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Synergistic Effectiveness of Crude Extract Leaves of Moringa Oleifera Lam and Atorvastatin-Treated Rabbits

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Abstract:
Moringa oleifera supplementation may help regulate hypercholesterolemic conditions in rabbits treated with atorvastatin, according to the data, which showed that the novel nutraceutical combination had a substantial influence on cholesterol metabolism. The purpose of this study was to examine the potential synergistic effects of Moringa oleifera in rabbits receiving atorvastatin treatment, which is commonly used to decrease cholesterol. Following a 28-day period of 1% cholesterol in rabbits, we gave them oral atorvastatin 50 gm/kg and/or Moringa oleifera Lam 200 mg/kg/day. In comparison to the corresponding control groups, the results demonstrated that dietary supplementation with Moringa oleifera Lam and atorvastatin significantly decreased blood levels of cholesterol and oxidant (MDA), liver enzymes (AST and ALT), and certain kidney function tests (urea, creatinine), while upregulating antioxidants. Our goal was to find out how the extraction of Moringa oleifera Lam demonstrated the plant's synergistic efficacy and provided a potentially useful therapeutic strategy that might be used with atorvastatin therapy.

Keywords: Cholesterol; Atorvastatin; *Moringa oleifera* Lam, Liver enzymes, Kidney function test, Antioxidant.

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Introduction:

A diet rich in fat or cholesterol frequently causes hypercholesterolemia. Since LDL cholesterol is a sign of increased risk for cardiovascular illnesses, a diet high in 1% cholesterol has been shown to increase LDL-C, lower HDL fractions, and have less of an impact on hepatic function [1]. By preventing endogenous cholesterol production, atorvastatin, a synthetic HMG-CoA reductase inhibitor, decreases plasma cholesterol levels. Statins block HMG-CoA, which is involved in cholesterol biosynthesis [2]. Triglyceride, low-density lipoprotein (LDL) cholesterol, and total cholesterol levels have all decreased in dose-dependent ways and have been noted in individuals

with hypercholesterolemia using atorvastatin. Statin drugs reduce the liver's production of cholesterol by blocking the creation of mevalonic acid, a rate-limiting step in the manufacture of cholesterol, and inhibiting HMG-CoA reductase [3]. As a result, intracellular cholesterol is decreased, which raises the number of low-density lipoprotein (LDL) receptors and increases the clearance of LDL cholesterol [4].

Moringa oleifera's nutritional and therapeutic qualities make it one of the most prized and grown plants in the world. The plant is a member of the Moringaceae, a single-generic family. There are fourteen species of trees and shrubs in the genus Moringa. Moringa oleifera (Moringa oleifera Lam) is the species' true botanical name [5]. Numerous nutritional and medicinal uses have been connected to its roots, bark, leaves, flowers, fruits, and seeds [6].

The antioxidant components in Moringa oleifera seed extract should be confirmed [4]. The presence of β-sitosterol, a bioactive phytoconstituent, maybe the reason why the crude extract of When rats are given a high-fat diet, moringa leaves dramatically reduce the amount of cholesterol in their blood [7]. The atherogenic principle in blood, comprising serum cholesterol, phospholipids, triglycerides, LDL, and VLDL, was found to be significantly reduced by Moringa fruit, according to Mehta et al. [8]. It also decreases the liver's lipid profile and increases the excretion of faecal cholesterol.

Whole pods and their components coat, pulp, and seed have also been shown to lower blood pressure in ethanolic and aqueous extracts. Of these, the seed exhibits the strongest activity [9], antispasmodic activity [10], and gastrointestinal motility disorder is treated by the spasmolytic activity of various plant constituents [11]. In rats, Moringa leaf methanol and aqueous extract demonstrated hepatoprotective and antiulcerogenic properties [12]. It has also been stated that the roots offer hepatoprotective properties. Significant hepatoprotective effects were also seen in the alcohol and aqueous extracts of Moringa flowers [13].

This effect might be attributed to the presence of quercetin, a well-known flavonoid having hepatoprotective properties [14]. Moringa leaves exhibit antibacterial activity [15]. has been postulated as an effective chemopreventive agent in chemical carcinogenesis [16]. It also includes flavonoids, alkaloids, kaempherol, rhamnetin, isoquercitrin, and kaempferitrin [9; 13, 17].

