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ORIGINAL ARTICLE

Effects of Moringa Oleifera leaves powder on lipid parameters of adult male rabbits.

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ABSTRACT... Objective: To observe the effects of moringa oleifera on lipid parameters of rabbits. **Study Design:** Experimental Trial. **Setting:** was carried out on healthy animal models using adult male rabbits in indoor Patient Ward of Pakistan Veterinary Clinic and Pet Centre Nawabshah. **Period:** January 2023 to June 2023. **Methods:** Thirty adult male rabbits age ranging from 16 to 24 months having weight between 2 to 3 kg were selected and randomly divided in to three groups comprising 10 rabbits in each group. Data was analyzed by using SPSS version 25.0. **Results:** The mean Cholesterol, LDL, HDL and Triglycerides level of the rabbits were compared between Group A (Control), Group B (250 mg/Kg MO) and Group C (500 mg/Kg MO) and data was collected on four scheduled days (Day 0, 15, 30 and 45). Statistical significant test repeated measure ANOVA was applied and there was statistically significant difference among means Cholesterol level in each time period in both Group B and C with P-value 0.002 on groups, mean LDL level with P-value 0.014 and 0.000 on both groups and mean HDL level with P-value 0.000 and 0.015on both groups. There were no statistically significant differences between means in Serum Triglyceride level in both groups with P-value 0.063 and 0.149. **Conclusion:** It was concluded in our study that Moringa Olifera has potential role in lowering serum Cholesterol, LDL and HDL level with regular use. But we cannot find any significant difference in lowering triglyceride level.

Key words: Hypocholesterolemia, Lipid Parameters, Moringa Oleifera.

INTRODUCTION

For many years, herbal plants have been employed as dietary supplements and extra medicinal therapies. Specific Moringa species and herbal treatments have been used to treat ailments from the dawn of mankind.[1] Moringa Olifera belonging to the Moringaceae family is produced as a versatile crop in over 60 countries and is dubbed "Nature's Gift" due to its several evident benefits.[2] Moringa Oleifera is a plant native to India and Pakistan that is extensively dispersed throughout the tropics and subtropics of Asia and Africa.[3] The major phytochemicals identified in various parts of M. oleifera: (i) Leaf powders: tannins, alkaloids, saponins, phenols, flavonoids and glycosides. (ii) Flowers: flavonoids, alkaloids, glycosides, tannins, steroids, quercetin, and terpenoids. (iii) Seeds: gallic acid, epicatechins, catechins, ferulic acid, caffeic acid, vanillin, protocatechuic acid, quercetin, cinnamic acid, phytosterols, glycosides and phenols. (iv) Roots: procyanidins, aurantamide acetate, 3-dibenzylurea, quercetin glycosides, quercetin rhamnoglucosides and chlorogenic acid, and (v) Stem bark: procyanidins, tannins, triterpenoids, sterols, glycosides, alkaloids (moringin and moringinine), S-sitosterol, S-sitostenone and octacosanoic acid. Glycosides are among the most frequently identified and researched chemicals in M. oleifera.[4]

Several clinical studies have investigated the pharmacological effects of Moringa oleifera on metabolic syndrome, type 2 diabetes, osteoporosis, anemia, and dyslipidemias. Eating foods rich in polyphenols has been associated in epidemiological research to a reduced risk of acquiring neurodegenerative diseases. In addition, vitro and vivo studies have demonstrated the wide variety of therapeutic

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benefits that can be derived from Moringa oleifera leaves. These include antimicrobial, antifungal, antiviral, cytotoxic, anti-hyperglycemic, anti-hyperlipidemic, antioxidant, anti-inflammatory, anti-parasitic, and cardio-protective properties.[5]

