HYPOLIPIDEMIC ACTIVITY OF THE AQUEOUS EXTRACT FROM THE *MORINDA CITRIFOLOA* LEAVES IN TRITON INDUCED HYPERLIPIDEMIC RATS

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Summary

Experimental and epidemiological studies have shown that the plasma hypercholestrelomic state is a main contributor of the development of many cardiovascular diseases. In the present study an aqueous extract from *Morinda citrifolia* leaves was evaluated for its hypocholesterolaemic and hypertriglyceridaemic activities. *Morinda citrifolia* leaf extract has not shown any side effects during acute toxicity studies Hyperlipidemia was developed by intraperitonial injection of Triton WR – 1339 400mg/kg.The animals were divided into Normal, Triton treated group, Triton plus Atorvastatin, Triton plus herb extract 150 mg/kg, Triton plus herb extract 300 mg/kg, treated groups. Oral administration of *Morinda citrifolia* leaf extract (150 mg/kg and 300 mg/kg) in both groups At 24 hrs after treatment with TRITON WR 1339 caused a significant decrease in serum lipid parameters like Triglycerides (TG), cholesterol (CH), LDL- cholesterol, Atherogenic index (AI), LDL/HDL Ratio and Total proteins as like in atorvastatin treated groups. The both extract treated groups and atorvastatin treated group caused a significant increase in HDL-cholesterol levels levels. Significant reduction in lipid parameters indicates that this herb may contain products that lowers the Serum lipid concentrations and might be beneficial in treatment of hyperlipidemia.

Key Words: *Morinda citrifolia,* Triton WR – 1339, Serum lipid parameters, Atherogenic index (AI), Total proteins, Atorvastatin, LDL/HDL ratio

Introduction

Hyperlipidemia has been ranked as one of the greatest risk factors contributing to the prevalence and severity of coronary heart disease (CHD). Hyperlipidemia is usually characterized by elevated serum total cholesterol and decrease in High density lipoprotein (HDL) cholesterol and increase in Low density lipoprotein (LDL) cholesterols. It is observed that there is also an association between hyperlipidemia, and serum Triglycerides [(1)]. It is now established that hyperlipidemia represents a major risk factor for the premature development of atherosclerosis [(2)].

Medicinal plants since times immemorial have been used in virtually all cultures as a source of medicine. The wide-spread use of herbal remedies for different ailments has been described in ancient texts such as the Vedas and bible [(3)]. In the recent times, focus on plant research has increased all over the world and large body evidence has collected to show immense potential of medicinal plants used in various traditional systems [(4)].

Morinda citrifolia Linn (Noni) is one the traditional folk medicinal plants that has been reported to have a broad range of therapeutic and nutritional value [(5)]. Major components in noni such as potassium, anthraquinones, Vitamin A & C, carotene, rutin, linoleic acid, alizarin, amino acids [(6)]. New iridoid glycosides named as citrifoliniside is identified along with five known flavonol glycosides recently [(7)] [(8)].

It was also utilized in the folk medicine for different kinds of illness for arthritis, diabetes, blood pressure, muscle aches and pains, gastric ulcers and sprains and drug addiction and also used for cancers. This plant is already proved for anti-inflammatory, anti-viral, anti-tubercular, analgesic, hypotensive, anti-hyperglycemic, and also for wound healing and immunological activities [(9)].

The present study was aimed at the assessment of the possible hyperlipidaemic activity of aqueous extract from *Morinda citrifolia* leaves in Triton WR-1339 induced hyperlipidaemic rats.

Material and Methods

Plant material

The leaves of the plant *Morinda citrifolia were* collected from the Sri Venkateswara government Poly Technique campus, Tirupati and auhentified by Dr.N.Yasodamma, Prof of Botany, Sri Venkateswara University, University, Tirupati.

Preparation of the aqueous extract

The aqueous extract from the leaves of *Morinda citrifoila* was prepared by the same method used in folk medicine with some improvements. These were then powdered coarsely. The powder was decocted in purified boiling water in the ratio of 1:16. The decoction was then kept for an overnight and filtered. To it 1-2 drops of chloroform was added and stored at 8º C in screwed glass vials. The weight/ml was estimated randomly and used for oral administration to animals.

Animal husbandry

All experiments and protocols described in the present study were approved by the Institutional Animal Ethical Committee (IAEC) of Sri Padmavathi School of pharmacy (No:1016/a/06/CPCSEA/004/2009), Tiruchanoor, Tirupati and with permission from Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Animals:

Male Wister rats (100 ± 25) were housed in groups of 6 animals and maintained under standardized condition (12-hours light/dark cycle, 24^0C) and fed with normal diet from Amruth feeds, (Pranav agro bio Ltd.Mumbai, Maharastra) and purified drinking water ad libitum.

