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ANTIOXIDANT AND ANTIHYPERLIPIDAEMIC ACTIVITY FOR METHANOLIC EXTRACT OF NYCTANTHES ARBOR-TRISTIS LEAVES

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Abstract:
Objective: *The present study was to evaluate the antihyperlipidemic activity for methanolic extract of Nyctanthes arbor-tristis (Linn.) leaves.*
Methodology: *Methanolic extract of Nyctathes arbor-tristis (MENA) was evaluated for in vitro antioxidant assay by reducing power assay and hydrogen peroxide assay and in vivo models of antihyperlipidemic study was carried out by 3 methods those are cholesterol diet, triton 100 X and fructose. The serum was collected and analyzed for lipid profile total cholesterol, triglyceride, high density lipoprotein, low density lipoprotein, very low density lipoprotein.*
Results: *Methanolic extract of Nyctanthes arbor-tristis leaves at the doses of 200 and 400 mg/kg bd. wt showed significance ($p < 0.01$) decrease in lipid profile like TC, TG, LDL, VLDL and showed significance ($p < 0.01$) increase in HDL.*
Conclusion: *Mena showed significant antihyperlipidemic activity with specific and non-specific mechanism which may be due to the presence of phytochemical constituents like phenols, triterpenoids and flavonoids.*
Keywords: *Nyctanthes arbor-tristis, Hyperlipidemia, cholesterol diet, triton 100 X and fructose.*

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INTRODUCTION:

Lipids are very diverse in both their respective structures and functions. These are insoluble in water. They are however soluble in other organic solvents such as ether, acetone and other. Major lipid groups include fats, phospholipids, steroids and waxes[1]. The main biological functions of lipids include energy storage, signaling, and acting as structural components of cell membranes [2]. In living cells, processes of carbohydrate metabolism, lipid metabolism and energy metabolism are closely related. Metabolic syndrome (MS), such as diabetes, obesity, hyperlipidemia and hypertension is more or less, associated with abnormal lipid metabolism. The accumulation of nutrients such as lipids and caloric surplus leads to abnormal lipid and ectopic fat accumulation, which is a fundamental component of metabolic disease [3]. Elevated serum total cholesterol (TC), low density lipoproteins (LDL), very low density lipoprotein (VLDL) and decrease high density lipoprotein (HDL) are the major risk factors for coronary heart diseases and chronic degenerative disease such as atherosclerosis Recent findings indicated that some of medicinal herbs or drugs, in addition to their lipid lowering ability,[4] can also reduce the production of reactive oxygen species and increase the resistance of plasma lipoprotein to oxidation that may contribute to their effectiveness at preventing atherosclerotic disease[5]

The term hyperlipidemia can be defined as increased level of lipid in blood. Hyperlipidemia is the major risk factor for many complications in human being. It is the first and for most factor that leads to diseases like atherosclerosis, coronary heart disease,[6] ischemic cerebro vascular disease, hypertension, obesity and diabetes mellitus (Type-II) etc.[7]

Nyctanthes arbor-tristis Linn. is a plant native to south Asia and southeast Asia belongs to the family Oleaceae commonly called as coral jasmine in English The plant has a wide range of medicinal value and has been used in Ayurvedic medicine for the treatment of various diseases such as sciatica, chronic fever, rheumatism, and internal worm infections, and as a laxative, diaphoretic and diuretic. Juice of the leaves is used as digestives, antidote to reptile venoms, mild bitter tonic, laxative, diaphoretic and diuretic[8]. Phytoconstituents isolated from *Nyctanthes arbor-tristis* were β-monogentiobioside, Nyctanthic acid, Tannic acid, Arbortristoside A & B, Glycerides, 3-4 secotriterpene acid, D-glucose, D-mannitol, Astragaline, Nicotiflorin, Oleanolic acid, Methyl salicylate, Flavonoids, Lupeol.[9]

MATERIALS AND METHODS:

Plant material collection and authentication

Nyctanthes arbor-tristis L. (leaves) was collected from BHEL, Hyderabad, Telangana state in the month of December 2015, identified and authenticated by Dr. S. Sureka (Medical officer, Government. Ayurvedic hospital near charminar Hyderabad).

