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An Evaluation of Anti-hyperlipidemic Activity of Ethanolic Extract of *Moringa oleifera* on High Fat Induced Hyperlipidemic Rat Model

Humayra Zaman Himi [a*], Md.Mahbubur Rahman [b], Syed Abir Hasan [a], Lia Rose Merry D. Cruze [c], Tasnuva Sharmin Zaman [d] and Md. Mustafiz Chowdhury [e]

[a] Department of Pharmacy, University of Asia Pacific, Farmgate, Dhaka, Bangladesh.
[b] Department of Pharmacy, Stamford University Bangladesh, Dhaka, Bangladesh.
[c] Department of Pharmaceutical Sciences, North South University, Plot # 15, Block # B., Bangladesh.
[d] Department of Pharmacy, Atish Dipankar University of Science and Technology, Bangladesh.
[e] Department of Pharmacy, University of Chittagong, Bangladesh.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Background: The art or practice of herbal remedies refers to the use of herbs and herbal treatments for the purpose of maintaining health and preventing, treating, or curing sickness. In some areas, herbal treatments can also be referred to as herbal medicine.
Aim: The present study was designed to investigate the antihyperlipidemic and antiatherogenic potentiality of ethanolic extract of *Moringa oleifera* extract in high fat diet-induced hyperlipidemic rats.

**Corresponding author: E-mail: himizaman16@gmail.com;*

Materials and Methods: *Moringa oleifera* ethanolic extract was prepared using Soxhlet apparatus. Male rats were made hyperlipidemic by giving high fat diet. M. oleifera was administered in a dose of 250, 500 and 750 mg/kg.b.w./day for 30 days in high fat diet-induced hyperlipidemic rats. The SGPT, SGOT, total cholesterol,triglyceride, LDL,HDL , Urea and creatinine levels were measured after the treatment.

Results: For both the SGPT and the SGOT, it was seen that groups 5 and 6 exhibited statistically significant ($p< 0.05$) outcomes in the case of the SGPT. When conducting the renal function test, it was observed that the levels of creatinine and urea were statistically significant ($p< 0.05$) in the cases of groups 4, 5, and 6. In the case of high-density lipoprotein (HDL) and low-density lipoprotein (LDL), groups 4, 5, and 6 showed statistically significant findings ($p< 0.05$) in HDL levels, while groups 5 and 6 showed statistically significant LDL levels. The triglyceride levels in the group were found to be statistically significant ($p< 0.05$), while the findings obtained from groups 5 and 6 were also found to be statistically significant.

Conclusion: The study revealed that *M. oleifera* has anti hyperlipidemic activity.

Keywords: Herbal medicine; moringa oleifera; HDL; LDL; phytochemicals.

1. INTRODUCTION

The liver, the biggest glandular organ, regulates the bulk of an individual's physiological processes. Throughout the day, the liver receives the whole blood supply of a person on many occasions. It plays a crucial role in human metabolism [1]. The abundance of lipoproteins and triglycerides (TG), which are subject to the effects of hereditary and environmental variables, serves as an indicator of the lipid metabolism process. Dyslipidemia may result from either intrinsic factors, exogenous factors, or a combination of genetic predisposition and external effects [2]. Statins are the most recognised and widely accepted therapy for hypercholesterolemia. Their efficacy is well acknowledged, and when used at the maximum allowable dose in combination with ezetimibe, they often facilitate the achievement of LDL-C goals [3]. But these drugs associated with damage of liver enzymes, cancer, diabetes, renal alternation and stroke etc [4]. So medicinal plant researcher are searching for new treatment with lower cost and fewer side effects. Some chemical components derived from medicinal plants may have therapeutic uses, say specialists in the subject. It follows that researchers are always on the lookout for new herbal cures and other plant-based therapies to treat a wide range of illnesses [5]. While phytotherapy is based on scientific study, herbalism is more concerned with the practical applications of medicinal plants. Plants have played a significant role in human medicine for thousands of years due to the wide diversity of chemicals they contain, many of which have medicinal characteristics [6]. The vast variety of chemical components found in medicinal plants allows them to exert a broad spectrum of pharmacological and therapeutic effects. Tanning agents, glycosides, alkaloids, saponins, polysaccharides, essential oils, terpenoids, resins, and plant lipids are all examples of such components [7-9]. Modifying plants genetically allows for the precise regulation of chemical concentrations, allowing for the desired therapeutic effect. Reverse genetics has many potential applications, one of which is to enhance the production of secondary metabolites like alkaloids [10]. Recent scientific progress around the world has led to more research into the healing properties of plants [11]. This is because plants are safe, have strong pharmacological activity, and are more cost-effective than man-made drugs.

