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Evaluation of Antihyperlipidemic Activity of Ethanolic Extract of *Syzygium cumini* in Triton X-100 Induced Hyperlipidemic Rats

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HUMAN

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Keywords: Antihyperlipidemic activity, Atorvastatin, *Syzygium cumini*, Triton x-100 induced model.

ABSTRACT

Syzygium cumini is a potent medicinal plant in the Indian systems of medicine. *Syzygium cumini* having high amount of phenolic contents which are used as anti-oxidant activities. The aim of present study was to determine the Anti-hyperlipidemic activity of ethanolic pulp extract of plant *Syzygium cumini* (Linn.). Flavonoids, triterpenoids, and tannins was the first amide to be isolated from *Syzygium* species. Ethanolic extracts of *Syzygium cumini* with a dose of 200and 400 mg/kg exhibited significant anti-hyperlipidemic activity in Triton X-100 induced hyperlipidemia in rats (P<.05). It was found that flavonoids, triterpenoids, and tannins are the active constituents of the plant were responsible for the Anti-hyperlipidemic activity because this constituent has the ability to reduce the Total Cholesterol & Total Triglyceride level in rats. Atorvastatin was used as standard drug (10 mg/kg). The total time period of this study was two weeks.

INTRODUCTION

Hyperlipidemia is a condition when abnormally high levels of lipids i.e. the fatty substance are found in the blood. This condition is also called hypercholesterolemia /hyperlipoproteinemia. The human body is complex machinery and for maintaining the homeostasis of various organ and organ system. Any undesirable change will disturb the balance resulting in the diseased state (Arun Kumar et al, 2013).

Lipids are fats in the bloodstream, commonly divided into cholesterol and triglycerides. Cholesterol circulates in the bloodstream and is involved in the structure and function of cells. Triglycerides (TG) are best viewed as energy that is either used immediately or stored in fat cells.TG is manufactured in the liver from the foods or by being absorbed from the intestine (Dhaliya Salam. A et al, 2013).

Cardiovascular diseases, especially coronary heart disease (CHD), are epidemic in India. The Registrar General of India reported that CHD led to 17% of total deaths and 26% of adult deaths in 2001-2003, which increased to 23% of total and 32% of adult deaths in 2010-2013. The World Health Organization (WHO) and Global Burden of Disease Study also have highlighted increasing trends in years of life lost (YLLs) and disability-adjusted life years (DALYs) from CHD in India. (Niharika Verma et al, 2017).

Syzygium cumini (Family Myrtaceae) is also known as *Syzygium jambolanum* and *Eugenia cumini*. Today these trees are found growing throughout the Asian subcontinent, Eastern Africa, South America, Madagascar and have also naturalized to Florida and Hawaii in the United States of America.

Different parts of the *jamun* were also used for its anti-diabetic, antioxidant, anti-inflammatory, neuro psycho-pharmacological, anti-microbial, anti-bacterial, anti-HIV, antileishmanial and antifungal, nitric oxide scavenging, free radical scavenging, anti-diarrheal, antifertility, anorexigenic, gastroprotective and anti-ulcerogenic and radioprotective activities.

This research work is novel and works on *Syzygium cumini* on Anti-Hyperlipidemic activity is not carried out by anyone previously. There are no scientific studies in support of this traditional claim. Hence in the present study, an attempt has been made to investigate Anti-Hyperlipidemic effects of *Syzygium cumini.*

MATERIALS AND METHODS

Plant material

The pulp of *Syzygium cumini*, (2 kg) were collected from local area supplier of Delhi. The sample of plant material was sent to Dr. K Madava Chetty, Assistant Professor Dept. of Botany, Sri Venkateswara University, for identification and taxonomic authentication.

Chemicals

All the chemicals like Atorvastatin, Triton X-100, Cholesterol & Triglyceride Kits were brought from the Chandni Chowk, Delhi, India.

Experimental Animals

Male Wistar albino rats (b.w.150-200 gm.) maintained in the Animal-House, Ram-Eesh Institute of Vocational & Technical Education, Gr. Noida, AKTU University, Lucknow, U.P, India at room temperature of 25 ± 18°C, relative humidity of 45-55% and a 12:12 hrs light/dark cycle. Food and water were given ad libitum. Each experimental group consisted of Six animals housed in separate cages. The animals had access to standard laboratory feed.