Chemicals:

Epinephrine, DTNB (sigma), Thiobarbituric acid (TBA) and Trichloroacetic acid, Hydrogen peroxide (SD fine chemicals Ltd). Sodium dihydrogen phosphate, potassium dihydrogen phosphate, Tris buffer and all other reagents used were of analytical grade.

Triton model of hyperlipidemia

Triton WR 1339 (Tyloxapol, Sigma – Aldrich, USA) was dissolved in normal saline (pH 7.4) and administred intraperitonially to the rats (400mg/kg B.W) in order to develop an acute hyperlipidemia in them.

Experimental design:

The experiment was conducted for 28 days. Male Wister rats (n= 30) were divided in to 5 groups of 6 animals each. Normal group which Received normal diet. Triton WR 1339 400mg/kg, i.p, Extract 150 mg/kg oral + Triton WR 1339 400mg/kg, i.p, Extract 300 mg/kg oral + Triton WR 1339 400mg/kg, i.p, 5mg/kg Atorvastatin + Triton WR 1339 400mg/kg, i.p.

Instruments

UV spectra were recorded in analytical UV-Visible spectrophotometer.

Analytical procedures

Serum triglycerides, total cholesterol, HDL and LDL-cholesterol and total proteins

Serum triglycerides, total cholesterol, HDL and LDL-cholesterol and total proteins were quantified by an enzymatic method using SPAN Diagnostics kits.(Surat,India).

The LDL- cholesterol was calculated by the Friedwalds formula (Harnafai et al., 2008) :

LDL- cholesterol = total cholesterol- [HDL-cholesterol + (triglycerides/5)].

Atherogenic index (AI) and LDL-C/HDL-C ratio

The AI was calculated by following formula:

AI = (total cholesterol-HDL-C)/HDL-C

The LDL-C/HDL-C was calculated as the ratio of serum LDL-C to HDL-C levels.

Histopathology

Liver and aorta were collected after the rats were sacrificed in 10% formal saline solution and utilized for the histopathological studies.

Statistical analysis

All the data expressed as mean ± SEM. Statistical significance between more than two groups was tested using one way ANOVA followed by the Tukey test using computer based fitting program (Prism, Graph pad.). Statistical significant was determined at $P < 0.05$.

Induction of Hyperlipidemia by Triton WR -1339:

Hyperlipidemia was induced by using intraperitonial injection of Trion WR-1339 at the dose of 400 mg/kg diluted in normal saline on 24hrs before the experiment. After treatment (24 h), animals were anaesthetized briefly with diethyl ether and blood was taken from their retroorbital plexus. The blood samples were immediately centrifuged (2500 rpm/20 min) and the serum was used for lipid analysis

Results

Preliminary phytochemical screening:

Preliminary phytochemical screening of the aqueous extract of M.citrifolia leaves was shown that the presence of alkaloids, carbohydrates, steroids, flavonoids, tannins and resins. Where as saponins and glycosides were absent.

Serum lipid parameters

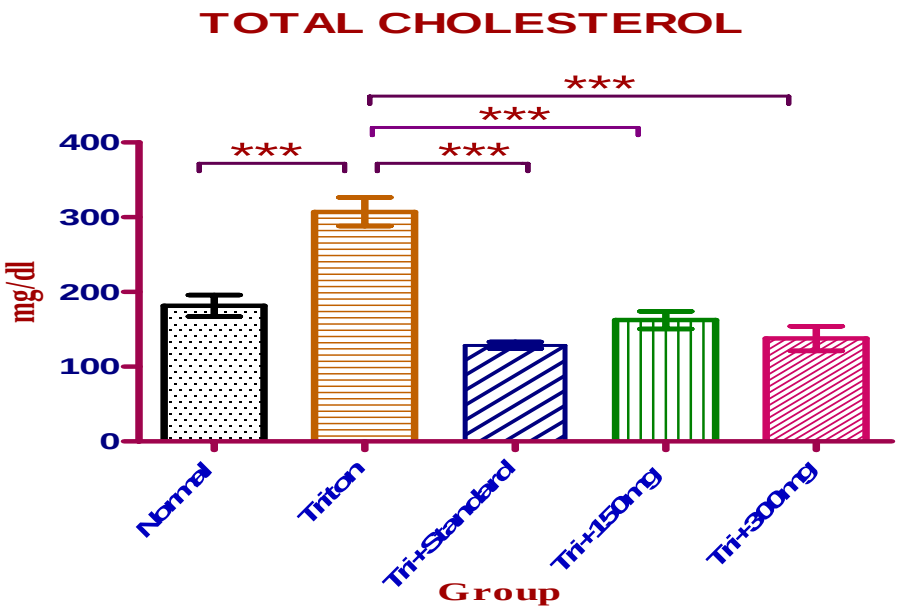
After 24 hrs of the induction of hyperlipidemia with Triton WR 1339 at the dose of 400 mg/kg i.p., marked increase in cholesterol ($p<0.001$), triglyceride concentrations ($p<0.01$) in serum was noted. The plasma cholesterol increased by all most two folds higher than the normal levels. A significant increase ($p<0.001$) in the total protein levels

and LDL-levels (p<0.001) were also observed in the rats treated with triton alone. Along with all the above changes, triton caused a drastic reduction in HDL-levels (p<0.05), when compared to normal animals. Atorvastatin was significantly decreased the all the above parameters except HDL when compared with the group which is treated with triton alone, it was observed that there is a significant increase in HDL levels in atorvastatin treated group when compared with that of the group which was treated with triton alone .

