Hypolipidemic Activity Of*ManjalNoikuKudineer*Against Atherogenic Diet-Induced Hyperlipidemia In Experimental Rats

Abstract

Hyperlipidemia, which is increased level of cholesterol and triglycerides in the blood, is a major risk factor the development of serious diseases like atherosclerosis, Coronart Artery Diseases, Stroke. Unhealthy food and lifestyle is the major cause for this condition to develop. Siddha system of Medicine with its uniqueness of treating the diseases has *ManjalNoikuKudineer*, a Siddha poly herbal formulation indicated for *Manjal Noi*. This study deals with the Hypolipidemic activity of *ManjalNoikuKudineer*against atherogenic diet induced hyperlipidemia in experimental Wistar Albino Rats, conducted at ArulmiguKalasalingam College of Pharmacy, Virudhunagar. This study shows the results of decreased body weight, blood lipid profile, liver lipid profile of hyperlipidemic rats on treating with the study medicine, which possess hypolipidemic activity. Histopathologic images of rat liver also shown normal parenchyma and parenchyma with minimal necrosis and minimal inflammatory changes on treating with study medicine MNK. This results stands as a support for MNK hypolipidemic activity, which in turn can be used as a antihyperlipidemic therapeutic agent from the Siddha System of Medicine.

Keywords

Manjal Noi, Siddha, Atorvastatin, *Karisalai*, *Keezhanelli*, Hyperlipidemia.

1. Introduction

Siddha system of medicine has numerous medicinal preparations in the literature for treating various ailments in the human body. Apparently it heals the body but it also heals the soul to attain longevity and eternity[1]. Liver is the largest organ that plays a major role in the bodily metabolisms. Liver is injured by various toxic chemicals, infections and unhealthy diet and alcohol consumption. Herbs and herbal medicines were widely used in the treatment of liver injury by rejuvenating its cellular pathology[2]. Deaths due to liver diseases accounts for about 2 million annually, which is 1 out of every 25 deaths, and among which Non alcoholic fatty liver disease is the second leading cause of end stage liver disease, according to global burden of liver disease: 2023 update[3]. Liver regulates cholesterol metabolism. Abnormality in the lipid level can alter liver metabolism and hepatic tissues. Hyperlipidemia is the most important cause for the risk of coronary heart disease and atherosclerosis[4]. In Siddha system of Medicine, liver disease comes under *ManjalNoi(Kaamalai)* with its yellowish discoloration of skin and mucous membrane[5]. *ManjalNoikuKudineer*(MNK)is one among the Siddha Medicines which is indicated for *Manjal Noi, Velluppu Noi, OodhalNoi*[6,9]. MNK is a six ingredients based Siddha poly herbal formulation comprises of *Phyllanthus Amarus (Keezhanelli), Ecliptaprostrata (Karisalai), TrichosanthesCucumerina (Peipudal), Piper nigrum (Ven milagu), Foeniculum vulgare (Sombu), Aegle marmelos (Vilvam)*[6,7]. Analysing hypolipidemic activity of*ManjalNoikuKudineer* would be greatest significance in the scientific world among hyperlipidemia. The present study deals with the Hypolipidemic activity of Siddha medicine *ManjalNoikuKudineer*against atherogenic diet induced Hyperlipidemia in experimental animals, which will contribute to further research.

2. Materials and Methods

2.1 Drug authentication

The herbal raw drugs of MNK were procured from a reputed raw drug store. Herbs were identified and authenticated by the faculties of Department of PG Gunapadam and Botanist, Department of Botany, Government Siddha Medical College, Palayamkottai.

2.2 Medicine preparation

The herbal raw drugs were cleaned and purified as mentioned in Siddha literature *Sikitcha Rathna Deepam in VaithiyaNool*[8]. Purified ingredients of MNK was chopped and coarsely powdered. The MNK decoction was prepared by adding 650ml of water to the coarse powder, boiled, reduced to 85ml of its volume and filtered. It is indicated for *Manjal Noi* with the dosage of 85ml, thrice a day, for three days.