Methods

Preparation of extract

The pulp of plant *Syzygium cumini* were collected and were authenticated. The pulp of the plant was collected and subjected to shade drying. The size was reduced and made to the coarse powder and then further passed through the appropriate sieve no. to obtain uniform particle size. The powdered pulp part extracted with ethanol and water by soxhlet apparatus. The pulp extracts were filtered and collected and concentrated by using Rotatory Flash Evaporator. The extract was used for the further experimental models.

Qualitative chemical tests

Ethanolic extract of the plant was subjected to chemical tests for the identification of their active constituents.

Acute toxicity study

Acute toxicity studies were performed according to OECD-423 guidelines (acute toxic class method). (Organization for Economic Co-operation and development, 2001). Animals were individually observed for changes in skin, mortality, general behavioral pattern, tremors, convulsions, salivation, diarrhea, lethargy, sleep, and coma a for the time period of 14 days.

Experimental Models for Evaluation of Anti-hyperlipidemic Activity

The antihyperlipidemic activity was performed by experimental model, triton x 100 induced hyperlipidemia.

Induction of Hyperlipidemia

Hyperlipidemia was induced in Male Wistar albino rats by single intraperitoneal injection of the freshly prepared solution of Triton X-100 (100 mg/kg) in physiological saline solution after overnight fasting for 18 hours. The animals were divided into five groups of six rats each. The Group I was marked as normal received standard pellet diet water. GROUP II, III, IV, and V were made Hyperlipidemic by Triton X-100 at a single dose of 100 mg/kg, i.p.Group II was marked as diseased group while GROUP III was administering with the standard drug Atorvastatin (10mg/ kg body weight) p.o for 14 days. GROUP IV and V were administering a daily dose of *Syzygium cumini* 200 and 400 mg/kg/ day p.o for 14 days, after inducing hyperlipidemia.

Collection of blood sample and analysis

On the 15^{th} day, blood was collect by heart puncture, under mild Diethyl ether anesthesia. The collected samples were centrifuged for 10 minutes. Then serum samples were collected and used for various biochemical experiments.

Biochemical analysis

The serum extract was assayed for Total Cholesterol (TC), Triglycerides (TG), High-Density Lipoprotein Cholesterol (HDL-C), Low-Density Lipoprotein Cholesterol (LDL-C) and Very Density Lipoprotein Cholesterol (VLDL-C), using standard protocol methods.

Histopathological studies

At the end of the study period, animals from experimental group were sacrificed liver and heart were collected. The transverse section of liver and heart were prepared using the usual techniques for preparation of permanent slides and these sections were observed for histopathological changes in liver and heart cells. Histopathological analysis of liver and heart were examined under light microscope.

Statistical analysis

The results were expressed as mean ± S.E.M. Statistical analysis was carried out by using ANOVA followed by Dunnet's multiple comparison tests using Graph pad PRISM software version 5.0. P values <0.05 were considered as statistically significant.

RESULTS

Effect of Triton-X-100 Induced Hyperlipidemia on Lipid Profile

1. Effect on serum Total cholesterol levels given with Triton x-100 intraperitoneal injection had increased serum TC level (182.2±5.67) when measured on the 15th day. This was significantly higher ($p<0.001$) when compared to serum TC levels in normal control rats (76.77±3.05. Triton x- 100 animals treated with Atorvastatin (10mg/kg, p.o. once daily) had serum TC level of (102.33±6.13) when measured on the 15th day. This was significantly lower ($p<0.001$) when compared to serum TC levels in Triton x-100 rats (182.2±5.67). Triton x-100 animals treated with extract 200 and 400mg/kg p.o. once daily had the serum level of (152.33±6.60 and 145.66±5.15) when measured on the 15th day. These values were significantly lower ($p<0.001$ and $p<0.001$) when compared to serum TC levels in triton x-100 rats (182.2±5.67).

2. Effect on serum Triglycerides level Rats given with Triton x-100 intraperitoneal injection had increased serum TG level (149.92±7.53) when measured on the 15th day. This was significantly higher ($p<0.001$) when compared to serum TG levels in normal control rats (71.15±7.16). Triton x-100 animals treated with Atorvastatin (10mg/kg, p.o. once daily) had serum TG level of (98.22±6.28) when measured on the 15th day. This was significantly lower ($p<0.001$) when compared to serum TG levels in Triton x-100 rats (149.92±7.53). Triton x-100 animals treated with extract 200 and 400mg/kg p.o. once daily had serum TG level

(125.36±7.05 and 120.60±8.54) when measured on the 15th day. These values were significantly lower ($p<0.001$ and $p<0.001$) when compared to serum TC levels in Triton x-100 rats (149.92±7.53).

