Antihypercholesterolemic Activity of Fruits of *Terminalia chebula* Retz. (Phanga) in Triton induced Hyperlipidemic Rats

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This study was conducted to investigate antihypercholesterolemic activity of 70% ethanolic extract from fruits of *Terminalia chebula* Retz. on triton induced hyperlipidemic rats. Phytochemical tests and acute toxicity test of 70% ethanolic extract of the fruits on albino mice by using OECD 423 guideline were done. A laboratory based experimental animal study with control group was carried out. Thirty albino rats of both sexes for antihypercholesterolemic activity study of fruits of *Terminalia chebula* were used from 2016 to 2017. After 18 hours fasting, each rat was given single dose of triton WR- 1339 (400 mg/kg body weight) intraperitoneally to induce hyperlipidemia. Group1 was hyperlipidemic control group which received distilled water only. Group 2, 3 and 4 were orally given 3 different doses (2 g/kg, 4 g/kg and 5 g/kg) of the extract of the fruits respectively. Group 5 (standard drug group) was given atorvastatin (30 mg/kg) orally. Determination of blood lipid levels from tail veins of the rats were done by using SD Lipidocare Analyzer at baseline (before triton injection) and 48 hours after triton injection. Phytochemical study of this fruit showed the presence of alkaloids, flavonoids, glycosides, steroids/terpene, polyphenol, tannin and saponin. In acute toxicity test, it was found that the median lethal dose (LD_{50}) of the extract was more than 5 g/kg body weight. It was observed that there were significant increases in mean blood total cholesterol level (327.67±19.24) mg/dl and triglyceride levels (>650 mg/dl) in hyperlipidemic control group. The extract (5 g/kg) receiving group only showed significant decrease in mean total cholesterol level (260.33±65.49 mg/dl) when compared with hyperlipidemic control group ($p<0.05$). Mean percent reduction of blood total cholesterol level was 20.55% .There were no reductions of blood triglyceride levels. Therefore, 70% ethanolic extract of fruits of *Terminalia chebula* Retz. (5 g/kg) had significant antihypercholesterolemic activity in triton induced hyperlipidemic rats.

Keywords: Antihypercholesterolemic activity, *Terminalia chebula* Retz., hyperlipidemic rats

INTRODUCTION

Hyperlipidemia is a disorder of lipid metabolism manifested by raised plasma levels of one or more of total cholesterol, low density lipoprotein cholesterol (LDL), triglyceride or both total cholesterol and triglyceride (Joint European Society of cardiology guideline, 2012).[1]

Hyperlipidemia is a major cause of atherosclerosis and atherosclerosis associated conditions such as coronary heart disease (CHD), ischaemic cerebrovascular disease and peripheral vascular diseases.[2] Raised

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DOI: https://doi.org/10.34299/mhsrj.009110

cholesterol increase the risk of ischaemic heart disease and stroke. Raised total cholesterol is a major cause of cardiovascular disease burden in both the developed and developing world as a risk factor for ischaemic heart disease and stroke. WHO estimated that almost 20 % of all stroke and more than 50 % of all heart attack can be linked to high cholesterol level.[3]

In the present day, lipid lowering drugs are used to treat hyperlipidemia. However, all the drugs have side effects. Because of their side effects, researchers make great efforts in the production of new drugs which will effectively decrease the plasma lipid levels with least side effects.

There are many medicinal plants and herbal drugs which have been used throughout the world for the treatment of hyperlipidemia. Myanmar is rich in plants and many of which have medicinal properties.

Terminalia chebula Retz. (Family-Combretaceae) is known as Phanga (ဖန်ခါး) which widely grows in middle region and hilly region of Myanmar and also widely grow in India. In Myanmar and Indian traditional medicine, the plant is useful in the treatment of indigestion, jaundice, skin infection and cough.[4] In India, it is component of the classic Ayurvedic combination called "Triphala"(fruits of *Terminalia chebula*, *Terminalia bellirica* and *Emblica officinalia*) having hypoglycaemic and antiinflammatory activity.[5] It was reported that the fruits extracts of Triphala were found to have antioxidant activity.[6]