Effect of the aqueous extract of *Morinda citrifolia* leaves on rat plasma lipid profile:

The serum total cholesterol and triglyceride levels of rats treated with aqueous extract of *M. citrifolia* are shown in figures 1 and 2.

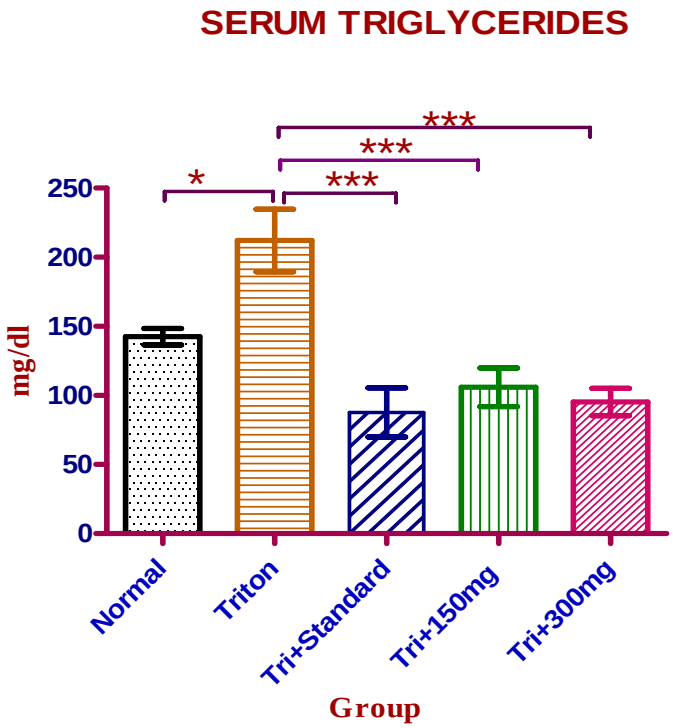
FIGURE 1:



All values shown are mean ± SEM and n = 6

- Comparisons were between normal Vs Triton treated groups, Remaining All groups were compared to Triton treated group.
- * p< 0.05, ** p<0.01,*** p < 0.001.

FIGURE 2:



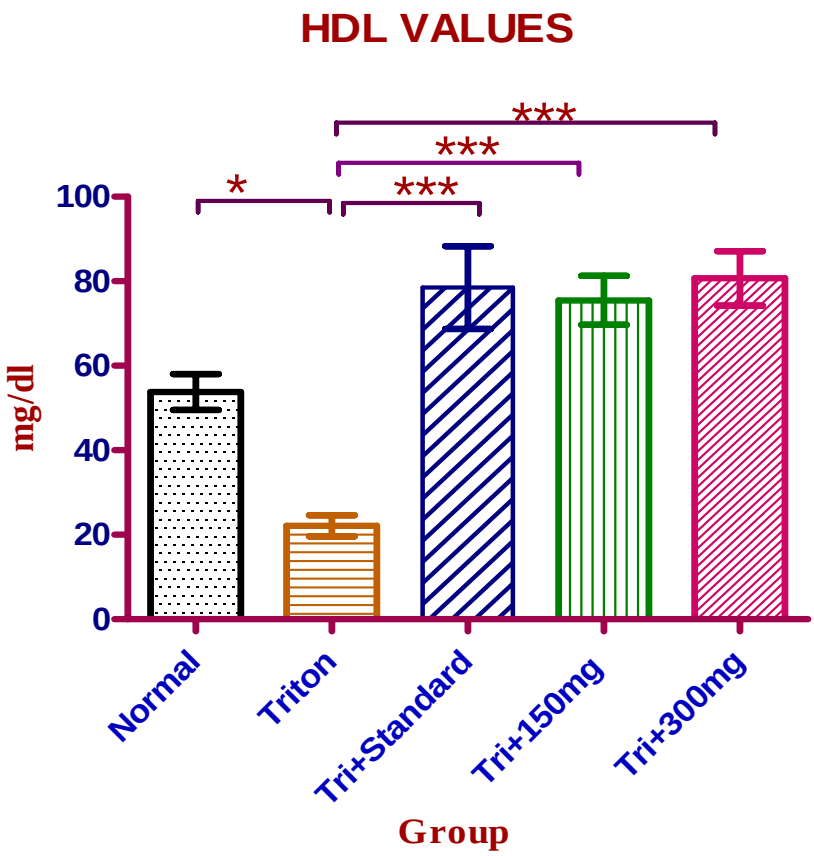
All values shown are mean ± SEM and n = 6

- Comparisons were between normal Vs Triton treated groups, Remaining All groups were compared to Triton treated group.
- * $p< 0.05$, ** $p<0.01$,*** $p < 0.001$.