3. Effect on serum HDL-C level Rats given with Triton x-100 intraperitoneal injection had increased serum HDL level (19.51±2.36) when measured on the 15th day. This was significantly lower ($p<0.01$) when compared to serum HDL levels in normal control rats (35.81±2.58). Triton x-100 animals treated with Atorvastatin (10mg/kg, p.o. once daily) had serum HDL level of (32.47±3.54) when measured on the 15th day. This was significantly higher ($p<0.01$) when compared to serum HDL levels in Triton x-100 rats (19.51±2.36). Triton x-100 animals treated with extract 200 and 400mg/kg p.o. once daily had serum HDL level (23.58±2.66 and 28.65±2.72) when measured on the 15th day. The extract 200 mg/kg was showed no significant changed and 400 mg/kg was significantly higher $p<0.05$ when compared to serum HDL levels in Triton x-100 rats (19.51±2.36).

4. Effect on serum LDL-C level Rats given with Triton x-100 intraperitoneal injection had increased serum LDL level (136.04±4.47) when measured on the 15th day. This was significantly higher ($p<0.001$) when compared to serum LDL levels in normal control rats (28.08±1.91). Triton x-100 animals treated with Atorvastatin (10mg/kg, p.o. once daily) had serum LDL level of (52.80±2.97) when measured on the 15th day. This was significantly lower ($p<0.01$) when compared to serum LDL levels in Triton x-100 rats (136.04±4.47). Triton x-100 animals treated with extract 200 and 400mg/kg p.o. once daily had serum LDL level (106.96±5.06 and 97.15±3.88) when measured on the 15th day. These values were showed no significant changed in LDL level when compared to serum LDL levels in Triton x-100 rats (136.04±4.47).

5. Effect on serum VLDL-C level Rats given with Triton x-100 intraperitoneal injection had increased serum VLDL level (28.77±0.30) when measured on the 15th day. This was significantly lower ($p<0.001$) when compared to serum VLDL levels in normal control rats (13.03±0.23). Triton x-100 animals treated with Atorvastatin (10mg/kg, p.o. once daily) had serum VLDL level of (18.60±0.03) when measured on the 15th day. This was significantly lower ($p<0.01$) when compared to serum VLDL levels in Triton x-100 rats (28.77±0.30). Triton x-100 animals treated with extract 200 and 400mg/kg p.o. once daily had serum VLDL level (23.86±0.21 and 22.86±0.52) when measured on the 15th day. These values were

significantly lower ($p<0.01$ and $p<0.001$) when compared to serum VLDL levels in Triton x-100) rats (28.77±0.30).

Table 1: Various parameters of antihyperlipidemic activity in Wistar rats, using ethanolic pulp extracts of *Syzygium cumini*

Treatments groups	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Normal control	76.77± 3.05	71.15± 7.16	35.81± 2.58	13.03± 0.23	15.83±0.41
Triton X- 100	182.33 ±5.67	149.92± 7.53	19.51± 2.36	136.04± 4.47	28.77± 0.30
Atorvastatin (10mg/kg)	102.33±6.13*	98.22± 6.28*	32.47± 3.54*	52.80± 2.97*	18.60±0.03*
Eth. (200mg/kg)	152.33±6.60*	125.36±7.05*	23.58± 2.66*	106.96 ±5.06*	23.86±0.21*
Eth. (400g/kg)	145.66± 5.15*	120.60±8.54*	28.65± 2.72*	97.15± 3.88*	22.86±0.52*

All values were expressed as Mean ± SEM, n.s. (nonsignificant) as compared to vehicle control group.

 ** $p<0.01$, when compared with normal control group (i.e., group I), ANOVA followed by Dunnett's t-test.

 *$p<0.05$, when compared with normal control group (i.e., group I), ANOVA followed by Dunnett's t-test.

 $p>0.05$, ns (not significant) when compared with the group I.