Plant extraction

The crude plant material was cleaned, made into small pieces, dried under sun and coarsely powdered. The powdered material was extracted by simple distillation process using methanol as solvent. Filtrate obtained was evaporated to dryness and extract obtained was stored in air tight containers for further use.

Preliminary phytochemical screening

Methanolic extract of *Nyctanthes arbor-trisis* was subjected to preliminary phytochemical investigation.[10]

Animals used

Wistar albino rats (Approx 150 to 180 g) were procured from Albino labs Hyderabad. Present study was carried out in CPCSEA approved animal house of Gokaraju Rangaraju College of Pharmacy, Bachupally, Hyderabad, India (Reg. No. 1175/PO/Ere/S/08/CPCSEA).

Assay kits

Kits for detecting total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL), very low density lipoprotein (VLDL), and high density lipoprotein (HDL) were purchased from K. K. Diagnostics.

Acute toxicity studies

The methanolic extract of *Nyctanthes arbor-tristis* leaves whole plant was tested for acute toxicity studies as per procedure given in OECD guidelines 425 and limit test method was followed. Mice were starved for 4h and fed orally with methanolic extract of *Nyctanthes arbor-tristis* at doses 2000 and 5000 mg/kg bd.wt. Animals were observed for 14 days for mortality.[11]

Experimental treatment design

***In vitro* antioxidant Assay**

Reducing Power Assay

1 mL of MENA (20 µg/mL) was mixed with phosphate buffer (2.5 mL) and potassium ferricyanide (2.5 mL). The mixture was incubated at 500°C for 20 min. Aliquot of trichloroacetic acid (2.5 mL) was added to the mixture, and centrifuged at 3000 rpm for 10 min. The upper layer of resultant solution (2.5 mL) was mixed with distilled water (2.5 mL) and ferric chloride solution (0.5 mL). The absorbance was measured at 700 nm. Ascorbic acid (20 µg/mL) was used as standard. Increased absorbance of the reaction mixture indicates increase in reducing power.[12]

Hydrogen Peroxide Assay

The ability of MENA to scavenge hydrogen peroxide was determined according to the method given by Ruch *et al*. A solution of hydrogen peroxide (2 mmol/L) was prepared in phosphate buffer (pH 7.4). MENA (1– 10 µg/mL) were added

to hydrogen peroxide solution (0.6 mL). Absorbance of resultant solution was determined after 10 min at 230 nm against a blank solution, and ascorbic acid was used as reference compound.[13]

***In vivo* antihyperlipidemic models**

The experiments was done according to the CPCSEA guidelines and approved by the Institutional Animal Ethical Committee. In the present study methanolic extract of *Nyctanthes abor-tristis* was dissolved distilled water. Doses selected of methanolic extract of *Nyctanthes arbor-trisis* was 200 and 400 mg/kg body weight. Albino rats were divided into groups containing of six animals each.

Cholesterol diet induced hyperlipidemic rat model

Group I: served as normal pellet diet and distilled water

Group II: served as cholesterol diet 500 mg/kg bd. wt/day [butter, sugar and coconut oil]

Group III: received MENA at a dose of 200 mg/kg bd. wt along with cholesterol diet

Group IV: received MENA at a dose of 400 mg/kg bd. wt along with cholesterol diet

Group V: received atorvastatin at a dose of 10 mg/kg bd. wt along with cholesterol diet

Group I was considered as control which received distilled water. Group II was considered as high cholesterol diet group and received the cholesterol diet. Group III was considered as test group and received the test extract of *Nyctanthes arbor-tristis* **(**MENA) at the dose (200 mg/kg. bd. wt/day, *p.o*) and high cholesterol diet. Group IV was also considered as test group and received the test extract of MENA *at* the dose (400 mg mg/kg. bd. wt/day, *p.o*) and high cholesterol diet. Group V was considered as standard group which received the standard drug Atorvastatin at the dose (10 mg/kg. bd. wt/day, *p. o*) along with the high cholesterol diet. All the groups other than control group received the cholesterol diet for 14 days and on the 15st day blood serum were withdrawn from the retro orbital plexus after overnight fasting for the study of biochemical parameters. Serum was estimated for the total cholesterol, triglycerides, LDL, VLDL and HDL cholesterol.