Materials and Methods:

Preparation of the plant.

The Moringa oleifera ethanolic extract was made according to: The plant leaves were washed with DW. and then dried for 3 weeks. The dry. A high-speed milling machine crushed the M. oleifera leaves into a fine powder. After extracting 1000 mg of the powder in 1000 mL of 100% ethanol for 48 hours, the sample was filtered twice using filter paper with 2 µm pores. The resultant extract was evaporated using a rotary evaporator at 50 °C. The residual yield of the M. oleifera ethanolic extract was 78.3 g per one kilogram of dry powder. The obtained extract was reconstituted in a brown container (1 g extract: 10 mL DW) and kept at 4 °C.

Experimental design

Thirty adult male rabbits were split into two groups: the experimental group consisted of five rabbits given normal saline as a negative control group. In contrast, the other group consisted of twenty-five rabbits given 1% cholesterol over twenty-eight days. The second group was then split into five groups at random (experimental two). The rabbits were divided into the following groups for the about two-month-long experiment.

(5 rabbits in each group): Group (G1): five rabbits will be administered by normal saline only; in group (G2) in this group five rabbits will be administered cholesterol 1% only; in group (G3) in this group five rabbits will be administered by cholesterol 1% /BW for twenty-eight days then treated with Atorvastatin 50 gm/kg dosage orally every day for four weeks; group (G4) experimental group: in this group, five rabbits will be administrating by cholesterol 1%/BW for 28 days then treated with Moringa Oleifera Lam 200 mg/kg/day orally every day for 4 weeks, group (G5) in this group five rabbits will be administrating by cholesterol 1%/BW for twenty-eight days then treated with Atorvastatin 50 mg/kg dosage orally with Moringa oleifera Lam every day for four weeks.

Biochemical traits: -

Serum cholesterol level (mg/dl): -

The serum cholesterol levels were determined using the reagents. After mixing the sera and reagents in the tube, the findings were let to stand for 5 minutes at 37°C. A sample was read against a blank with a spectrophotometer (Biotech, British) at a wavelength of 505 nm, and the standard absorbance was determined.

Total Cholesterol concentration = (O.D sample)/ (O.D/ standard) × nn =200 mg/dl.

Liver Enzymes Determination

The activity of Aspartate aminotransferase (AST) was determined using the AST Kit (BioSystems, Spain) [18]. Serum Alanine Aminotransferase (ALT) activity is measured using a particular kit (SPECTRUM ALT - kit, Egypt-IFUFCC25) and equipment (Spectrophotometer Sesil, England).

Antioxidant Estimation: -

The serum Catalase concentration is determined using the spectrophotometric technique of Hadwan and Abed (2018) [19]. Malondialdehyde was measured using a spectrophotometer and the Thiobarbituric acid (TBA) test technique [20].

Kidney Function Test

A unique kit called Spectrum–creatinine kit, Egypt-IFUFCC10, was used to test the amount of creatinine in serum using a semi-auto chemical analyzer [21] and a spectrophotometer made by Sesil, England. The Semi-auto chemistry analyzer [22] was used to test the serum urea using the Egypt-IFUFCC40 Spectrum-urea kit.

Results:

Liver enzymes

The results obtained shown in Table (1) exhibited a higher significant difference in liver enzymes (AST and ALT) in the cholesterol treaded group (40.81±3.47, 23.65±3.43), respectively, as compared with other groups. Also, the same table revealed a significant reduction in the groups treated with Moringa oleifera Lam and combined Atorvastatin +Moringa oleifera compared to the cholesterol group.

Table 1. Effect of Atorvastatin, Moringa oleifera Lam, and their combination on some serum liver enzymes in male rabbits (Mean±SE).