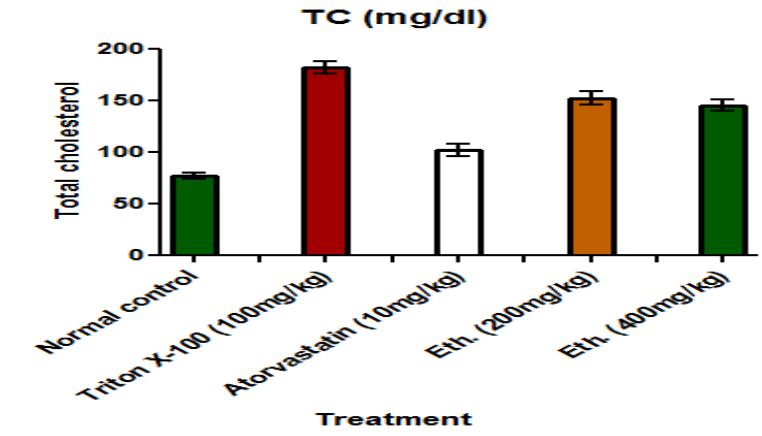

Figure No. 1: Effect of different quantity of different doses on Conc. of TC

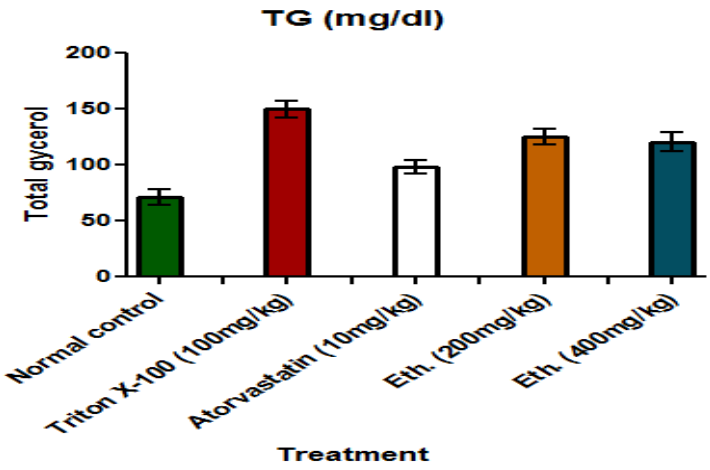

Figure No. 2: Effect of different quantity of different doses on Conc. of TG

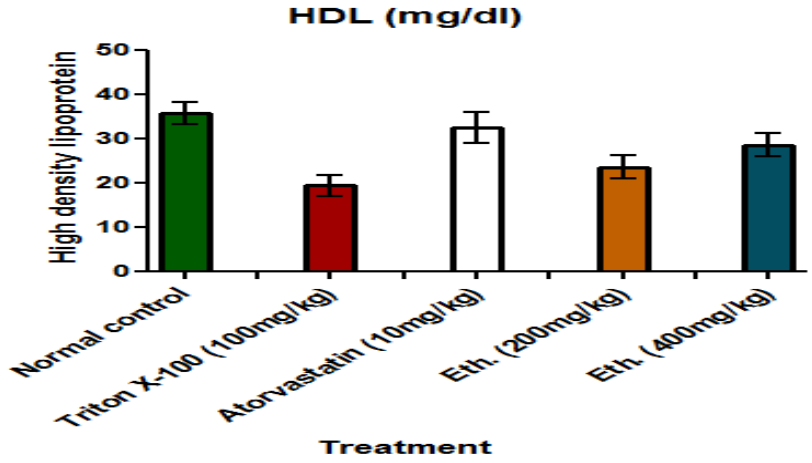

Figure No. 3: Effect of different quantity of different doses on Conc. of HDL

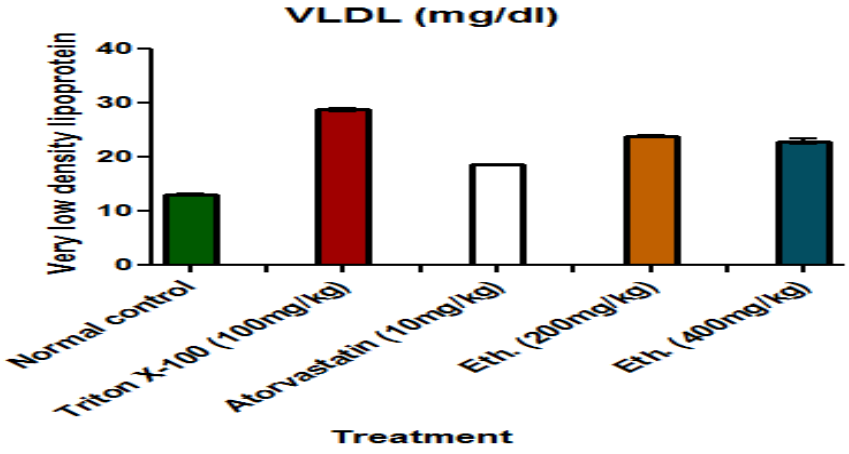

Figure No. 4: Effect of different quantity of different doses on Conc. of VLDL