Moringa oleifera has nutritional and therapeutic features as a result of its tremendous medical potential; however, this is only the case if the economic worth of the plant's nutritional value, medicinal applications, and animal feed is significant. This tropical deciduous tree, which is endemic to the southern Himalayas in northern India, is a perennial and belongs to the Moringaceae family. Antioxidant, anti-inflammatory, neuroprotective, hypoglycemic, and blood lipid-lowering are just a few of the nutritional and medicinal benefits of *Moringa oleifera* extracts. *Moringa oleifera's* rich phytochemical content—including flavonoids, glucosinolates, isothiocyanates, and phenolic acids—is strongly associated with its positive effects [12].

The purpose of our present study is to evaluate the anti-hyperlipidemic effects of *Moringa oleifera*.

2. MATERIALS AND METHODS

2.1 Plant Collection and Extract Preparation

Moringa oleifera were collected from local market of Dhaka. The material was authenticated by National herbarium, Bangladesh. Firstly *Moringa oleifera* was cleaned properly with water and it was then air-dried. Finally dried leaves were crushed in powder. The powder was soaked for 10 days in 70% ethanol. Vigorous shaking was also performed occasionally. Next, the solution was filtered. The collected filtrate was dried in a rotary evaporator at a low temperature and pressure. Finally, the crude residue was subjected to the required pharmacological testing.

2.2 Drugs and Chemicals

Atorvastatin drug was obtained from incepta pharmacetucals as a gift sample. Ethanol was sourced from Merck in Germany.

2.3 Experimental Animal Procurement, Nursing, and Grouping

A total of 90 male rats weighing between 120 and 150 grams were obtained from Jahangirnagar University in Savar, Dhaka. Each of them was housed in a climate-controlled environment (temperature 25±3°C, relative humidity 55±5%, and a 12-h light/dark cycle) at the University of Dhaka's Institute of Nutrition& Food Science (INFS). They were given a conventional food and were permitted to drink clean water. All of the animals were maintained in this habitat for at least one week prior to the research for adaptation. 90 rats were randomly distributed into 9 groups were each groups contain 10 rats.

High Fat Diet: The Levin and Dunn-Meynell composition served as the basis for adjusting the high-fat diet. The high-fat diet consists of 50% lipids, 40% carbohydrates, and 10% proteins. The dietary composition is shown in Table 1.

After mixing the ingredients thoroughly, the high fat diet was given to the rats to induce obesity for 10 weeks [13].

2.4 Experimental Design

Rats were individually weighed and then divided into nine independent groups for research on anti-hyperlipidemic action. The distribution of rodents among the groups was based on their body weight, with each group consisting of five rats. Group 1 received normal saline and group 2 received the standard drug atorvastatin at 10mg/kg body weight. The atorvastatin control group in Table 2 shows rats that were given atorvastatin with a high-fat diet since using simply atorvastatin would result in the animals dying. The rest of the groups received different dose of plant extract at 250,500 and 750mg/kg dose. N/A indicates that rats in this group did not receive any therapeutic treatment.

