifera leaves was recently found to have hypocholesterolemic and antioxidant properties [27].

Because of its natural antioxidant compounds, such as vitamins and carotenoids, the leaves of *M. oleifera* have medicinal potential [25]. The advantageous pharmacological effects of M. oleifera consist of anti-inflammatory, wound-healing, diuretic [28], antifungal [29] and antioxidant [30] activities.

Marketed under the brand name Zocor, simvastatin is a member of the statin drug class, which is used to lower blood cholesterol in a number of disorders [31]. Significant anticoagulant and endothelial cell protective effects were observed with simvastatin [32]. Among the many adverse effects of simvastatin are rash, headaches, and gastrointestinal issues [33], and A number of these side effects pose a serious risk to the patients' lives and health [34].

Material and Methods:

Plant material and preparation of the extract:

Leaves of *M. oleifera* were obtained from ISIS Company, Simvastatin was purchased from Global Napi Pharmaceuticals, Egypt. Cholesterol was purchased from Sigma chemical company.

The leaves of M. oleifera were washed, dried, and ground. For three days at 4 °C, the 40 g of leaf fragments were macerated in 70% ethyl alcohol while being constantly stirred. After filtering and lyophilizing the extract, a solid powder was produced and stored at 4 °C. The dose in this trial was 300 mg/kg, which was administered orally after being combined with distilled water [35].

Animals:

Thirty-five mature male Wistar rats weighing between 115 and 120 grammes were acquired and housed in the Laboratory Animal House of Zagazig University in Egypt's Faculty of Medicine. The animals were kept in standard laboratory settings with stainless steel cages and exposed to 12-hour light/dark cycles. Rats were kept at ambient temperature, exposed to the natural light cycle, and given libitum control diets for one week prior to study in order to facilitate adaptation and acclimatisation. Five groups of seven rats each—each consisting of four rats housed in a cage—were randomly assigned to the animals. Rats were housed at a temperature of 24 ± 2 °C and a relative humidity of $60 \pm 5\%$ for the duration of the 20-week experiment. The experimental protocol was reviewed and approved by ZU-IACUC committee (protocol No. ZU-IACUC/1/F/20/2020).

Experimental design:

The rats were splitted into seven groups as follows:

Group I (ND); received control diet and water ad libitum during the whole experimental period (10 weeks).

Group II (HFD); received high fat diet during the whole experimental period (10 weeks).

Group III (SIM); received high fat diet for and were given (40 mg/kg p.wt) Simvastatin daily by oral gavage for the 6 weeks [36]

Group IV (MOR); received high fat diet for and were given (300 mg/kg

p.wt) Moringa olifera extract daily by oral gavage for the 6 weeks [37]

Group V (SIM + MOR); received HFD and combination of simvastatin with *Moringa olifera extract* daily by oral gavage for the full six weeks at the same dosages and administration schedules as the other groups.

The HFD consisted of 2% cholesterol, 30% animal fats, and 68% regular chow [36]. Using a computerised weighing balance, body weights were taken on the first day of the experiment and then once a week for the next six weeks.

Sample collection

-Tissues homogenate:

Rats were measured for final body weight at the conclusion of the experiment. Following an overnight fast, the rats were put under anaesthesia and blood samples were taken from each rat's retro-orbital plexus while they were sedated with diethyl ether. In order to separate serum for the Lipid Profile, the first blood sample was taken and placed in a simple tube. THF overdosage resulted in the death of the animals.

Statistical analysis:

Data were expressed in tables and figures as mean (M) ± standard deviation (SD). The experimental results were analyzed statistically using SPSS version 20.0. One-way ANOVA was carried out to test the significant differences among all experimental groups. Moreover, Student t test was performed to determine the significant difference between control group and the rest of experimental groups and all differences were considered statistically significant when $p \leq 0.05$. The graphs of genes expression were created using GraphPad prism software (version 8).

Results

The Effect of Moringa Extract and SIM on body weight

Table 1 showed that the percentage of rats' body weight that changed from their starting weight among test groups. The HFD feed rats that obtained a combination of SIM and MOR had the greatest impact on body weight.

The effect of Moringa Extract and SIM on Lipid Profile Test

Based on data shown in tables 2 and 3, it was noted that *MOR* in combination of SIM has the best effect on lipid profile as when used individual highly significantly decrease cholest. by 52.38% and T.G by 32.18% ($p \leq 0.001$) compare to HFD-feed rats group but the best effect showed in combination with SIM as highly significantly deceased cholest. by 53.96% and T.G by 42.73% ($p \leq 0.001$).

Discussion

No pharmaceutical intervention produces a satisfactory reduction in weight with negligible side effects. Thus, the goal of the current study is to investigate how extract from Moringa olifera leaves may help treat metabolic abnormalities brought on by obesity. Rats were made obese in the current study using a high-fat diet in order to assess the effects of an ethanolic extract of Moringa olifera leaves on several parameters.

Over the past few years, the usage of natural treatments to manage obesity has expanded. This is brought on by the growing population, the high expense of medical care for common illnesses, the negative effects of many modern medications, and the emergence of drug resistance.

The nature of Moringa oleifera as an antioxidant is a direct possible mechanism for explaining the therapeutic action of M. oleifera in

improving the observed metabolic derangements. Moringa oleifera includes phenolic components such as quercetin, isoquercetin, kaempferol and rutin, as well as vitamins, minerals, amino acids, carotenoids, alkaloids, and flavonoids. Moringa oleifera's glucose-lowering action was attributed to these active components [25], reduced atherogenesis, hypolipidemic activity, and cardiovascular problems in the pre-clinical report as well [37].