2.3 Animal care and husbandry

The study protocol for MNK was reviewed and approved by Institutional Animal Ethics Committee (IAEC), ArulmiguKalasalingam College of Pharmacy, Virudhunagar. Wistar albino adult male rats weighing 150-200gm from animal

housing facility of Vels University were housed in polypropylene cages maintained with temperature 27°C ±1°C and 12 hrs light and dark cycle. The animals were allowed to adapt to the environment for seven days and supplied with a standard pellet diet (Sai Durga foods, Bangalore) and water ad libitum. The experimental protocol has got the approval IAEC bearing no----------. Chemicals used for the study include, Atorvastatin obtained from the local pharmacy, Tamil nadu (periyandavar medicals). Diagnostic kits for estimation were purchased from Merck Diagnostics India Ltd. Anesthetic ether, ethyl acetate, and ethanol (SD Fine Chemicals, Mumbai).

2.3.1 Atherogenic diet

Experimental hyperlipidemic diet: Experimental diet consists of well-pulverized mixture of cholesterol – 400 mg/kg, cholic acid – 50 mg/kg, and coconut oil. This mixture is made into paste-like molds and is fed to the rats.The prepared atherogenic diet was used in place of normal pellet diet to all the groups except control. Rats were exposed to atherogenic diet and water ad libitum for 20 days and were used to study the effect of MNK against experimental hyperlipidemia.

2.4 Pharmacological Evaluation

All animals starved for 18 hours and provided water ad libitum before the experiment. The animals were divided into five groups of six rats each. Group I served as normal control administered with 2% CMC only, Group II served as hyperlipidemic control rats received atherogenic diet, Group III and IV served as test groups received MNK 200mg/kg and MNK 400mg/kg respectively, Group V served as Atorvastatin (10mg/kg/day) considered as standard. All the groups except the normal control group administered atherogenic diet for two days. After inducing the hyperlipidemia, the respective treatment was continued for 7 days. Animals were given standard pellet diet and water ad libitum. The next day after the completion of experimental study, the blood was taken from the rats under mild anesthetic state by retro orbital sinus puncture. The collected blood samples were centrifuged (2500 rpm) for 10 minutes. Then serum samples were separated and it was used for various biochemical analyses. Then animals were sacrificed and the liver were taken for histopathological study. Liver lipid extraction includes in which the liver was homogenized in cold 0.15M KCl and extracted with CHCl3: CH3OH (2% v/v). This lipid extract was used for the estimation of lipid parameters. Biochemical analysis includes serum total cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL) by standard enzymatic calorimetric methods.

2.5 Histopathology

All rats were sacrificed after the collection of blood sample. Liver was excised from the rats to visually detect gross lesions, and weighed to determine weight variation and preserved in 10% neutral formalin for histopathological assessment. The tissue was embedded in paraffin, and then sectioned, stained with haematoxylin and eosin and were examined microscopically.

2.6 Statistical evaluation

All the values were expressed as mean ± standard error of mean. The data were statistically analyzed by one-way ANOVA followed by Dunnett's t-test, and value $P < 0.05$ was considered to be significant. *$p<0.001$;**$p<0.01$ vs control.