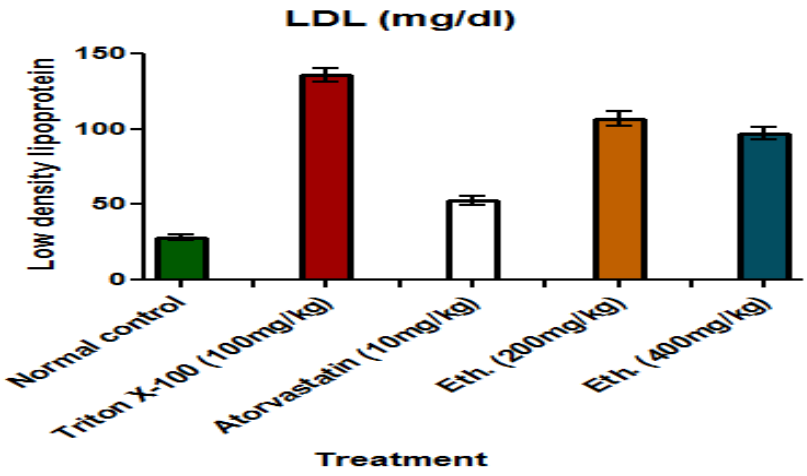

Figure No. 5: Effect of different quantity of different doses on Conc. of LDL

HISTOPATHOLOGY OF RAT HEART

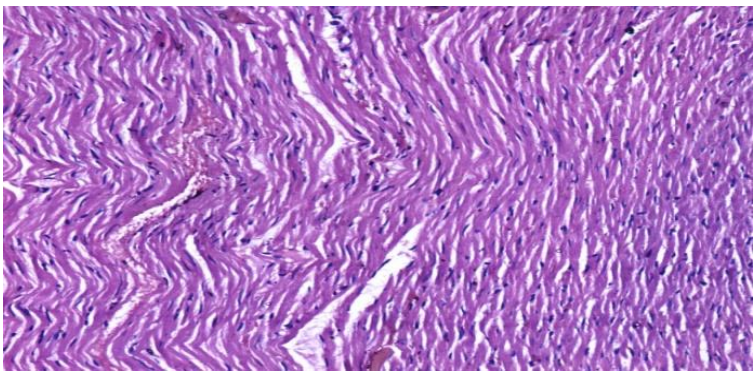

Figure No. 6: Rat heart of normal control group showing normal cardiac myocytes. (H&E X 200)

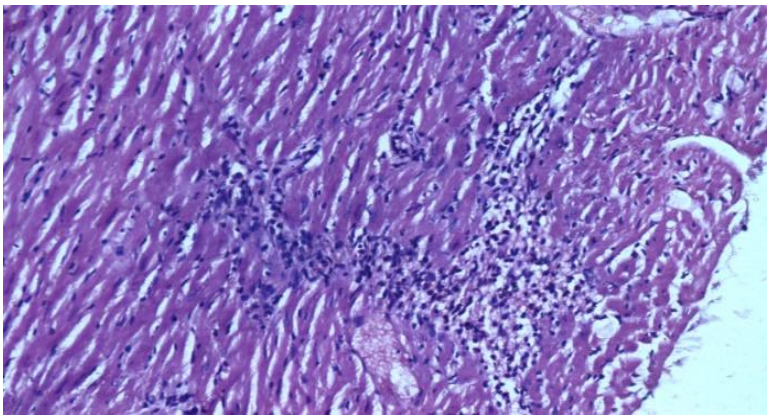

Figure No. 7: Heart of rat treated with Triton x-100 shows focal myocyte necrosis with inflammatory infiltrate and focal edema leading to mild separation of cardiac myocytes.