It was also reported that the fruit of *Terminalia chebula* Retz. possessed many pharmacological activities such as hypolipidemic, antibacterial, antistress and hypoglycaemic activities.[5, 7] The fruits also possessed hypocholesterolaemic activity against cholesterol induced hypercholesterolaemia and artherosclerosis in rabbits.[8] In atherogenic diet induced hyperlipidemic model of the rats, the rats receiving treatment with dried fruit powder of *Terminalia chebula* Retz. showed significant reduction in total cholesterol, triglycerides and elevation of high density lipoprotein cholesterol.[9]

As far as we know, there is no scientific report of hypolipidemic effect of fruits of *Terminalia chebula* Retz. in Myanmar. This study was focused on determination of antihypercholesterolemic activity of 70% ethanolic extract of *Terminalia chebula* Retz. fruits on triton induced hyperlipidemia in albino rats.

The specific objectives of the study were:

- To investigate the phytochemical constituents and physicochemical parameters of *Terminalia chebula* Retz. fruits,
- To investigate the acute toxicity of 70% ethanolic extract of *Terminalia chebula* Retz. fruits in albino mice,
- To determine the antihypercholesterolemic activity of 3 different doses of 70% ethanolic extract of *Terminalia chebula* Retz. fruits in triton induced hyperlipidemic rats,
- To compare the antihypercholesterolemic activity of 70% ethanolic extract of *Terminalia chebula* Retz. fruits with that of standard drug, atorvastatin in triton induced hyperlipidemic rats.

MATERIALS AND METHOD

Study design

Study design was laboratory based experimental animal study.

Place of study and study period

The study was in Pharmacology Research Division, Department of Medical Research and study period was in 2016-2017.

Plant materials and preparation for plant extraction

Terminalia chebula Retz. mature fruits were collected from Magwe Region and identified by the Botanist from Botany Department, Yangon University. *Terminalia chebula* Retz. fruits excluding seeds were cut into small pieces and air dried in the shade.

Air dried fruits (4 kg) were made into powder by using grinding machine. The air dried fruit powder were extracted with 70% ethanol to get 70% ethanolic extract.[10]

Phytochemical studies and physicochemical tests Terminalia chebula Retz. fruits

Phytochemical tests of *Terminalia chebula* Retz. fruits were performed qualitatively to investigate the phytochemical constiuents such as alkaloids, flavonoids, glycosides, polyphenol etc. by using the method of Harborne.[11]

For safety purpose of medicinal plants, the physicochemical tests of *Terminalia chebula* Retz. fruits were done to investigate the Quality Control Parameters of the fruits (such as swelling index, foaming index, moisture content, total ash, water soluble ash, acid insoluble ash, watery extract, ethanol extract, petroleum ether extract and pH) by using the method of WHO (2011).[12]

Acute toxicity study of 70% ethanolic extract of Terminalia chebula Retz. fruit in experimental animals

A total of 24 adult female albino mice (ddy strain) (12 weeks old) weighing between 25 g-30g were used for acute toxicity test of 70% ethanolic extract of *Terminalia chebula* Retz. fruit.

Acute oral toxicity test was performed by using 3 dose levels (300 mg/kg body weight, 2000 mg /kg body weight and 5000 mg/kg body weight) according to OECD guideline 423 (Organization for Economic Co-operation and Development).[13]

In this study, mice were observed after giving test substance at least once during the first 30 minutes, periodically during the first 24 hours with special attention for signs of toxicity given during the first 4 hours and daily thereafter up to 14 days. Signs of toxicity and mortality of the mice were recorded. Individual body weight of mice was measured and recorded before the test substance was administered and once weekly up to 2 weeks.

Determination of antihypercholesterolemic activity of 70 % ethanol extract of Terminalia chebula Retz. fruits in Triton WR-1339 induced hyperlipidemic rats

Thirty adult healthy albino rats (Wistar strain) of both sexes weighing (200g - 250g) were used in this study. For antihypercholesterolemic activity study in rats, 20 g of Triton WR-1339 was dissolved in 100 ml of 0.9 % physiological saline solution to obtain 20 % Triton WR-1339 solution.

Animals were divided into five groups and each group contained 6 rats. After keeping the animals in 18 hours fasting, each rat was given single dose of Triton WR-1339 (400 mg/kg) in 0.9 % physiological saline solution (i.e, 2 ml/kg) intraperitoneally to induce hyperlipidemia.