3. Result

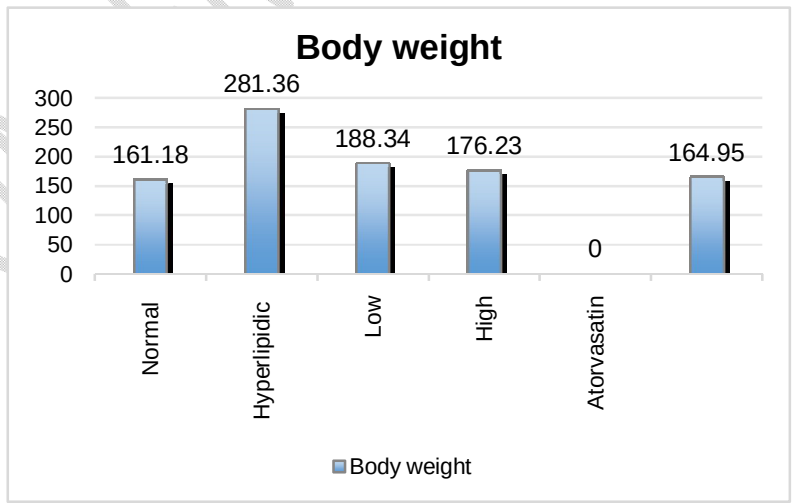
3.1 Body weight

The results of effect of MNK on body weight of atherogenic induced hyperlipidemic rats shows that the total body weight in the hyperlipidemia-induced group have significantly increased compared to normal rats. The values have risen to 281.36±1.15 mg/dl compared to Group I (normal rat group), in which values lie in the range 161.18±3.28 mg/dl. This indicates hypercholesterolemia. In the treatment group treated with MNK (200 mg/kg) and MNK (400 mg/kg), the values are reduced 188.34±2.19 ($P < 0.001$) and 176.23±0.66mg/dl ($P < 0.01$), respectively. There is a significant reduction in total cholesterol values in MNK treatment group. On the other hand, atorvastatin also has significantly reduced serum total cholesterol levels to 164.95±0.78mg/dl ($P < 0.001$) as shown in the Table 1 and Figure 1.

Table 1: Effect of MNKon body weight of hyperlipidemic rats.

S.no	Groups	Body weight
1	Normal control	161.18±3.28
2	Hyperlipidic Control	281.36±1.15
3	MNK 200mg/kg	188.34±2.19*
4	MNK 400mg/kg	176.23 ±0.66*
5	Atorvastatin(10mg/kg/day)	164.95±0.78**

Figure 1: Effect of MNKon body weight of hyperlipidemic rats.



3.2 Blood lipid profile

Total cholesterol levels in the hyperlipidemia-induced group have significantly increased compared to normal rats. The values have risen to 175.14 ± 1.35 mg/dl

compared to Group I (normal rat group), in which values lie in the range 70.85 ± 1.27 mg/dl. This indicates hypercholesterolemia. In the treatment group treated with MNK (200 mg/kg) and MNK(400 mg/kg), the values are reduced to 79.31 ± 1.26 ($P < 0.001$) and 70.53 ±1.42 mg/dl ($P < 0.01$), respectively. There is a significant reduction in total cholesterol values in MNK treatment group. On the other hand, atorvastatin also has significantly reduced serum total cholesterol levels to 66.5 ± 1.11 mg/dl ($P < 0.001$) as shown in the Table 2 and Figure 2.

The TG levels have reached as 183.17±1.53mg/dl in hyperlipedemia-induced group compared to normal rats where the values are 70.14±1.52mg/dl. This indicates triglyceridemia. In the group treated with MNK (200 mg/kg) and (400 mg/kg), the values are significantly reduced to 64.65±1.05*mg/dl ($P < 0.01$) and 62.81±0.58mg/dl ($P < 0.01$), respectively. In the atorvastatin treated group, the values are reduced to 58.85±1.76mg/dl ($P < 0.001$) [Table 2] [Figure 2].

LDL-cholesterol in atherogenic-induced group has significantly increased to 18.87±1.36 mg/dl compared to normal rat group, 47.15±1.29mg/dl. In the group treated with MNK(200 mg/kg) and (400 mg/kg), the values were reduced to 31.19±1.52and 29.31±1.05 mg/dl ($P < 0.001$), respectively. There is a significant reduction in LDL-cholesterol values in MNK treatment group. atorvastatin has significantly reduced LDL-cholesterol level to 18.65±1.28mg/dl ($P < 0.001$) [Table 2] [Figure 2].

