Original Research Article

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

Abstract

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. To assess the lipid profiles of *Moringa oleifera* extract, rats were studied. 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. 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.

Keywords: Herbal medicine, *Moringa oleifera*, HDL, LDL, Phytochemicals.

Comment [A1]: Methodology like how many rats used, what is the dosage studied should be mentioned

Comment [A2]: In results section, Since its about lipidemia it is better to mention the lipid levels followed by Liver and Kidney function

Introduction

The liver, the largest glandular organ, is responsible for controlling the majority of a person's bodily functions. Numerous times during the day, the whole blood supply of an individual goes through the liver. The liver is very critical for human metabolism. [1]. Hepatotoxicity, the most frequent form of liver illness, is a leading cause of death and impairment in both animals and humans. Several medications can cause it. [2]. Addiction to alcohol or drugs, exposure to harmful chemicals, infections with viruses or parasites, and high levels of reactive oxygen species (ROS) are among the most damaging factors that may affect liver cells (OH, H2O2, O2). [2]. The ability to neutralize free radicals is why ascorbic acid and the tripeptide L-glutathione (L-cysteine, glycine, and L-glutamate) are taken orally as dietary supplements. Because of their anti-inflammatory, antibacterial, and immune-enhancing qualities, they are highly prized by many. [3]. On the flip side, they might trigger an allergic response, such as dermatitis, or gastrointestinal issues, such as gas, diarrhea, indigestion, or even difficulties breathing due to airway narrowing. There are 1.5 billion individuals worldwide who suffer from chronic liver disease (CLD). Among Americans aged 45–64, the prevalence of CLD has risen by 31% in recent years. [4].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 hepatoprotective effects of *Moringa oleifera*.

Comment [A3]: Paper is about anti hyperlipidemic property but introduction discuss about liver & hepatoprotective. There is no mention about cholesterol, lipidemia etc.

Comment [A4]: The topic is antihyperlipidemic but its given hepatoprotective

MATERIALS AND METHODS

Plant Collection and Extract Preparation

Moringa oleifera were collected from local market of Dhaka. The material was authenticated by National hebarium, Bangladesh.1st*Moringa oleifera* was cleaned properly with water and it was then air-dried. Finally dired leaves were crushed in powder. The powder was soaked for 15 days in 70% ethanol. The solution was kept for 15 days. 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.

Comment [A5]: herbarium

Comment [A6]: Abbreviation cant be used it should be in words First

Drugs and Chemicals

Atorvastatin drug was obtained from incepta pharmacetucals as a gift sample. Ethanol were bought from Taj Scientific store.

Experimental Animal Procurement, Nursing, and Grouping

A total of 90 male rats weighing between 120and 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 adaption. All experimental methods followed the recommendations of the Institutional Animals Ethics Committee (IEAC).90 rats were randomly distributed into 9 groups were each groups contain 10 rats.

Comment [A7]: adaptation

Comment [A8]: IEAC approval no not mentioned

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. The atorvastatin control group in Table 1 shows rats that were given atorvastatin with a high-fat diet since using simply atorvastatin would result in the animals dying. N/A indicates that rats in this group did not receive any therapeutic treatment.

Table 1: Antihyperlipidemic activity analysis

Group number	Group Status	Treatment specimen & Dose	Group Abbreviation
1	Negative Control	Physiological Saline	N
2	Positive Control	High Fat Diet	P
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_{250}	HFD +MO_{750}
7	*M. oleifera*	MO_{250}	MO_{250}
8	*M. oleifera*	MO_{500}	MO_{500}
9	*M. oleifera*	MO_{750}	MO_{750}

High Fat Diet: The high-fat diet was modified based on the composition supplied by Levin and Dunn-Meynell. The high fat diet is composed of 50% lipid, 40% carbohydrate, and 10% protein. The diet's composition is shown in Table 2.

Table 2: 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%)

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

Comment [A9]: This should come first prior to Experimental design with induction of high fat diet as title

Evaluation of anti-hyperlipidemic Activity

For this experiment, 100 rats were randomly picked and equally divided into fourteen groups

Comment [A10]: Only 90 rats and 9 groups

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}

Biological Sample Collection.

We punctured a rat's tail to obtain blood samples for measuring blood glucose levels. On the other hand, 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.

Estimation of Biochemical Parameters

The blood glucose level was measured using a glucometer. Aside from the Humaluzer 3000, lipid profile, kidney, and liver function tests were performed. In addition, the gluconeogenic and glycolytic enzyme activity of kidney and liver samples was examined

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 ($p<0.5$).

Results and discussion:

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 and urea were statistically significant ($p< 0.05$) in the cases of groups 4, 5, and 6. Two separate investigations [16, 17] came to the same conclusions about the subject. 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. There were two other investigations that came to the same conclusions [18, 19].

Table 4: Lipid profile of *Moringa oleifera:*

	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.

Conclusion

Within the scope of this investigation, the hepatoprotective properties 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

Comment [A11]: Anti hyperlipidemic property

the ability to reduce hyperlipidemia and diabetes. After the active chemicals have been discovered, it is possible to conduct a comprehensive investigation.

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