Group 1 (Hyperlipidemic control group) was given distilled water (10 ml/kg) only. Fruit extracts (2 g/kg, 4 g/kg and 5 g/kg) and Atorvastatin (30 mg/kg) were dissolved in 10 ml of distilled water for each dose. Immediately after triton injection, Group 2, 3 and 4 were given orally fruit extract in different concentrations (2 g/kg, 4 g/kg and 5 g/kg), respectively. Group 5 (standard drug group) was orally treated with standard drug, Atorvastatin (30 mg/kg) immediately after triton injection.

The second doses of the extract and Atorvastatin were given at 22 hours after triton injection according to the method described in Rodolfo (1964) and Remo and Mukesh, Sikarwar and Patil (2012).[14, 15] The blood samples for determination of lipid profiles were taken from tail vein of the rats. Fasting blood lipid profiles (Total cholesterol, Triglyceride, High density lipoprotein, Low Density Lipoprotein) of all rats were measured before and at 48 hours after Triton injection for all groups by using blood lipid measuring device, SD Lipidocare ^{R} Analyzer (SD Biosensors Inc. Korea).[14, 15]

Data analysis

The results were shown in mean±SD. Unpaired ‘t” test was used to observe the significance of difference between means of

the control and the test groups. Unpaired 't' test was also used to observe the significance of difference between means of the test and the standard drug groups. Values with $p<0.05$ was considered as statistically significant.

RESULTS

Yield percentage of *Terminalia chebula* Retz. fruits was 20% after extraction with 70% ethanol.

Acute toxicity test of 70% ethanolic extract of Terminalia chebula Retz. fruits on albino mice

It was found that 70% ethanolic extract of *Terminalia chebula* Retz. fruits showed no toxic effect and lethality on all experimental groups upto the maximum dose level of 5g/kg during the observation period of 14 days. So, Medium Lethal Dose (LD_{50}) of the extract was more than 5 g/kg. There were no significant differences of weekly body weights of the control group and extract receiving groups up to 2 weeks.

Phytochemical analysis and physicochemical test of Terminalia chebula Retz. fruits

The results of phytochemical analysis of *Terminalia chebula* Retz. fruits showed that both dried powder and extract of the fruits contained alkaloid, flavonoids, glycoside, amino acid, polyphenols, saponin, carbohydrates, reducing sugar steroids/ terpene and resin .Tannin and cyanogenic glycosides were not present in both of them.

Table 1. Physicochemical test of dried powder of fruits of *Terminalia chebula* Retz.

No.	Physicochemical test	Results
1.	Swelling index	5.1ml
2.	Foaming index	100
3.	Moisture content	6%
4.	Total ash	7.02%
5.	Water soluble ash	4.74%
6.	Acid insoluble ash	0.8%
7.	Watery extract	85.9%
8.	Ethanol extract	69.2%
9.	Petroleum ether extract	0.41%
10.	pH (1%)w/v	3.63

The results of Physicochemical test were shown in Table 1.

Effect of 70% ethanolic extract of Terminalia chebula Retz. fruits on blood lipid levels of triton induced hyperlipidemic rats

Mean blood total cholesterol, Low density Lipoprotein (LDL), triglyceride and High Density Lipoprotein (HDL) levels of triton induced hyperlipidemic control group, Group (1) at baseline (0 hour) were 102±4 mg/dl, 36±7.54 mg/dl, 101.67±22.8 mg/dl and 45.33 ±6.86 mg/dl, respectively. At 48 hour after triton injection, mean blood total cholesterol was 327.67±19.24 mg/dl and triglyceride levels were more than 650 mg/dl. There was significant increases in blood total cholesterol and triglyceride levels of Group (1) when compared with those of the baseline levels ($p<0.001$).

Mean blood total cholesterol, LDL, triglyceride and HDL levels of triton induced hyperlipidemic group (Group 2) administered with 70% ethanolic extract of the fruits (2 g/kg) at baseline (0 hour) were 104.17±3.25 mg/dl, 32.33±5.79 mg/dl, 73.5 ±16.84 mg/dl and 55±9.03 mg/dl, respectively. At 48 hour after triton injection, mean blood total cholesterol was 338.17±44.8 mg/dl and triglyceride levels were ranged (from 579 to >650 mg/dl). No significant decrease in blood total cholesterol and triglyceride levels were found when compared with those of the hyperlipidemic control group. Mean blood total cholesterol, LDL, triglyceride and HDL levels of triton induced hyperlipidemic group (Group 3) administered with 70% ethanolic extract of the fruits (4 g/kg) at the baseline (0 hour) were 103.67±3.5 mg/dl, 35.33 ±10.3 mg/dl, 83.67±26.02 mg/dl and 51± 7.97 mg/dl, respectively.