HDL-cholesterol in atherogenic -induced group has significantly decreased compared to normal rats. The values have reduced to 41.34±1.49mg/dl compared to normal rat group, 20.13±1.12mg/dl. In the group treated with MNK(200 mg/kg) and (400 mg/kg), the values were 23.21±0.93 ($P < 0.01$) and 34.17±1.20mg/dl ($P < 0.01$), respectively. In atorvastatin treated group, the values were 40.23±1.45mg/dl ($P < 0.001$) [Table 2] [Figure 2].

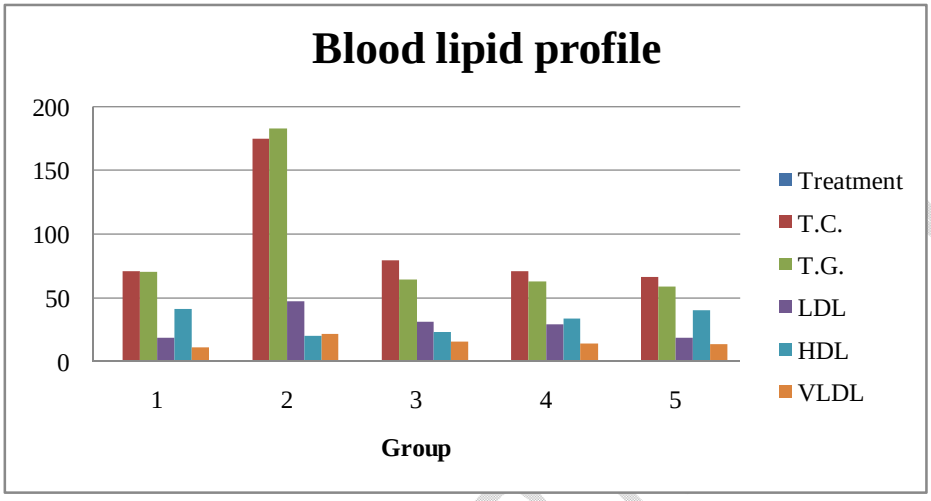
VLDL-cholesterol in atherogenic-induced group has significantly increased to 21.81±1.57mg/dl compared to normal rat group, 11.34±1.05mg/dl. In the group treated with MNK (200 mg/kg) (400 mg/kg), the values are reduced to 15.39±0.40 ($P < 0.01$) and 14.11±1.61mg/dl ($P < 0.01$), respectively. There is a significant reduction in MNK treatment group. atorvastatin has significantly reduced VLDL-cholesterol level to 13.62±1.91 mg/dl ($P < 0.001$) [Tables 2] [Figure 2].

Table 2: Effect of MNKon Blood lipid profile of hyperlipidemic rats.

Group	Treatment	T.C.	T.G.	LDL	HDL	VLDL
I	Normal Control	70.85±1.27	70.14±1.52	18.87±1.36	41.34±1.49	11.34±1.05
II	Hyper lipidimic Control	175.14±1.34	183.19±1.42	47.15±1.29	20.13±1.12	21.81±1.57
III	MNK 200mg/kg	79.31±1.26*	64.65±1.05*	31.19±1.52*	23.21±0.93*	15.39±0.40*

IV	MNK 400mg/kg	70.53±1.42*	62.81±0.58*	29.31±1.05*	34.17±1.20*	14.11±1.61*
V	Atorvastatin 10Mg/kg	66.5±1.11**	58.85±1.76**	18.65±1.28*	40.23±1.45*	13.62±1.91**

Figure 2 Effect of MNKon Blood lipid profile of hyperlipidemic rats.