Groups	parameters (Mean ± SD)	
	AST (U/L)	ALT (U/L)
Control	34.96± 3.66^{b}	17.48± 1.88^{d}
Cholesterol (U/L)	40.81± 3.47^{a}	23.65± 3.43^{a}
Atorvastatin (U/L)	36.40± 6.41^{b}	21.04± 2.25^{b}
M. oleifera Lam (U/L)	29.96± 2.69^{c}	19.08± 1.67^{c}
Atorvastatin+ M. oleifera Lam (U/L)	24.70± 5.92^{d}	19.20± 2.82^{c}
LSD	2.34	1.66

***The different small letters show significant difference at (P<0.05)**

Kidney Function

In comparison to the other groups, the blood levels of urea and creatinine in the cholesterol (U/L) treated group (23.97±2.72, 0.885±0.077) correspondingly showed greater significant (p≤0.05) values (Table 2). Furthermore, as compared to the cholesterol group, the urea and creatinine concentrations of the Moringa oleifera Lam and combination Atorvastatin + Moringa oleifera Lam groups (four and five) were significantly lower.

Table 2. Effect of Atorvastatin, Moringa oleifera, and their combination on some kidney serum function in male rabbits (Mean±SE).

Groups	parameters (Mean ± SD)	
	Urea (mg/dl)	Creatinine (mg/dl)
Control	17.41± 2.74^{b}	0.657±0.014^{b}
Cholesterol (U/L)	23.97± 2.72^{a}	0.885± 0.077^{a}
Atorvastatin (U/L)	21.31± 1.85^{a}	0.52± 0.050^{c}
M. Oleifera Lam (U/L)	16.96± 2.67^{b}	0.62 ± 0.033^{b}
Atorvastatin+ M. Oleifera Lam (U/L)	14.87± 1.46^{b}	0.628± 0.025^{b}
LSD	3.62	0.13

*The different small letters show significant difference at (P<0.05)

Antioxidants

The groups treated with atorvastatin, moringa oleifera lam, and atorvastatin + moringa oleifera lam had the lowest serum MDA levels. In contrast, the group treated with cholesterol had the highest significant (p≤0.05) levels (3.938±0.47) (Table 3). However, group three (2.774±0.36) and group five (2.541±0.56) did not vary significantly from the cholesterol group (3.938±0.47).

The findings demonstrated that, in comparison to the control group, rabbits that were exposed to Moringa oleifera Lam and a combination of atorvastatin and Moringa oleifera Lam saw a considerable rise in plasma antioxidant catalase (CAT) (Table 3). When compared to the other groups, the cholesterol-treated group showed a substantial (p≤0.05) decrease in serum CAT (0.776±4.36).

Table 3. Effect of Atorvastatin, Moringa oleifera, and their combination on some antioxidants in male rabbits (Mean±SE).

Groups	parameters (Mean ± SD)	
	MDA (nmol/ml)	CAT (KU/L)
Control	2.182±1.15^{c}	0.909±6.81^{a}
Cholesterol (U/L)	3.938±0.47^{a}	0.776±4.36^{b}
Atorvastatin (U/L)	2.774±0.36^{b}	0.807±4.99^{a}
Moringa Oleifera Lam (U/L)	2.217±0.40^{c}	1.247±5.29^{d}
Atorvastatin + Moringa Oleifera (U/L)	2.541±0.56^{b}	0.94± 5.88^{c}
LSD	0.4	0.14

***The different small letters show significant differences at (P<0.05)**

Discussion

High-fat or cholesterol diets are common causes of hypercholesterolemia. All statins can cause asymptomatic modest elevations in blood transaminases, with an incidence ranging from 1% to 1.5%, but this seldom necessitates cessation of medication [23,24]. Serum transaminase increase is frequently self-limiting and is assumed to be caused by hepatocyte cellular membrane changes with enzyme leakage rather than direct liver injury [25]. When rabbits were given a leaf extract of M. oleifera, the levels of the AST and ALT rose and then fell to near-control values. As a result, they hypothesized that pretreatment with M. oleifera leaf extract affects liver enzyme levels, perhaps improving liver function dysfunction (in 0.2, 0.6, 0.6, and 2.3% of patients taking atorvastatin 10, 20, 40, and 80 mg/day, respectively, high blood transaminase levels and elevated serum aspartate or alanine aminotransferase levels were noted). Persistent blood transaminase increases occurred in 0.7% of individuals using atorvastatin in clinical studies. Another important study found that the rate of abnormal transaminase levels was similar to that of atorvastatin after 52 weeks of therapy [26]. While aqueous leaf extracts also showed antiulcer activity [12], Rats showed hepatoprotective and antiulcerogenic properties in the methanol fraction of M. oleifera leaf extract, suggesting that the plant's antiulcer component is extensively disseminated.