From the 15st day the groups III and IV received the MENA and group V Atorvastatin drug along with the cholesterol diet. At the end of 21st day, blood serum was withdrawn from the retro orbital plexus after overnight fasting for the study of biochemical parameters. Serum was estimated for the total cholesterol, triglycerides, LDL, VLDL and HDL cholesterol.[14]

Triton 100 X induced hyperlipidemic rat model

Group I: served as normal pellet diet and distilled water.

Group II: received Triton 100-X (300 mg/kg bd.wt, i.p).

Group III: received MENA at a dose of 200 mg/kg bd. wt and also Triton 100 X (300 mg/kg bd.wt, i.p).

Group IV: received MENA at a dose of 400 mg/kg bd. wt and also Triton 100 X (300 mg/kg bd.wt, i.p).

Group V: received atorvastatin at a dose of 10 mg/kg bd. wt and also Triton 100 X (300 mg/kg bd.wt, i.p).

The animals were divided into groups randomly. Group I was served as normal pellet diet water and orally administrated with 1 % acacia. Group II, III, IV, V was administered with triton 100-X at a single dose of 300 mg/kg, i.p. Group II was considered as negative group provided with triton 100 X at 300 mg/kg, while Group III was administered with MENA at the dose of 200 mg/kg/ day p.o for 7 days after 72 h of triton administration. Group IV was administrated with MENA at the dose of 400 mg/kg day p.o for 7 days after 72 h of triton administration and Group V was administered with the standard drug Atorvastatin at dose of (10 mg/kg bd. wt. p.o) for 7days after 72 h of triton administration.

On the 8th day the blood was collected by retro orbital sinus puncture, under mild ether anaesthesia. Serum is separated and used for estimating the lipid profile like TC, TG, LDL, VLDL and HDL.[15]

Fructose induced hyperlipidemic rat model

Group I: served as normal pellet diet and distilled water

Group II: served as normal diet and water with 10% fructose for 20 days.

Group III: received MENA at a dose of 200 mg/kg bd. wt along with 10% fructose for 20 days.

Group IV: received MENA at a dose of 400 mg/kg bd. wt along with 10% fructose for 20 days.

Group V: received atorvastatin at a dose of 10 mg/kg bd. wt along with 10% fructose for 20 days.

Animals of all groups except normal control are administrated with 10% fructose in water for a period of 3 weeks for induction of hyperlipidemia. During this treatment schedule rats of Group III and IV received 200 and 400 mg/kg, bd wt *p.o* of MENA. Group V rats were treated with standard atorvastatin (10 mg/kg) bd. wt. *p.o* feeding of animals by fructose and respective drug treatments were done simultaneously from the beginning.

At the end of 21 days period, blood samples were collected by the retro orbital puncher. Serum were separated and used for biochemical estimation like TC, TG, LDL,VLDL and HDL. Physical parameters like body weight was evaluated before and after treatment.[16]

Collection of blood

After the experiment, blood was collected by retro orbital sinus puncture, under mild anaesthesia.

The collected samples were centrifuged for 15minutes. Then serum were collected and used for various biochemical experiments. The animals were then sacrificed and liver was collected.

Determination of serum lipid profile

Serum samples were analyzed for

- Total serum cholesterol (TC)
- Triglyceride (TG)
- High-density lipoprotein cholesterol (HDL-C)
- Low density lipoprotein (LDL-C)
- Very low density (VLDL-C).