3.3 Liver lipid profile

Total cholesterol levels in the hyperlipidemia-induced group have significantly increased compared to normal rats. The values have risen to 154.17±1.12mg/dl compared to Group I (normal rat group), in which values lie in the range 73.17±0.91mg/dl. This indicates hypercholesterolemia. In the treatment group treated with MNK (200 mg/kg) and MNK(400 mg/kg), the values are reduced 80.15±0.96 ($P < 0.001$) and 73.3±0.89mg/dl ($P < 0.01$), respectively. There is a significant reduction in total cholesterol values in MNK treatment group. On the other hand, atorvastatin also has significantly reduced serum total cholesterol levels to 71.05±1.01 mg/dl ($P < 0.001$) as shown in the Table 3 and Figure 3.

The TG levels have reached as 156.82±1.50 mg/dl in hyperlipedemia-induced group compared to normal rats where the values are 68.75±1.97 mg/dl. This indicates triglyceridemia. In the group treated with MNK (200 mg/kg) and (400 mg/kg), the values are significantly reduced to 82.15±1.07 mg/dl ($P < 0.01$) and 72.12±1.01/dl ($P < 0.01$), respectively. In the atorvastatin treated group, the values are reduced to 65.25±1.19 mg/dl ($P < 0.001$) [Table 3] [Figure 3].

LDL-cholesterol in atherogenic-induced group has significantly increased to 43.32±2.57mg/dl compared to normal rat group, 18.17±1.74 mg/dl. In the group treated with MNK(200 mg/kg) and (400 mg/kg), the values were reduced to 31.22±1.15and 20.7±1.22mg/dl ($P < 0.001$), respectively. There is a significant reduction in LDL-cholesterol values in MNK treatment group. Atorvastatin has significantly reduced LDL-cholesterol level to 18.98±1.45mg/dl ($P < 0.001$) [Table 3] [Figure 3].

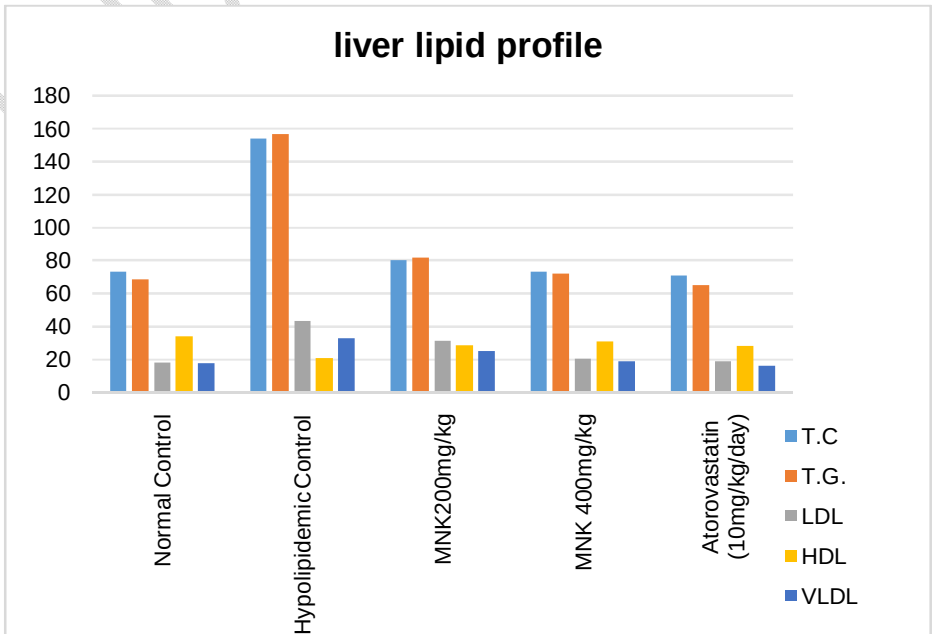
HDL-cholesterol in atherogenic -induced group has significantly decreased compared to normal rats. The values have reduced to 21.25±1.17mg/dl compared to normal rat group, 34.27±0.85mg/dl. In the group treated with MNK(200 mg/kg) and (400 mg/kg), the values were 28.96±0.98 ($P < 0.01$) and 31.15±1.54/dl ($P < 0.01$), respectively. In atorvastin treated group, the values were 28.4±1.13mg/dl ($P < 0.001$) [Table 3] [Figure 3].