Importantly the elevated total cholesterol concentrations ($p<0.001$) produced by Triton administration after 24hrs were significantly suppressed ($p<0.001$) by the test drug in both 150mg/kg and 300 mg/kg treated groups. In a similar manner a significant ($p<0.01$) reduction in triglyceride levels were also observed.

Aqueous extract of *M. citrifolia* caused a significant reduction ($p<0.001$) in the LDL-cholesterol levels and a significant increase ($p<0.001$) in HDL-cholesterol levels in the Triton WR 1339 treated rats when compared to the animals treated with triton alone (fig 3 and 4).

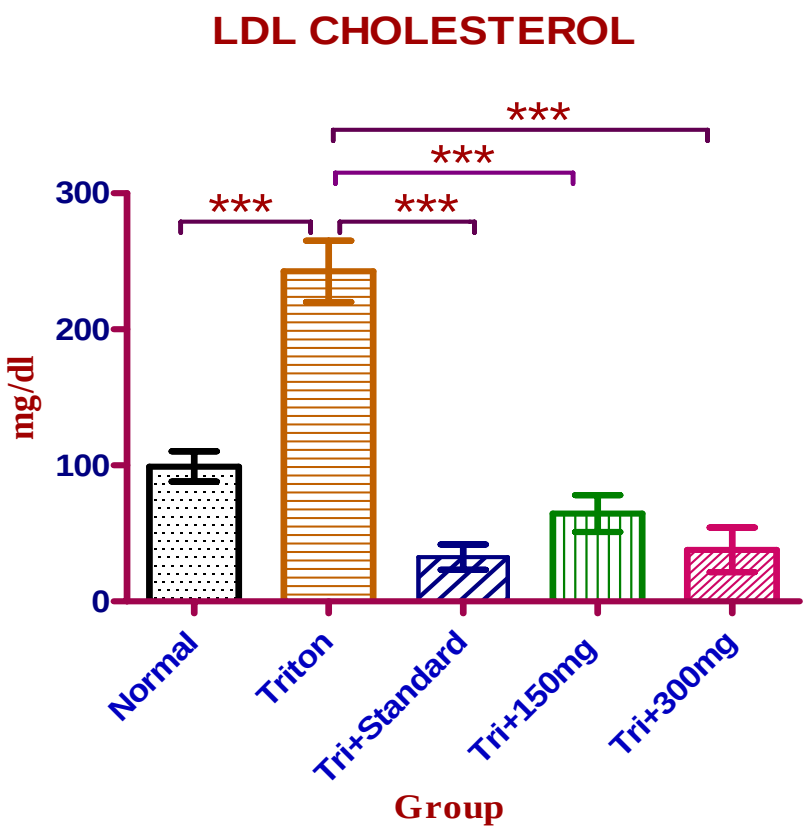
FIGURE 3:



All values shown are mean ± SEM and n = 6

- Comparisons were between normal Vs Triton treated groups, Remaining All groups were compared to Triton treated group.
- * $p < 0.05$, ** $p < 0.01$,*** $p < 0.001$.