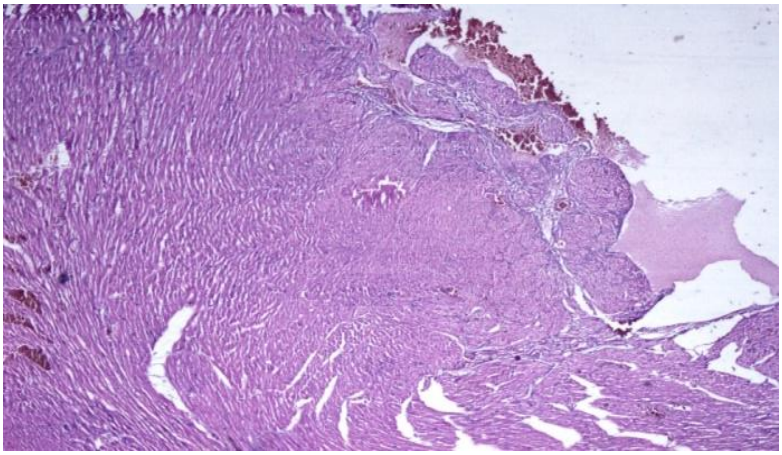

Figure No. 8: Heart of rat treated with low dose extract shows mild edema leading to separation of cardiac myocytes. (H&E X 400)

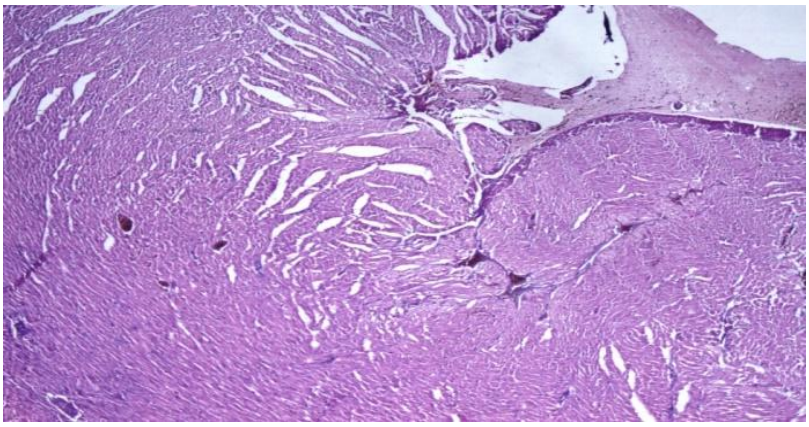

Figure No. 9: Heart of rat treated with high dose extract shows significant myocardial edema with separation of cardiac myocytes. (H&E X 40)

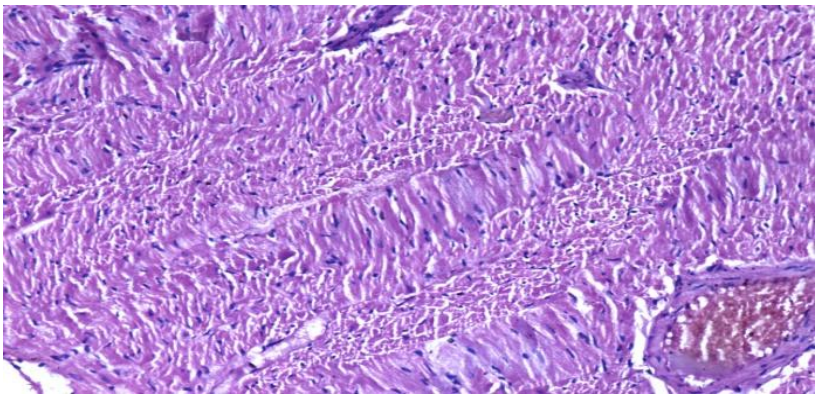

Figure No. 10: Heart of rat treated with Atorvastatin shows normalization of the myocardium. (H&E X 200)

HISTOPATHOLOGY OF RAT-LIVER

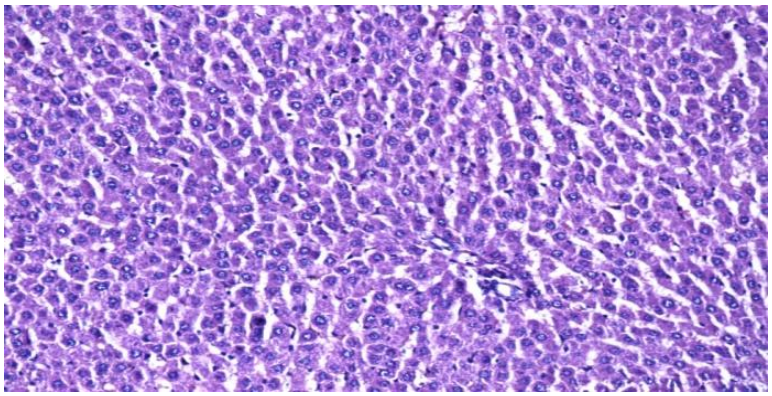

Figure No. 11: Rat liver of normal control group showing normal liver architecture with normal hepatocytes and portal tract. (H&E X 200)