VLDL-cholesterol in atherogenic-induced group has significantly increased to 33.1±1.07mg/dl compared to normal rat group, 17.69±1.38 mg/dl. In the group treated with MNK (200 mg/kg) and (400 mg/kg), the values are reduced to 25.32±0.39 ($P < 0.01$) and 18.98±1.26 mg/dl ($P < 0.01$), respectively. There is a significant reduction in MNK treatment group. atorvastatin has significantly reduced VLDL-cholesterol level to 16.5±0.98 mg/dl ($P < 0.001$) [Tables 3] [Figure 3].

Table 3 Effect of MNK on liver lipid profile of hyperlipidemic rats

Group	Treatment	TC	T.G.	LDL	HDL	VLDL
I	Normal Control	73.17±0.91	68.75±1.97	18.17±1.74	34.27±0.85	17.69±1.38
II	Hypo lipidemic Control	154.17±1.12	156.82±1.50	43.32±2.57	21.25±1.17	33.1±1.07
III	MNK 200mg/kg	80.15±0.96*	82.15±1.07*	31.22±1.15*	28.96±0.98*	25.32±0.39*
IV	MNK 400mg/kg	73.3±0.89*	72.12±1.01*	20.7±1.22*	311.15±1.54*	18.98±1.26*
V	Atorvastatin 10mg/kg/day	71.05±1.01*	65.25±1.19*	18.98±1.45*	28.4±1.13*	16.5±0.98*

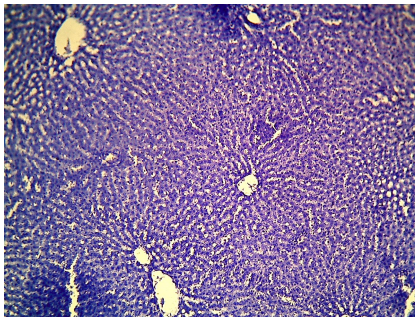
Figure 3 Liver lipid profile of effect of MNK on hyperlipidemic rats



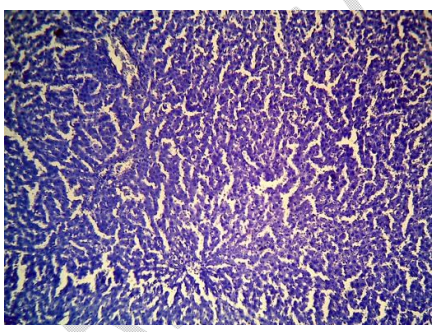
3.4 Histopathology

The liver section of normal control rats shown parenchyma with hepatocyte which appear normal and central vein, portal tract were also normal. Hyperlipedemic Control rats liver sections shown parenchyma with scattered focal area of necrosis of hepatocyte. The liver section of positive control shown normal parenchyma. Treatment group IV rats shown parenchyma with minimal necrosis, and minimal inflammation. Treatment group V shown parenchyma with hepatocyte which appear normal, and central vein & portal tract are normal. The following histopathologic images in the figure 4 shows the above results of MNK on rat groups.