Figure 4

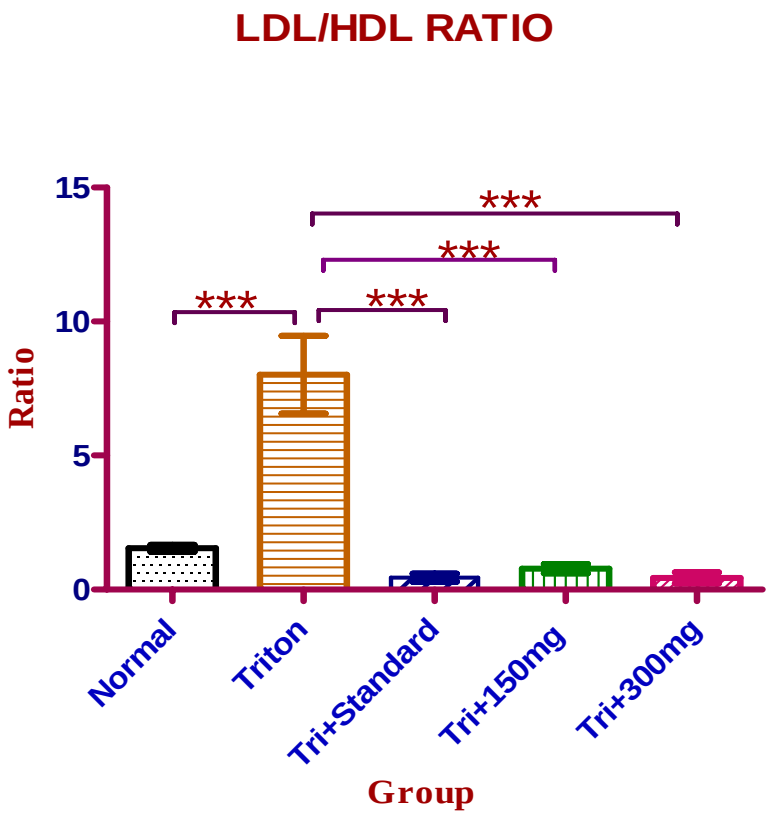


All values shown are mean ± SEM and n = 6

- Comparisons were between normal Vs Triton treated groups, Remaining All groups were compared to Triton treated group.
- * $p < 0.05$, ** $p < 0.01$,*** $p < 0.001$.

The ratio of LDL/HDL is predictive indicator of cardiovascular disease incidence. The triton injection produced a significant increase (p<0.001) of this marker. Whereas, treatment with aqueous extract of *M. citrifolia* caused a significant decrease (p<0.001) of this marker when compared to the triton treated group (fig 5).

FIGURE 5

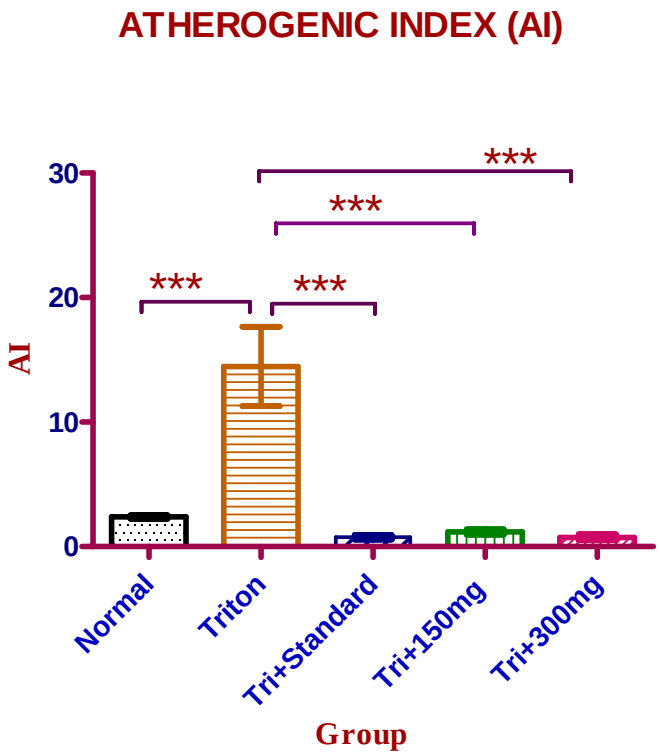


All values shown are mean ± SEM and n = 6

- Comparisons were between normal Vs Triton treated groups, Remaining All groups were compared to Triton treated group.
- * p< 0.05, ** p<0.01,*** p < 0.001.

Promising results were also observed in lowering the atherogenic index (p<0.001) and total proteins (p<0.001) when treated with the aqueous extract of *M.citrifolia* (150 mg/kg, 300 mg/kg), on comparison with the triton treated group (fig 6 and 7).