MO's potential as a hypolipidemic medicine has been shown to reduce lipid levels because it inhibits the production of cholesterol micelles and the activity of pancreatic cholesterol esterase, pancreatic lipase, and the binding of bile acids. There are some evidences that MO may help mitigate the chronic effects of illnesses including diabetes and atherosclerosis.[6] B-sitosterol, present in MO, is one of the plant sterols that reduces blood cholesterol through lowering LDL levels and blocking the body's absorption of cholesterol from its own tissues. To do this, neutral steroid excretion is increased in the feces, which in turn increases the quantity of cholesterol expelled.[7] Many studies on moringa species have found to reduce serum cholesterol, triglycerides, High-density Lipoprotein (HDL) and low-density lipoprotein (LDL) concentration.[8] Lipoproteins transport dietary lipids like cholesterol and triglycerides from the digestive tract to the rest of the body, where they are used for various metabolic processes like generating energy or forming hormones like testosterone and oestrogen. Cholesterol, low-density lipoprotein cholesterol (LDL-C), triglycerides, and high-density lipoprotein (HDL) cholesterol are all major players in these pathways (HDL).[9] Very low-density lipoprotein (VLDL) and chylomicrons are examples of primary triglyceride-containing TRLs. Liver and intestinal enterocytes, respectively, produce and secrete these substances. The breakdown of the core triglycerides in VLDL and chylomicrons is facilitated by lipoprotein lipase (LPL), leaving behind remnants of both lipoproteins that are comparatively high in cholesterol. While the liver may remove some of these particles from circulation, the ones that remain are further modified by hepatic lipase and LPL, ultimately giving rise to cholesterol-enriched LDL particles.[10]

Important structural alterations separate residual lipoproteins from non-remnant lipoproteins and may enhance atherogenicity as a result of the action of two enzymes, lipoprotein lipase (LPL) and hepatic lipase (HL), and a specific transfer protein, cholesterol ester transfer protein (CETP).[11] Two major low-density lipoprotein and high-density lipoprotein transport cholesterol (HDL). HDL particles carry cholesterol for cellular efflux, mostly removing it from peripheral cells and tissues and returning it to the liver for excretion in the bile.[12] Protein-coated molecular assemblies called lipoproteins are employed by the body to transport and store cholesterol and other lipids, and they mix easily with blood. The proteins called apolipoproteins have a role in this structure. High-density lipoprotein (HDL), sometimes known as "good cholesterol," is characterized by a high ratio of protein to lipid (cholesterol and other lipids).[13] The objectives of our study were to observe the effects of Moringa oleifera leaves powder on the Lipid parameters of animal models.

METHODS

This Experimental trial was carried out on healthy animal models using adult male rabbits in indoor Patient Ward of Pakistan Veterinary Clinic and Pet Centre Nawabshah. Duration of study was 06 months from January 2023 to June 2023 after approval from ethical committee (PUMHS/SBA/PVC/213-24/10/21). Thirty adult male rabbits age ranging from 16 to 24 months, were selected and randomly divided in to three groups comprising 10 rabbits in each group.

Animal Modeling and Grouping

Healthy adult male rabbits having ages range from 16 to 24 months and weighing 2.0 kg to 3.0 kg were selected for study purpose. Models were acquired from indoor ward of Pakistan Veterinary Clinic and Pet Centre Nawabshah, Sakrand which is registered with Pakistan Veterinary Medical Council Islamabad. After taking written permission from administration authorities of the concerned animal house, animals were kept and maintained in cages especially designed for research purpose, under standard condition of well aerated room at temperature of 20^0C to 30^0C with air-conditioning, light/dark cycle of 12 hours of the day under conventional laboratory

conditions throughout the study period. Five rabbits were housed in each cage and were allowed free access to fresh water and hay. Three groups having 10 rabbits in each were labeled as group A, B and C. Group A (control group) was served only with hay and fresh water. Group B and Group C containing ten rabbits each were served with 250 mg/kg and 500 mg/kg per day of Moringa Oliefera leaf powder respectively along with fresh water and hay for study purposes.

Product Preparation

Already air dried Moringa leaves were obtained from local market. They were ground into fine powder by using a domestic electrical grinder WestPoint model WF-314 grinder machine. The fine powders was packed in 250mg and 500mg plastic packets by the help of Electrical Balance and were refrigerated at a temperature below 3˚C for couple of days than was used.[14,15]

Product administration: In an aseptic atmosphere and handling with great care and taking precautions, already measured leaf powder was mixed initially with 5cc plain tap water and with the help of a feeding syringe the concoction was directly administered in the oral cavity of rabbits. Rabbit's oral cavity was checked for deglutition. MO was given daily at 10 am for 45 days.

Schedule for Blood Sampling and Extraction

The blood samples were extracted in a systematic, consistent manner. The first sample was extracted at zero time and was designated as day 0. Subsequent samples were extracted every two weeks, on days 15, 30, and 45. Total of 2cc blood sample was drawn through clear venipuncture from rabbit's ear, using the large veins because veins in ear are more prominent ad visible. 2cc blood drawn with the help of 3cc syringe and was collected in vacutainer blood collection tube already labeled with identification code of the subjects and was immediately carried to PUMHSW Diagnostic Laboratory Lipid Profile.