At 48 hour after triton injection, mean blood total cholesterol was 295.17±44.09 mg/dl and triglyceride levels were ranged from (369 to >650 mg/dl). No significant decreases in blood total cholesterol and triglyceride levels were found when compared with those of the hyperlipidemic control group.

Mean blood total cholesterol, LDL, triglyceride and HDL levels of triton induced hyperlipidemic group (Group 4) administered with 70% ethanolic extract of the fruits (5 g/kg) at baseline (0 hour) were 116.5 ±26.58 mg/dl, 57.5±37.28 mg/dl, 80.67 ±21.44 mg/dl and 42.5±10.78 mg/dl, respectively. At 48 hours after triton injection, mean blood total cholesterol was 260.33±65.49 mg/dl and triglyceride levels were ranged (from 397 to >650 mg/dl).

Significant decrease in blood total cholesterol was found when compared with those of the hyperlipidemic control group ($p<0.05$). Reduction of total blood cholesterol level was 20.55%. There were no decreases in blood triglyceride level when compared with the hyper-lipidemic group. Mean serum total cholesterol, LDL, triglyceride and HDL levels of triton induced hyperlipidemic group (Group 5) administered with standard drug, atorvastatin (30 mg/kg) at baseline (0 hr) were 105.67±5.47mg/dl, 47.33±7.11mg/dl, 85.5 ±16.54 mg/dl and 40.83± 3.25 mg/dl, respectively. At 48 hours after triton injection, mean blood total cholesterol was 284.67±90.83 mg/dl and triglyceride levels were ranged from (235 mg/dl to >650 mg/dl). The results were shown in Table 2. Blood total cholesterol and triglyceride levels were lower than those of the controls but they were not statistically different. Reduction of total cholesterol level was 13.13 % when compared with those of the control. There was no significant difference between mean total cholesterol level of atorvastatin and the extract of *Terminalia chebula* Retz. fruits.

This study was done to determine antihypercholesterolemic activity of 70% ethanolic extract of *Terminalia chebula* Retz. fruit in triton induced albino rats. So, the results of blood lipid levels such as total cholesterol, LDL, HDL and triglyceride levels had to be obtained in this study. But, exact values of triglyceride levels of some triton induced hyperlipidemiac rats could not be determined because triglyceride levels were more than 650 mg/dl. The reason was that this SD Lipidocare.[R] Analyzer apparatus can measure triglyceride levels up to 650 mg/dl. Moreover, LDL and HDL levels can be measured when triglyceride levels are not more than 650 mg/dl (Limitation of this study).

Table 2. Blood total cholesterol and triglyceride levels among the control, test and Atorvastatin receiving groups of triton induced hyperlipidemic rats

Treatment (Groups)	Baseline level (0 hour)		After 48 hours triton injection	
	Total Cholesterol (mg/dl)	Triglyceride (mg/dl)	Total Cholesterol (mg/dl)	Triglyceride (mg/dl)
Triton induced hyperlipidemic control (Distilled water only)	102 ±4	101.67 ±22.8	327.67 ±19.24	>650
Triton+plant extract (2 g/kg)	104.17 ±3.25	73.5 ±16.84	338.17 ±44.8	(579 - >650)
Triton+plant extract (4 g/kg)	103.67 ±3.5	83.67 ±26.02	295.17 ±44.09	(369 - >650)
Triton+plant extract (5 g/kg)	116.5 ±26.58	80.67 ±21.44	260.33 ±65.49*	(397 - >650)
Triton+standard drug (Atorvastatin 30 mg/kg)	105.67 ±5.47	85.5 ±16.54	284.67 ±90.83	(235 - >650)

Results were shown in mean±SD.
*= $p<0.05$,**=$p<0.01$,***=$p<0.001$
Unpaired t- test was used.

Statistical comparison was carried out between the triton induced hyperlipidemic control group and the extract of *Terminalia chebula* Retz. fruits receiving groups as well as standard drug, atorvastatin (30 mg/kg) receiving groups.