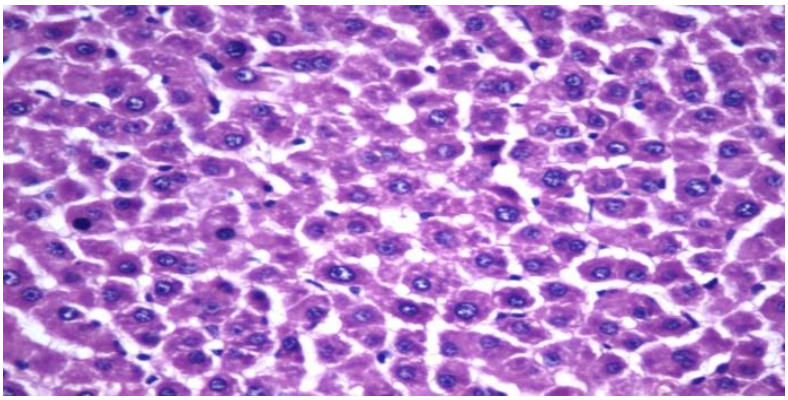

Figure No. 12: Rat liver treated with TritonX-100 shows fatty infiltration and granular degeneration with edema. (H&E X 400)

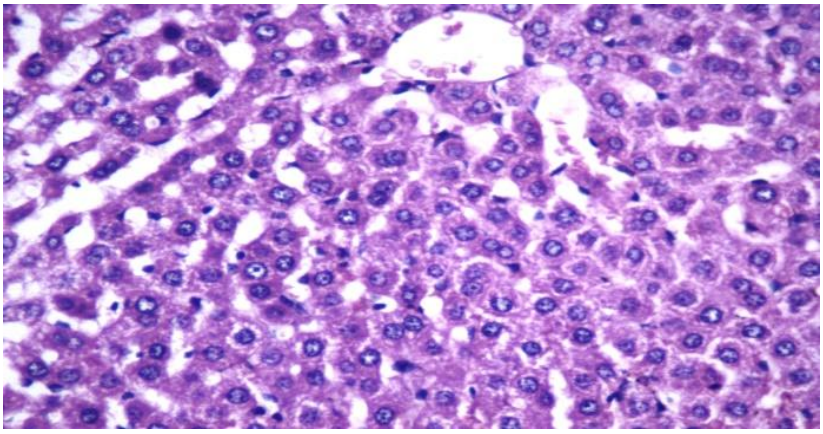

Figure No. 13: Rat liver treated with low dose extract shows mild fatty infiltration and granular degeneration. (H&E X400).

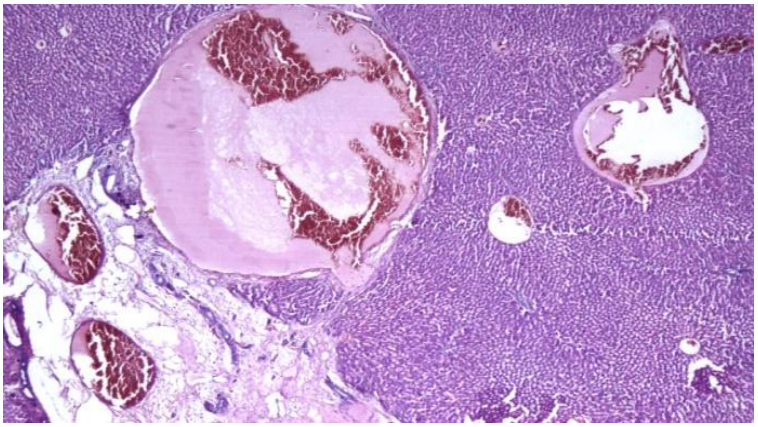

Figure No. 14: Rat liver treated with high dose extract shows marked congestion with the presence of dilated and congested blood vessels. (H&E X 40)