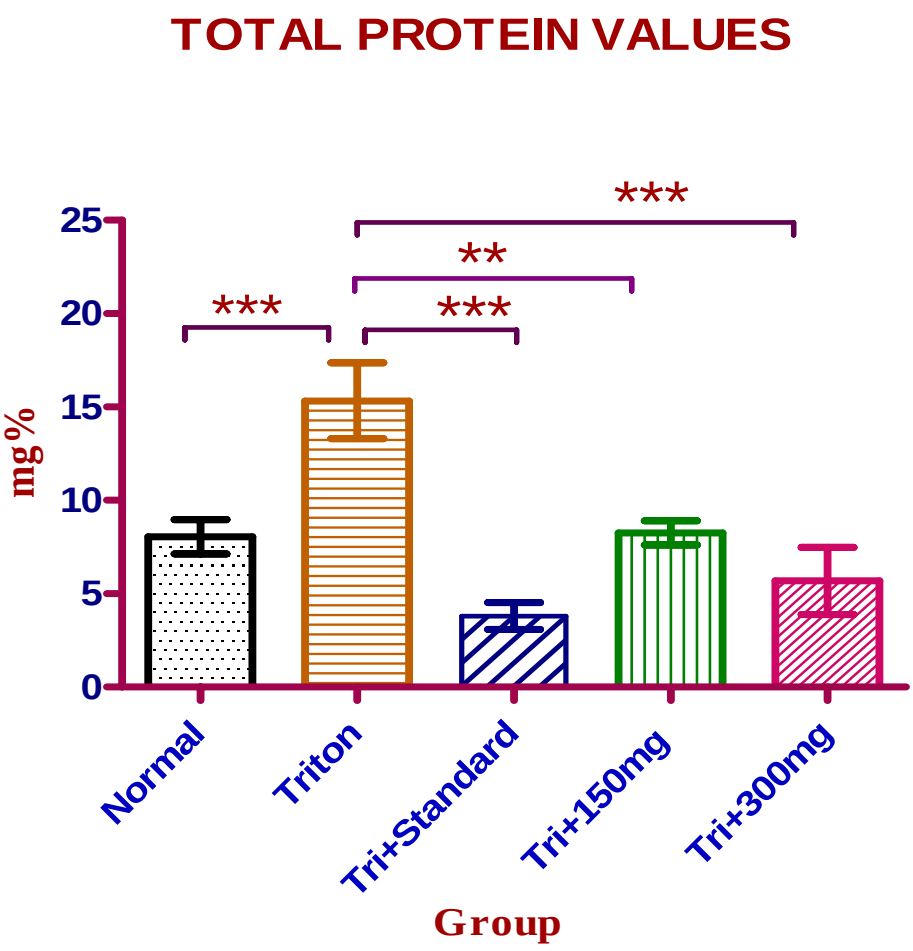
FIGURE 6



All values shown are mean ± SEM and n = 6

- Comparisons were between normal Vs Triton treated groups, Remaining All groups were compared to Triton treated group.
- * $p < 0.05$, ** $p<0.01$,*** $p < 0.001$.

FIGURE 7

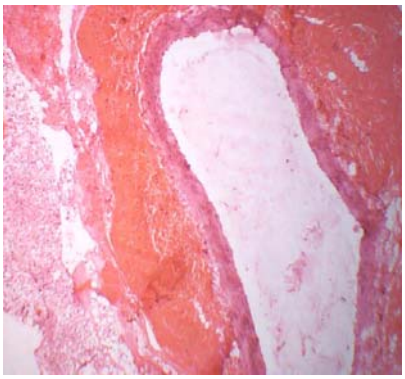


All values shown are mean ± SEM and n = 6

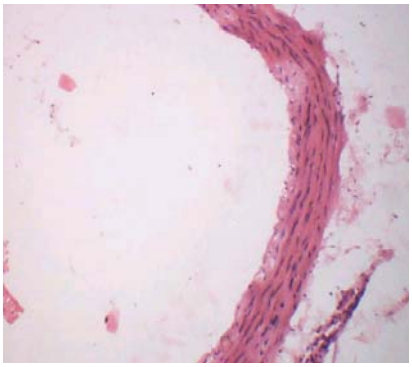
- Comparisons were between normal Vs Triton treated groups, Remaining All groups were compared to Triton treated group.
- * $p < 0.05$, ** $p < 0.01$,*** $p < 0.001$.