Table 1. Composition of high fat diet

Food Ingredients	Composition
Lipid (50%)	Milk powder (10%) Ghee (30%) Mutton fat (40%) Coconut oil (10%) Butter (10%)
Carbohydrate (40%)	Boiled rice (40%) Smashed potato (40%) Boiled corn (20%)
Protein (10%)	Dry powdered prone (40%) Dry boiled mutton (20%) Cheese (20%) Egg (20%)

2.5 Evaluation of Anti-Hyperlipidemic Activity

For this experiment, 90 rats were randomly picked and equally divided into nine groups.

2.6 Biological Sample Collection

Blood was drawn from the animal as soon as its heart was punctured and transferred to a micro centrifuge tube after the killing. The samples were centrifuged at 5,000 rpm for 5 minutes to create the supernatant fluid. Biochemical testing subsequently required the transfer of this fluid to an additional micro centrifuge tube. We carefully took the kidney and liver from the animal after sacrifice and cleaned them in ice-cold saline to assess their function.

2.7 Estimation of Biochemical Parameters

For the evalution of lipid profile total cholesterol, triglyceride,HDL and LDL level were examined. For kidney function test the level of Urea and Creatinine in blood ware measured and for liver function tests SGOT and SGPT levels were evaluated. All the test were performed by using Humaluzer 3000.

Table 2. Antihyperlipidemic activity analysis

Group number	Group Status	Treatment specimen & Dose	Group Abbreviation
1	Negative Control	Physiological Saline	N
2	Positive Control	High Fat Diet	HF
3	High Fat Diet + Atrovastatin	High Fat Diet + Atrovastatin	HFD + ATV
4	High Fat Diet + *M. oleifera*	High Fat Diet+ MO_{250}	HFD + MO_{250}
5	High Fat Diet + *M. oleifera*	High Fat Diet + MO_{500}	HFD + MO_{500}
6	High Fat Diet + *M. oleifera*	High Fat Diet + MO_{750}	HFD + MO_{750}
7	*M. oleifera*	MO_{250}	MO_{250}
8	*M. oleifera*	MO_{500}	MO_{500}
9	*M. oleifera*	MO_{750}	MO_{750}

Table 3. Application of treatment efficacy

Group Number	Group Specification	Treatment species	Dose treatment species (mg/kg)	Abbreviation of Groups
1	Negative control	Physiological saline	10 ml/kg	N
2	High Fat	N/A	N/A	HF
3	HF+At_{10}	Atrovastatin 10mg/kg	10	At_{10}
4	HF +MO_{250}	*Moringa oleifera*	250	MO_{250}
5	HF+MO_{500}	*Moringa oleifera*	500	MO_{500}
6	HF+MO_{750}	*Moringa oleifera*	750	MO_{750}
7	MO_{250}	*Moringa oleifera*	250	MO_{250}
8	MO_{500}	*Moringa oleifera*	500	MO_{500}
9	MO_{750}	*Moringa oleifera*	750	MO_{750}

2.8 Statistical Analysis

All of our findings (raw data) in terms of numerical parameters were recorded and analyzed on a broadsheet using the MS Excel application. The gathered data were subjected to descriptive statistics, with the findings reported as mean SD. To evaluate statistical significance, we used the SPSS 16 software's "One-way Anova test" to interpret inter-group heterogenicity in terms of several biological factors. The occurrences are considered statistically significant since the 'p' value was less than 0.05 ($p<0.5$).

3. RESULTS AND DISCUSSION

The global use of herbal medicines for addressing diverse health conditions is seeing significant growth. There is a significant increase in the acceptability and public interest in natural medicines in both emerging and established nations. These herbal remedies are now not only accessible in pharmacy shops but also in grocery stores. For both the SGPT and the SGOT, it was seen that groups 5 and 6 exhibited statistically significant ($p< 0.05$) outcomes in the case of the SGPT. However, in the case of the SGOT, there were no statistically significant findings. There were two other investigations that came to the same conclusions [14,15]. When conducting the renal function test, it was observed that the levels of creatinine levels were statistically significant ($p< 0.05$) in the cases of groups 4, 5, and 6 (Table 4). Also in case of urea the results were statistically significant in groups 4, 5 and 6. Two separate investigations [16,17] came to the same conclusions about the subject which provide as the same results of our experiment. When examining the levels of high-density lipoprotein (HDL) and low-density lipoprotein (LDL), it was seen that groups 4, 5, and 6 exhibited statistically significant discoveries ($p< 0.05$) in HDL levels. On the other hand, groups 5 and 6 further showed statistically significant levels of LDL. It was determined that the triglyceride levels in Group 4 were statistically significant ($p< 0.05$) Additionally, the data obtained from groups 5 and 6 were also found to be statistically significant in the case of total cholesterol. Two further studies produced the same findings of our investigation [18,19].