Data Collection and Analysis Procedure

After collecting laboratory investigation reports from PUMHSW Diagnostic Laboratory, the data was processed by hand sorting techniques, Calculator, Microsoft office and using statistical program for social sciences (SPSS version 25.0). The data collection form was prepared to study each variable separately and data was analyzed and finally findings were recorded and presented in the form of charts and tables. $P \leq 0.05$ was designated as the significance level, and a 95% confidence level was used.

RESULTS

Moringa Olifera was given to the animal models daily for 45 days as per schedule and samples were collected fortnightly on four scheduled days (Day 0, 15, 30 and 45). The mean Cholesterol, LDL, HDL and Triglycerides level of the rabbits were obtained and were compared between Group A (Control), Group B (250 mg/Kg MO) and Group C (500 mg/Kg MO).

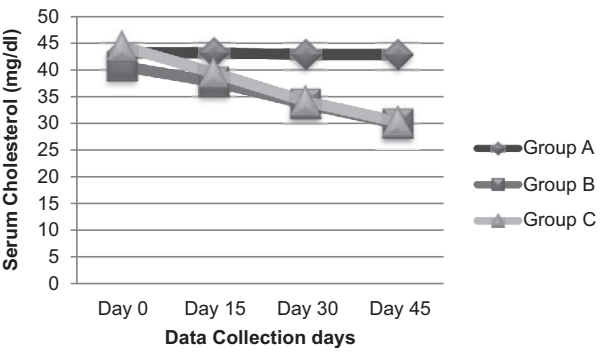

Figure-1. Comparison between mean serum cholesterol level of all groups of rabbits on scheduled days

There was significant difference among means in each time period in both Group B and C with P-value 0.002 on both groups.

There was significant difference among means in each time period in both Group B and C with P-value 0.014 and 0.000 on both groups.

There was significant difference among means in each time period in both Group B and C with P-value 0.000 and 0.015on both groups.

There was insignificant difference among means in each time period in Group B and C with P-value 0.063 and 0.149 on both groups.

Sr. No.	Group	Group Pairs		Difference	P-Value
1	B	1	2	2.9*	0.000
2		1	3	7.0*	0.000
3		1	4	10.7*	0.000
4		2	3	4.1*	0.000
5		2	4	7.8*	0.000
6		3	4	3.7*	0.002
There was significant difference among means in each time period with P-value 0.000 which indicates highly significant difference among means of Cholesterol level in group B.					
	Group	Group Pairs		Difference	P-Value
7	C	1	2	4.9*	0.000
8		1	3	10.2*	0.000
9		1	4	14.0*	0.000
10		2	3	5.3*	0.000
11		2	4	9.1*	0.000
12		3	4	3.8*	0.002
There was significant difference among means in each time period with P-value 0.002 which indicates highly significant difference among means of Cholesterol level in group C.					

Table-I. Showing the paired difference in mean serum cholesterol level within the Groups and compared the mean.

Sr. No.	Group	Group Pairs		Difference	P-Value
1	B	1	2	1.34*	0.010
2		1	3	4.14*	0.000
3		1	4	6.92*	0.000
4		2	3	2.80*	0.000
5		2	4	5.58*	0.000
6		3	4	2.78*	0.014
There was significant difference among means in each time period with P-value 0.000 which indicates highly significant difference among means of Low Density Lipoprotein (LDL) in group B.					
	Group	Group Pairs		Difference	P-Value
7	C	1	2	1.26	0.213
8		1	3	4.84*	0.000
9		1	4	8.14*	0.000
10		2	3	3.58*	0.000
11		2	4	6.88*	0.000
12		3	4	3.30*	0.000
There was significant difference among means in each time period with P-value 0.000 which indicates highly significant difference among means of Low Density Lipoprotein (LDL) in group C.					

Table-II. Showing the paired difference in mean serum Low density lipoprotein level within the Groups and compared the mean.