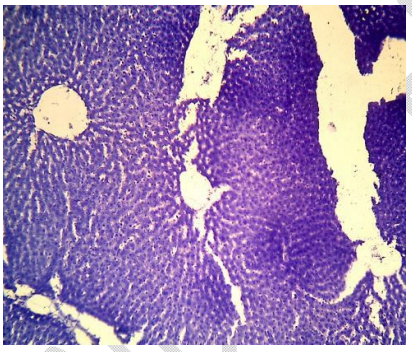
Figure 4 Histopathologic images of rat liver



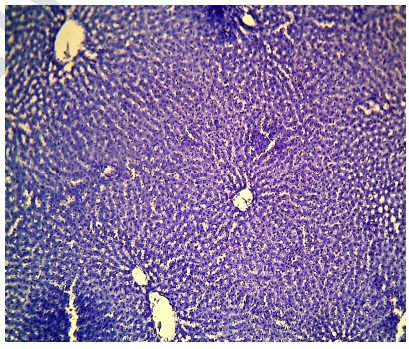
Normal Control Rat



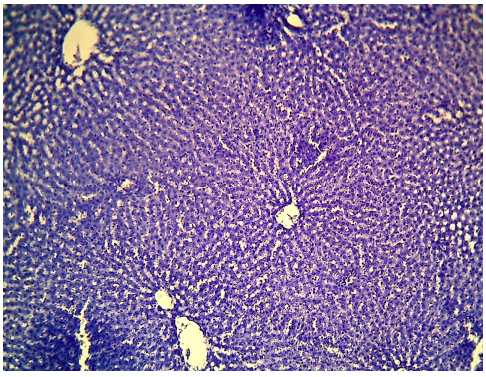
Hyperlipidemic Control Rat



Positive Control



Treatment Group IV



Treatment Group V

4. Discussion

The present study shown that there is increase in body weight in atherogenic diet induced hyperlipidemic rats, which shows Hypercholestrolemia whereas reduction in body weight in MNK treated group. Also, all atherogenic diet-induced rats had hyperlipidaemia, as evidenced by increased liver and blood cholesterol, triglyceride, VLDL, and LDL levels, as well as a decrease in HDL. According to previous studies and researches, there is a correlation between a lower risk of ischaemic heart disease and elevated HDL cholesterol and decreased levels of TC, LDL cholesterol, and TG. A higher VLDL causes the highest possible quantity of LDL to develop, which may stick to blood vessel walls and obstruct regular blood flow. The decrease in cholesterol may indicate that lipolysis or inhibition has enhanced the oxidation of fatty acids that have been mobilized[10]. There is a well-established correlation between elevated levels of low-density lipoprotein (LDL-C) and elevated risk of coronary artery disease (CAD) and low High-density lipoprotein (HDL-C)[11].

Atherogenic diet which induce acute hyperlipidemia, particularly in rats has been used for screening natural or chemical hypolipidemic drugs. The results showed that MNK produced a significant reduction in cholesterol level and also it reversed atherogenic induced hyerlipidemic effect in rats. Similarly, MNK at a dose of 200 and 400mg/kg significantly lowered both plasma triglycerides and cholesterol levels. The reduction of total cholesterol by the MNK at the dose level of 200 and 400 mg kg may be associated with a decrease of LDL, which is the ultimate aim of many hypolipidemic agents.

Also, other research evidence include that there is hypolipidemic activity, hepatoprotective activity, nephroprotective activity in *Phyllanthus Amarus (Keezhanelli)*[12], and there is hypolipidemic activity, anti inflammatory activity, hepatoprotective activity, rejuvenative property in *Ecliptaprostrata (Karisalai)*[13] which are all the ingredients of MNK which also supports the results of the present study. The study of hypolipidemic activity of MNK, which is lowering cholesterol levels, may increase the fecal bile excretion with the consequent reduction of hepatic cholesterol because of its metabolism. This shows the slow down of the rate of diffusion through the intestinal mucosa which in turn reducing the absorption of cholesterol and triglycerides.

5. Conclusion

The results obtained from the pharmacological screening have led to the conclusions that the study medicine *ManjalNoikuKudineer*(MNK) have significant hypolipidemic activity. Hence it can be exploited as antihyperlipidemic therapeutic agent or adjuvant in existing therapy for the treatment of hyperlipidemia. Further study by measurement of heparin-releasable plasma LPL activity and LCAT activity is significant can be undertaken.

6. References

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