M. oleifera has many potential pathways via which it reduces serum lipid levels. Some people think that stopping 3-hydroxy-3-methylglutaryl coenzyme-A reductase (HMG-CoA reductase)

Table 4. Lipid profile of *Moringa oleifera*

Groups	SGPT	SGOT	Creati-nine	Urea	TC	HDL	LDL	TG
C	36.28±4.25	36.52±3.12	0.5±0.05	29.49±2.32	94.82±3.19	66.25±3.62	35.22±3.26	54.28±3.29
HF	95.32±6.80	88.73±8.18	2.24±0.06	87.72±6.91	177.84±9.53	38.54±3.84	69.32±5.69	104.24±6.26
HF+At_{10}	74.59±2.09	74.33±6.28	1.1±0.08	59.51±6.39	119.26±7.56	57.36±4.58	45.40±4.28	70.64±5.94
HF +MO_{250}	93.28± 6.51	88.18±3.59	1.97±0.08*	82.39±4.81*	172.42±6.21	42.60±2.81*	37.54±3.57	97.29±6.82*
HF+MO_{500}	90.27±6.58*	87.29±8.13	1.70±0.07*	78.30±3.59*	166.17±7.53*	47.84±5.33*	41.66±4.58*	93.10±5.28
HF+MO_{750}	86.81±5.29*	85.52±3.61	1.29±0.05*	74.53±5.60*	162.23±6.18*	54.54±4.28*	45.08±3.28*	90.25±6.73
MO_{250}	34.67±2.62	39.42±2.85	0.60±0.08	28.08±3.06	90.18±4.51	63.28±4.20	37.30±2.21	56.29±4.10
MO_{500}	36.37±4.12	34.73±2.80	0.70±0.06	32.53±2.84	92.30±2.81	66.53±3.29	35.18±3.26	53.29±3.10
MO_{750}	34.12±3.29	35.79±3.18	0.9±0.08	30.80±2.35	94.95±3.22	65.19±4.24	38.42±4.20	55.53± 4.23

*Note: The results were expressed in Mean±SEM (standard mean error) *p< 0.05, **p< 0.01, and ***p< 0.001 were considered as statistically significant. The statistical analysis followed by one-way analysis of variance (Dunnett's test) compared to the control.*

from working could lower the production of cholesterol. HMG-CoA reductase is the main enzyme that makes cholesterol. MEPA may also enhance the activity of lecithin-cholesterol acyl transferase (LCAT). This enzyme has a crucial function in integrating unbound cholesterol into HDL. This will enhance the process of reverse cholesterol transport and effectively block the absorption of LDL by endothelial cells via competition [20,21].

4. CONCLUSION

Within the scope of this investigation, the anti-hyperlipidemic property of *Moringa oleifera* ethanolic extract were investigated. Based on the findings of this research, it seems that an ethanol extract derived from the plant *M. oleifera* may be able to provide protection against excessive cholesterol, damage to the liver, and impaired kidney function. As a result, more research is necessary in order to determine the active components in the entire extract that have the ability to reduce hyperlipidemia. After the active chemicals have been discovered, it is possible to conduct a comprehensive investigation.

ETHICAL APPROVAL

All experimental methods followed the recommendations of the Institutional Animals Ethics Committee (IEAC) with the ethical approval no (301/LUB.Pharm).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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