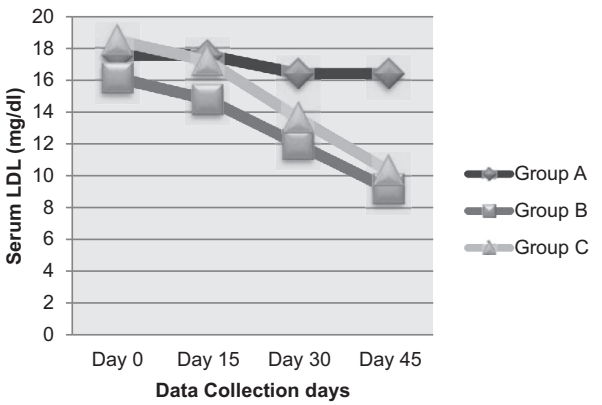

Figure-2. Comparison between Mean LDL level of All groups of Rabbits on scheduled days

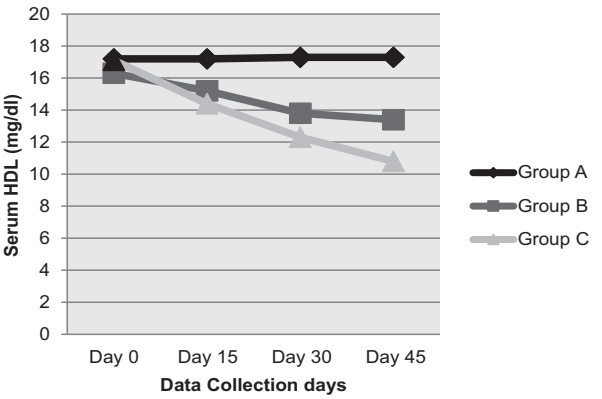

Figure-3. Comparison between Mean HDL Level of All groups of Rabbits on scheduled days

DISCUSSION

Among its many names are Moringa, drumstick tree, horseradish tree, ben oil tree, and miracle tree. Moringa oleifera (MO) is a member of the Moringaceae family and grows quickly. Specific Moringa species and herbal treatments have been used to treat ailments from the dawn of mankind. It has demonstrated bioactivity, including antibacterial, nutraceutical, hepatoprotective, antihypertensive, cholesterol-lowering, anti-urolithiasis, antifertility, and antioxidant activity. Pharmacological and Phytochemical properties of Moringa oleifera described by Anzano A. et al[16] in a study that are Cardiovascular properties and Antihypertensive properties by causing reduction of total cholesterol, triglycerides, low-density lipoprotein, and very-low-density lipoprotein.

Sr. No.	Group	Group Pairs		Difference	P-Value
1	B	1	2	1.1*	0.000
2		1	3	2.5*	0.000
3		1	4	2.9*	0.000
4		2	3	1.4*	0.000
5		2	4	1.8*	0.000
6		3	4	0.4*	0.037
There was significant difference among means in each time period with P-value 0.000 which indicates highly significant difference among means of High Density Lipoprotein (HDL) in group B.					
	Group	Group Pairs		Difference	P-Value
7	C	1	2	2.7*	0.000
8		1	3	4.8*	0.000
9		1	4	6.3*	0.000
10		2	3	2.1*	0.000
11		2	4	3.6*	0.000
12		3	4	1.5*	0.015
There was significant difference among means in each time period with P-value 0.000 which indicates highly significant difference among means of High Density Lipoprotein (HDL) in group C.					

Table-III. Showing the paired difference in mean serum high density lipoprotein within the Groups and compared the mean.

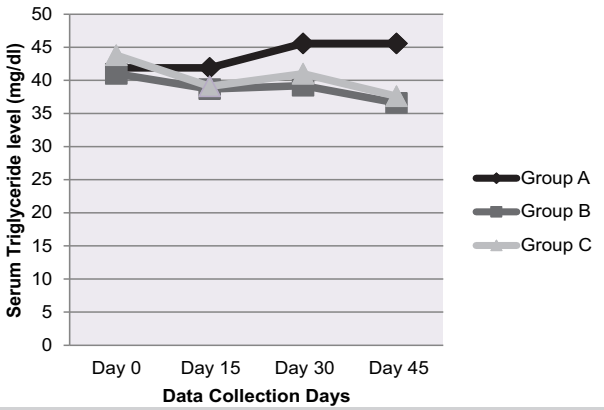

Figure-2. Comparison between Mean LDL level of All groups of Rabbits on scheduled days

Additionally, it has been demonstrated that powdered moringa oleifera leaves causes a hypocholesteromic effect by increasing the excretion of neutral steroids and bile acids. Blood total lipids and serum cholesterol levels are significantly reduced by the use of MO and these blood cholesterol and triglyceride are the foremost risk factors for heart diseases. In our study there was significant difference among means in each time period with 250mg/kg and