Histopathological studies

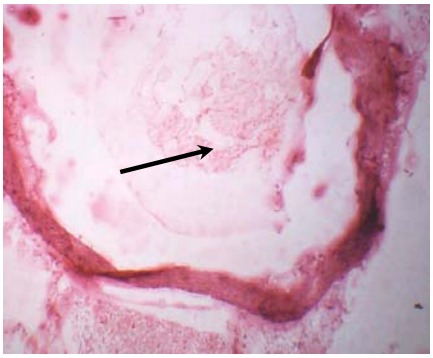
Histopathology of Aorta



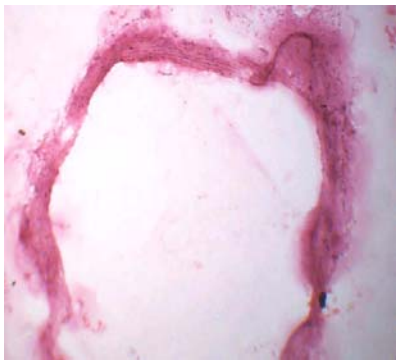
A) Normal



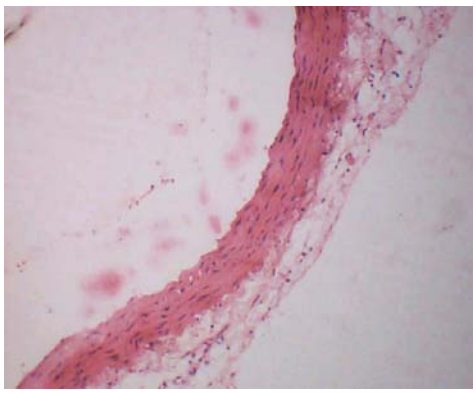
B) Atorvastatin + Triton treated



C) Triton treated

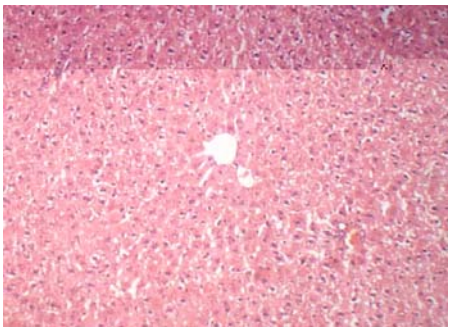


D) Triton + 150 mg extract treated

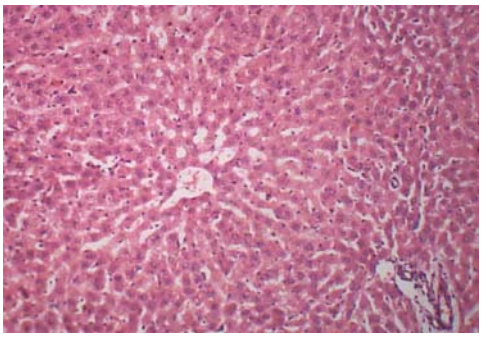


E) Triton + 300mg extract treated

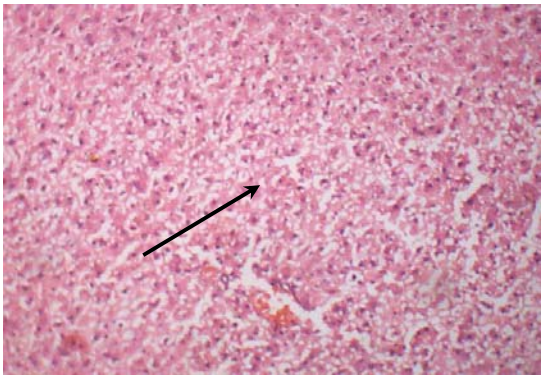
HISTOPATHOLOGY OF LIVER



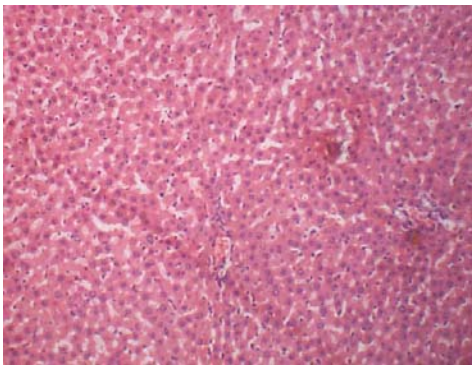
A) Normal



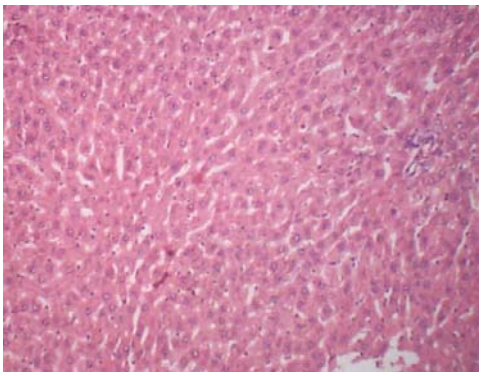
B) Triton + Atorvastatin treated



C) Triton treated



D) Triton + 150 mg /kg extract treated



E) Triton + 300mg/kg extract treated

Discussion

Hyperlipidemia has been already documented as one of the vital coronary risk factors. Hyperlipidemia, especially hypercholesterol is positively related to morbidity and mortality of coronary heart disease [(10)].

Triton WR-1339 has been widely used to block triglyceride rich proteins to induce hyperlipidemia in several animals. This model is widely used for a different aims and in particular in rats it has been used for screening natural and chemical hypolipdemic drugs [(11)].

In our hand, same model similar pattern of lipid profile changes 24hrs after triton injection in rats the feasibility of using it assess the hypolipidemic activity of aqueous *Morinda citrifolia* leaves extract.

The present study clearly suggests that the aqueous extract of the" *Morinda citrifolia*" leaves in two different doses 150 mg/kg, 300 mg/kg in rat significantly lowered the both serum total cholesterols and serum triglycerides levels after 24hrs of the administration of the triton WR-1339. Mostly from increase of VLDL secretion by the liver accompanied by a strong reduction of VLDL and LDL catabolism [(12)].

Since the portion of triglyceride in VLDL is several times lower than the cholesterol. It is not surprising that the hypolipidemic activity of the test drug is markedly higher for the cholesterol than the TG's. The Underlying mechanism of this activity is not elucidated by the present study.

The reduction of the total cholesterol by the test drug was associated with a decrease of its LDL fraction, which is the target of several hypolipidemic drugs. The underlying mechanism may be the reduction of its preferential deposition in Atherosclerotic tissues.

This result suggest that cholesterol lowering activity of the herb extract can be result from the rapid catabolism of LDL- cholesterol through its hepatic receptor for final elimination in form of the bile acids[13]. However this hypothesis is needed to be validating by experimental study.