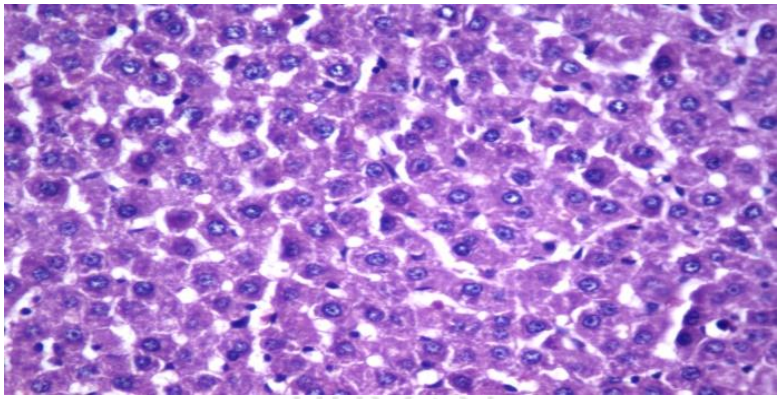

Figure No. 15: Rat liver treated with atorvastatin shows negligible fatty infiltration and granular degeneration. (H&E X400)

DISCUSSION

Cardiovascular diseases, especially coronary heart disease (CHD), are epidemic in India. The Registrar General of India reported that CHD led to 17% of total deaths and 26% of adult deaths in 2001-2003, which increased to 23% of total and 32% of adult deaths in 2010-2013. The World Health Organization (WHO) and Global Burden of Disease Study also have highlighted increasing trends in years of life lost (YLLs) and disability-adjusted life years (DALYs) from CHD in India.

The plant has been chosen for this study as it was easily available and the antihyperlipidemic activity has not been reported earlier.

Phytochemical analysis of the plant extract showed different phytoconstituents viz. glycosides, phytosterols, triterpenoids, alkaloids and flavonoids, Saponins are known to have anti-hyperlipidemic properties.

The major chemical constituent present in this plant is Malic acid, Galic acid, and Tannins which are responsible for the antihyperlipidemic activity.

Flavonoids are reported to increase HDL-C concentration and decrease in LDL and VLDL levels in hypercholesteremic rats.

The presence of flavonoids and polyphenols found in *Syzygium cumini* extracts could, therefore, be considered responsible for increasing HDL and decreasing LDL and VLDL in *Syzygium cumini* treated rats. Atorvastatin which was used as positive control in this study is a HMG-CoA reductase inhibitor. HMG-CoA reduces serum triglyceride levels through the modulation of apolipoprotein C-III and lipoprotein lipase.

The Triton x-100 used in this study contain saturated fatty acids which increase the activity of HMG Co-A reductase, the rate determining enzyme in cholesterol biosynthesis. This may be due to higher availability of acetyl Co-A, which stimulated the cholesterogenesis rate.

At the end of the study period, animals from experimental group were sacrificed liver and heart were collected. The transverse section of liver and heart were prepared using the usual techniques for preparation of permanent slides and these sections were observed for histopathological changes in liver and heart cells. Histopathological analysis of liver and heart were examined under light microscope.

Antihyperlipidemic activity observed with Atorvastatin (10mg/kg) and the *Syzygium cumini* ethanolic extracts (400 mg/kg) showed better activity than *Syzygium cumini* ethanolic extracts (200 mg/kg).

CONCLUSION

Hyperlipidemia has been affecting mankind since ages. Though many drugs are available to treat Hyperlipidemia, the problem of enhanced cholesterol levels in the blood is still prevailing and is being a cause for many coronary disorders.

Recently, certain chemicals (drugs) of natural origin have seen light in treating these cholesterol levels, reducing the risk of heart attacks. To reduce the risk due to hyperlipidemia, the various ways are to follow a healthy lifestyle and drugs that can reduce the cholesterol and triglyceride levels in the blood.

The present study suggests that post-treatment with ethanolic extract of *Syzygium cumini* showed dose-dependent antihyperlipidemic action against Triton x-100 induced hyperlipidemia.

The *Syzygium cumini* extract at a dose of 200, 400 mg/kg body weight orally showed Antihyperlipidemic activity which may be due to the presence of Saponins, Flavonoids, Phenols, alkaloids, Tannis (Phenolic compounds) and Triterpenoids found in the preliminary phytochemical screening.

From these results, it can be concluded that ethanolic extract of the pulp of *Syzygium cumini* contains active compounds which decrease serum lipid profiles ($P<0.05$) and lowers the risk of atherosclerosis in hyperlipidemic rats and 400mg/kg extract show better activity compare to 200mg/kg.

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