The high level of quercetin, a popular flavonoid having hepatoprotective properties [14], maybe the reason why the aqueous and alcohol extracts from Moringa flowers were also shown to have a considerable hepatoprotective impact [27]. There have also been reports of hepatoprotective effects from moringa roots. When lovastatin or M. oleifera was administered to hypercholesterolemic rabbits, the lipid profiles of the liver, heart, and aorta were reduced [28].

The pretreatment with M. oleifera decreased the levels of cholesterol (CHOL), HDL-c, and LDL-c in animals that were not fed Pb and increased total protein but decreased total bilirubin and triglycerides after M. oleifera treatment and Pb. In comparison to G1, M. oleifera demonstrated a protective effect by lowering total bilirubin (TBIL) and triglycerides (TG) while increasing total protein (TP) [29].

Antioxidants are molecules that scavenge free radicals and reduce oxidative stress, preventing, slowing, or delaying the oxidation of oxidizable products. Oxidative stress occurs when reactive oxygen and/or nitrogen species surpass endogenous antioxidant capability, resulting in the oxidation of various biomacromolecules such as proteins, enzymes, lipids, and DNA. Antioxidant molecules

are critical in the body's defence against Reactive Oxygen Species (ROS), which are toxic byproducts of normal cell aerobic respiration [30].

According to Kaur and Kapoor [31], antioxidants are highly valued for their potential to reduce anti-ageing effects and free radical damage. It works as an enzyme, mineral ion, and a source of nutritional value in the body, as well as an industrial addition. Plant antioxidant activity varies substantially depending on the floral components [32].

Polyphenols [17] are antioxidants that include phenolic acid, flavonoid/bioflavonoid, and tannic acid (tannins). These chemicals are primarily obtained from medicinal plants and vegetables [30, 33]. The antioxidant activity of dried seed oil exceeds that of BHT and alpha Tocopheryl. Freeze-dried leaf extracts in aqueous methanol (80%) and ethanol (70%) demonstrated radical scavenging and antioxidant activity. Drumstick leaves are thought to be a possible source of natural antioxidants. The primary phytochemical features of Moringa oleifera leaves are quercetin-3-O-glucoside and kaempferol-3-O-glucoside, which have strong antioxidant activity by scavenging free radicals and decreasing oxidative stress [34]. M. oleifera is also an effective antioxidant [35,36]. Atorvastatin lowers inflammation and oxidative stress [37]. Kidney dysfunction is characterized as the kidneys' inability to remove muscle metabolic waste products from the bloodstream, resulting in high creatinine levels in serum [38,39].

In this study, Moringa oleifera ethanolic extract administered with atorvastatin showed nephroprotective efficacy by improving kidney function and protein production. Other xenobiotics have been shown to provide similar protection, including heavy metals in rats, gentamicin in rabbits, and acetaminophen in mice [40, 41, 42]. Abdel-Daim et al. [43] show the protective efficiency of Moringa oleifera ethanolic extract treatment in rats, demonstrating protective benefits against CoCl2-induced kidney injury [44].

As a result, it is anti-nephrotoxic, as evidenced by investigations on acetaminophen-induced nephropathy [45]. According to Sreelatha and Padma [46], Moringa oleifera leaf extract enhanced antioxidant enzymes and decreased lipid peroxidation. Moringa oleifera leaf extract has been proven to reduce renal dysfunction and enhance renal function, with virtually normal blood urea and creatinine levels indicating improved renal function [44].

Conclusions

In the current study, cholesterol increased the liver enzymes of rabbits and caused oxidative stress and inflammation in renal function (urea and creatinine), which suggests a nephrotoxic effect. Nonetheless, the administration of Moringa oleifera leaf extract may be a viable option for a supplement to reduce oxidative damage and inflammatory reactions, which arise in pathological liver diseases and renal toxicities.

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