Sr. No.	Group	Group Pairs		Difference	P-Value
1	B	1	2	2.3	0.063
2		1	3	1.8	0.085
3		1	4	4.4	0.087
4		2	3	-0.5	0.651
5		2	4	2.1	0.181
6		3	4	2.6	0.291
There was insignificant difference among means in each time period with P-value 0.063, or 0.085 which indicates insignificant difference among means of serum triglycerides in group B.					
	Group	Group Pairs		Difference	P-Value
7	C	1	2	4.7*	0.043
8		1	3	2.8	0.149
9		1	4	6.2*	0.010
10		2	3	-1.9	0.240
11		2	4	1.5	0.500
12		3	4	3.4	0.210
There was insignificant difference among means in each time period with P-value 0.149, or 0.240 which indicates insignificant difference among means of serum triglycerides in group C.					

Table-IV. Showing the paired difference in mean serum triglycerides level within the Groups and compared the mean.

500mg/kg and P-value was 0.000 and 0.002 respectively which indicates highly significant difference among means serum cholesterol level. A study conducted by Mehta LK et al[17] to see the effects of MO on serum cholesterol and lipid profile and the results of this study showed that the administration of M. oleifera at the dose of 200 mg/kg orally or lovastatin 6 mg/kg orally daily for a period of 120 days to rabbits significantly lower than equivalent control groups serum levels of total cholesterol, phospholipid, triglycerides, LDL, VLDL, total lipids, cholesterol balance, and atherogenic index. When M. Oleifera was administered to healthy rabbits, the HDL levels were lowered in comparison to the normal control group.

Lipid metabolism syndrome or dyslipidemia is an abnormal condition inside the body obvious with increasing or decreasing the segments of plasma lipid. This condition can be treated with an antioxidant found in many herbal and natural products. Moringa Oleifera has also potent anti-oxidant properties that have strong saponin and flavonoid content and the ability to regulate the

body's lipid levels. We used MO in rabbits to observe its effects on serum triglyceride level with different doses. The mean Serum Triglyceride Level was significantly decreased in some pairs after 45 days of treatment with MO in both study groups with P-value for pairs was p= 0.063 and 0.043 respectively. Another experimental trial conducted by Setyawati T et al[18] on rats showed the same results. They divided rats in six groups to observe the effects of MO with different doses and durations. The data were analyzed revealed that there was no discernible change in the triglyceride levels following the treatment, with a P-value of 0.827 ($p > 0.05$). This is in accordance with our study as well.

In recent years, Moringa oleifera is being considered as a subject of interest due to its many characteristics, which are found to be beneficial to human health. In this context the use of Moringa oleifera, as supplements in food in the recent times is being disseminated because of its wide range of benefits that have been observed. In our study the effects of MO was observed to see its effects on Lipid profile of rabbits like Low density lipoprotein level (LDL) and High Density Lipoprotein (HDL) on rabbits. There was significant difference among means in each time period with P-value 0.010 in group served with 250mg/kg and significant difference among means in each time period with P-value 0.014 which in group served with 500mg/kg of MO. An interventional trial conducted by Seriki SA[19] et al to see the effects of MO on lipid profile, Blood pressure and BMI in animals also concluded that there were significant difference in Cholesterol and Triglycerides level but no significant reduction in lipid profile component Low density lipoprotein (LDL), High density lipoprotein (HDL). Another experimental study conducted by Mardewi NK et al[20] also revealed a significant difference, the Duncan's multiple-range test was used to continue the investigation. From this study it was established that the adding moringa leaf meal was the optimal level to reduce the triglyceride of meat without troubling the HDL.

CONCLUSION

It was concluded in our study that Moringa Olifera has potential role in lowering serum Cholesterol, LDL and HDL level with regular use. But we cannot find any significant difference in lowering triglyceride level.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHORSHIP AND CONTRIBUTION DECLARATION

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2	Ghulam Mustafa Dahri	Data analysis and Critical review.	
3	Yasmin Shaikh	Manuscript drating and data Interpretation.	
4	Noreen Irum	Literature and Drafting.	
5	Zaheera Yousif Memon	Literature and Drafting.	
6	Rukhsana Malik	Data analysis and Critical review.	
7	M. Siddique Rajput	Data analysis and Critical review.