Otherwise this finding suggests that the cholesterol lowering activity of these extracts appears to be due to the enhancement of LDL-C catabolism through hepatic receptors [13].

In addition to this aqueous extract also showed protective action by the increase in HDL-C, which is reported to have a preventive function against Athrogenisis. Since an independent inverse relationship between blood HDL-C levels and cardiovascular risk incidence has been documented and reported beyond any doubt [14]. This lipoprotein is called "good cholesterol", facilitates the metabolism of triglycerides and cholesterol from plasma to liver. Where it is catabolised and eliminated in the form of bile acids.

It was also recently reported that triglycerides play an important role in the regulation of lipoprotein metabolism. Indeed, the elevated plasma TG levels were associated with an increased incidence of CAD [15]. Moreover these higher plasma TG levels have been attributed mainly to an increased population of small dense LDL deposits which are very Atherogenic [16] and enhanced cholesterol ester mass transfer from apolipoprotein - B containing lipoproteins [11]. TG's also been reported to be a major determinant of cholesterol esterification, its transfer and HDL remodeling in human plasma [17]. Aqueous extract of *Morinda citrifolia* leaves significantly suppressed the elevated blood concentrations of TGs. This result suggests that the extract was able to restore at least partially the catabolism of triglycerides. The underlying mechanism of this activity is not elucidated in present study.

However, as hypothesized by many works with other plants the catabolic restoration of catabolic metabolism of TG's could be due to an increased stimulation of lipoprotein lipase (LPL) [18] [19].

Administration of aqueous extract of the test drug provides a beneficial action on rat lipid metabolism. In regard to the reduction of AI was decreased in all two test drug treated groups. Similar results were reported by others when studying the hypolipidemic effect of natural products [(20)]. It is also desirable to have higher plasma HDL and lower LDL- cholesterol to prevent Athrogenisis, since there is a positive co-relation between an increase LDL-C/HDL-C ratio and development of Athrogenisis.

Again the administration of the aqueous extract significantly suppressed the elevated values of LDL-C/ HDL-C ratio showing the beneficial effect of this plant in prevent in incidence. Histopathologocal findings also suggesting that the decreased lesion formation in the test drug treated groups in rats. The results found clearly demonstrate that the bioactive compound like flavones glycosides (flavonoids) exhibit different pharmacological activities including hypolipidemic and anti-atherogenic effects [(21)].

Noni appears to be cardioprotective by reducing risk of atherosclerosis and lowering blood pressure that regulates the LDL- receptor, which leads to accelerated LDL clearance from the circulation [(22)]. Major components have been identified in the Noni plant such as vitamin C and vitamin A [(6)]. Some evidences also suggests that vitamin A and vitamin C having strong anti-oxidant properties.

Conclusion

Diseases of the cardiovascular system are the most common causes of death in industrialized countries. It is now established that hyperlipidemia represents a major risk factor for the premature development of atherosclerosis and its cardiovascular complications. A logical strategy to prevent or treat atherosclerosis and reduce the incidence of cardiovascular disease is to target the hyperlipidemia by diet and or lipid lowering drugs. All synthetic drugs like statins, fibrates, and bile acid binding resins have the adverse effects like hepatic dysfunction, myostitis, rhabdomyolysis, constipation and pre-existing hemorrhoids and GIT distress, reversible myopathy.

Along with this these drugs also have tendency to produce interactions with the drugs like erythromycin, azole anti-fungals, cyclosporine, which will lead to the uncompensated adverse events in some times. In this respect many individuals disenchanted with the worth of allopathic treatments and the adverse effects that can be anticipated, to overcome this we prefer the herbal drugs to treat hyperlipidemia. The present study finds out the role of anti–hyperlipidaemic activity of aqueous extract of *Morinda citrifolia* leaves.

Administration of triton increased the lipid profile in plasma and fat formation and fat deposition in liver and aorta in rats. Administration of Triton WR–1339 in rats also increased the hepatic cholesterol biosynthesis in two folds. The increase in serum cholesterol, triglycerides, LDL cholesterol and also increased levels of total proteins has observed with decrease in HDL cholesterol levels in surfactant administered rats.

Treatment with the aqueous extract of the *Morinda citrifolia* at a dose of 150 mg/kg and 200 mg per kg in rats for 28 days significantly bought about a reduction in the all lipid parameters associated with increase in HDL. Histopathology also confirms a reduction in fat deposition in the liver and aorta in both animal species.

The present study indicates that administration of Triton WR-1339 in rats may cause hyperlipidemia and atherosclerosis, in long term usage *Morinda citrifolia* leaves may be useful in the management of above specified conditions.

Hence from the present study we can conclude that the *Morinda citrifolia* leaves may be useful in the management of hyperlipidemia and hyperlipidemia induced atherosclerosis.

Aknowledgements

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