Hyperlipidaemia is a characteristic of obesity pathogenesis, and it is defined by hypertriglyceridemia and hypercholesterolemia, as well as a reduction in HDL levels. Hyperlipidemia is considered to be a major risk factor for cardiovascular diseases including atherosclerosis [24].

The current investigation has shown that the lipid profile of HFD-fed rats was considerably different from that of the HFD-fed rats group, leading to dyslipidemia and elevated glucose levels. These effects were also found by Liu, C., et al. ,2018. [7]

The combination of extract of Moringa with Simvastatin showed maximize the efficacy of SIM when used alone as when used Mor extract with SIM reduced T.C and T.G and LDL highly significant decreased ($p \leq 0.001$) and highly significant increased HDL level ($p \leq 0.001$) this effect agree with the result that reported from [38] this result as that reported in [36] that showed better effect when used combination Moringa extract with Simvastatin. The current observations match with a study wherein administration of a crude extract of ME resulted in an increase in blood HDL levels and a decrease in LDL, triglycerides, and cholesterol levels. Based on these findings, MOR may be a helpful treatment for dyslipidemia and its atherogenic effects. It may also offer some protection against hypertension and cardiovascular illnesses.

Similar results were also shown in [36], who showed that In a rat model of HFD, MOR leaves moderated the decreased insulin sensitivity and dyslipidemia.

Our findings showed that rats given a high-fat diet for six weeks experienced a notable increase in body weight, indicating that they were obese. One interesting approach that has been suggested is the use of plant extracts containing a variety of polyphenols to prevent obesity and related illnesses. In comparison to the HFD group of rats, the current study showed that Moringa Extract decreased body weight gain, ceased the increase in cholesterol, LDL, and TG, and boosted HDL.

Conclusion

The Extract of both *Moringa Oleifera* with combination with Simvistatin when combined with HFD, managed body weight gain is observed, indicating a decreased level of obesity. Improvements in liver function, glucose, serum lipid fractions, and metabolic hormones followed this response. The potential ameliorative mechanism associated with obesity may be ascribed to the antioxidant properties of the polyphenols found in MOR Extract. Enhancing redox balance pathways is an effective treatment approach for obesity and the negative effects that go along with it. So, the extract of *Moringa Oleifera* leaves provide an additive potential to Simvastatin as a hypolipidemic agent in HFD- fed rat.

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Table (1): The effects of *Moringa leaves extract* (MOR), simvastatin (SIM) and their combination on body weight gain (g) for 6 weeks. All values are means ± SD (n = 7).

Group/ weeks	Normal control	HFD	HFD + SIM	HFD + MOR	HFD + SIM+MOR
1st week	151 ± 4.3	240 ± 5.4	240 ± 4.6	240 ± 3.7	240 ± 4.3
2nd week	157 ± 3.2	257 ± 4.2	248 ± 4.1	250 ± 4.7	245 ± 3.3
3rd week	168 ± 3.9	275 ±6.1	253 ± 5.9	258 ± 4.2	250 ± 4.8
4th week	183 ± 4.3	296 ± 4.3	260 ± 4.6	267 ± 5.3	257 ± 4.1
5th week	195 ±3.5	319 ± 4.8	268 ± 3.8	275 ± 4.1	265 ± 4.6
6th week	200 ± 3.9	344 ± 5.1	272 ± 3.7	284 ± 3.7	270 ± 5.2
% of change from initial weight	32.4	43.33	13.3	18.33	12.50

Table (2): The effects of Moringa leaves extract (MOR), simvastatin (SIM) and their combination on Lipid for 6 weeks. All values are means ± SD (n = 7)

Group/ Parameter	Cholest. (mg/dl)	T.G (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Neg. Control	108 ± 4.9	65 ± 3.8	68 ± 2.8	26.2 ± 2.7	12.9 ± 3.7
HFD	314 ± 4.4	129 ± 3.27	37.2 ± 3.1	245 ± 6.8	26 ± 2.36
HFD+SIM	150^{***} ± 4.3	80^{***} ± 3.34	54.3^{***} ± 2.58	73.6^{***} ± 2.16	17.16^{***} ± 2.85
HFD+MOR	150^{***} ± 3.74	86.8^{***} ± 2.78	44.6^{***} ± 3.55	92.16^{***} ± 2.92	19.3^{***} ± 2.16
HFD+SIM +MOR	145^{***} ± 3.88	73.3^{***} ± 2.8	54.83^{***} ± 2.31	66.5^{***} ± 3.08	14.83^{***} ± 2.31

Superscripted stars indicate to the significant differences between test groups and control group, where (*) indicates to $p < 0.05$ mildly significant, (**) $p < 0.01$ significant, (***) $p < 0.001$ highly significant, NS = Non- significant. HFD (high fat diet), SIM (Simvastatin), Mor (Moringa oleifera), Cholest. (Cholesterol), T.G (Triglyceride), HDL (high-density lipoprotein), LDL (low-density lipoprotein), VLDL (very-low-density lipoprotein).

Table (3): Variations of the percent of change among the experimental groups compared to HFD control group

Group/ Parameter	Cholest. % change (decrease)	T.G % change (decrease)	HDL % change (increase)	LDL % change (decrease)	VLDL % change (decrease)
HFD+SIM	52.2	37.98	45.96	69.95	34.0
HFD+MOR	52.2	32.71	19.89	62.38	25.76
HFD+SIM +MOR	53.82	42.17	47.39	72.85	42.96

Percentage of change was calculated compared to HFD control group