DISCUSSION

In this study, hyperlipidemia was induced by intraperitoneal injection of Triton WR 1339. Triton WR 1339 is one of the many well-known nonionic detergents (surfactant) that induces the elevation of plasma cholesterol and triglyceride levels by increasing the hepatic cholesterol synthesis. The sustained hyperlipaemia and hypercholesterolaemia are induced by interference with the uptake of plasmalipids by tissues. As a reaction to the impaired uptake of plasma cholesterol, the rate of endogenous cholesterol bio-

synthesis is rapidly increased in the liver. The administration of the surface active agent, triton WR 1339 induces hyper-choLesterolaemia and hyperlipaemia in many species. Triton is used to induce hyperlipidemia in rats because it has rapid onset of action and cause marked hyperlipidemia. Triton-induced hyperlipi-demia in rat model is simple and rapid for evaluation of test substances and can be considered as useful method for screening of new antihyperlipidemic drugs.[14, 15]

It was reported that oral administration of watery extract of *Terminalia chebula* Retz. fruits had no acute toxicity on mice and chronic toxicity on albino rats.[16] In this acute toxicity study, 70% ethanolic extract of *Terminalia chebula* Retz. fruits showed no toxic effect and lethality on mice up to the maximum dose level of 5 g/kg. Therefore, LD50 value was found to be more than 5 g/kg.In this study, 70% ethanolic extract of *Terminalia chebula* Retz. fruits was used because phytochemical constituents which can give antihyperlipidemic activity can be dissolved in 70% ethanol.

In this study, blood total cholesterol levels and triglyceride levels of the rats were used for data analysis because this SD Lipido-care^{R} Analyzer can measure blood lipid levels such as total cholesterol, LDL, HDL, triglyceride when blood triglyceride level of the sample is not more than 650 mg/dl. If blood triglyceride level is more than 650 mg/dl, the analyzer can measure blood total cholesterol level only and it cannot measure blood LDL and HDL levels.

In this study, blood triglyceride levels of the hyperlipidemic control rats and most of the extract and atorvastatin receiving rats were more than 650 mg/dl. In this study, in the hyperlipidemic control group which received distilled water only, there was marked increase in the levels of blood total cholesterol (TC) and triglyceride (TG) in the rats administered intraperitoneally with triton WR 1339 (400 mg/kg) ($p<0.001$).

Diapa, Kirti and Tejal (2010) reported that watery extract of fruits of *Terminalia chebula* Retz. had antihyperlipidemic activity in high cholesterol diet fed rats.[17] In this study, it was observed that extract of the fruits (5 g/kg) decreased significantly the levels of total cholesterol (TC) but blood triglyceride (TG) levels were not decreased when compared with hyperlipidemic control group. The extract of the fruits (2 g/kg and 4 g/kg) did not decrease significantly the levels of blood total cholesterol (TC) and blood triglyceride (TG) levels when compared with hyperlipidemic control group.

In the present study, atorvastatin was used as standard drug to compare the antihyperlipi-demic effect with that of the extract of *Terminalia chebula* Retz. fruits. Atorvastatin showed no significant decrease in serum TC and TG levels in this rat model. The previous researchers reported that hypolipidemic activity of medicinal plants such as *Aloe vera* gel, leaves of *Hisbiscus cannabinus* L. fruits of *Terminalia chebula* Retz. stem of *Tinospora cordifolia, Bauhinia purpurea* leaves were stuided in hyper-lipidemic animals. It was observed that plant constituents such as saponin, polyphenol, flavonoids, tannin and steroids might be responsible for hypolipidemic activity of these plants.[18] In the present study, the antihypercholesterolemic effect of the extract of *Terminalia chebula* Retz. fruits can be due to the presence of polyphenols, flavonoids, steroid and saponin obtained in it.

Conclusion

This study scientifically proved the traditional use of *Terminalia chebula* Retz. fruits for antihypercholesterolemic activity. Therefore, *Terminalia chebula* Retz. fruits can be a potential herbal medicine for treatment of hyperlipidemia.

Recommendation

Subacute toxicity study of *Terminalia chebula* Retz. fruits in albino rats should be carried out and if there is no subacute toxicity, antihypercholesterolemic activity of this fruit will be continued for clinical study in human subjects.

ACKNOWLEDEMENT

We would like to thank Director-General, Department of Medical Research for allowing us to perform this research.

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