Histopathological examination

Rats were sacrificed by cervical dislocation and their liver were dissected out and used for histological study. Liver obtained from all the experimental groups were washed immediately with saline and then fixed in 10% buffered neutral formalin solution.

Statistical analysis

Graph Pad prism 3 software was used for statistical analysis of data. All the results were expressed as mean±standard error of mean (SEM), analyzed for ANOVA and Student t-test (Multiple). Differences between groups were considered significant at $p<0.01$, $p<0.05$ levels.

RESULTS:

Results of preliminary phytochemical analysis

The methanolic extract of *Nyctanthes arbor-tristis* leaves were found to possess different phytoconstituents like phenolics, flavonoids, triterperinoids, glycosides, saponins, tannins, carbohydrates, steroids.

Results of Acute Oral Toxicity Studies

Acute oral toxicity studies of *Nyctanthes arbor-tristis*. leaves extracts were carried out according to OECD-425 guidelines. The study was carried out in Wister rats at a dose of 2000 mg/kg, p.o. The animals were observed for 14 days for mortality and acute toxicities. They exhibited normal behaviour, without any signs of toxicity. Their motor activity and secretary signs were also normal and no sign of depression was observed.

Results of *In vitro* antioxidant studies

Methanolic extract of *Nyctanthes arbor-tristis* showed good antioxidant activity by reducing power assay and hydrogen peroxide assay with IC_{50} value 33 and 22 µg/mL respectively. Ascorbic acid was used as reference standard showed the IC_{50} value 22 and 19 µg/mL with reducing power assay and hydrogen peroxide assay respectively (table 1 and table 2- figure 1 and figure 2).

Table 1: Anti-oxidant activity for methanolic extract *Nyctanthes arbor-tristis* leaves by reducing power assay method.

S. No	Compounds	Concentration (µg/ mL)	% inhibition	IC_{50} value (µg/ mL)
I	MENA	10 20 30 40 50	21.51±0.581 31.42±0.280 41.14±0.247 54.45±0.313 67.60±0.767	33
II	Ascorbic acid	10 20 30 40 50	22.12±0.408 37.90±0.690 46.89±0.245 53.59±0.652 75.06±0.784	22

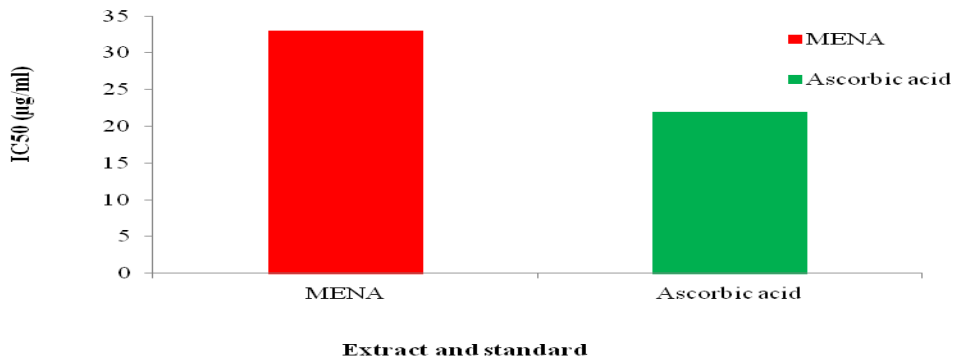


Fig 1: Reducing power assay for methanolic extract of *Nyctanthes arbor-tristis* leaves.

Table 2: Anti-oxidant activity of methanolic extract of *Nyctanthes arbor-tristis* by H_2O_2 radical scavenging activity.

S. No	Compounds	Concentration (µg/ mL)	% inhibition	IC_{50} value (µg/ mL)
I	MENA	10	20.05±0.863	22
		20	48.47±0.901	
		30	57.87±0.823	
		40	64.30±0.063	
		50	70.95±0.238	
II	Ascorbic acid	10	32.97±0.273	19
		20	52.57±0.402	
		30	60.67±0.828	
		40	68.71±0.240	
		50	73.25±0.113	

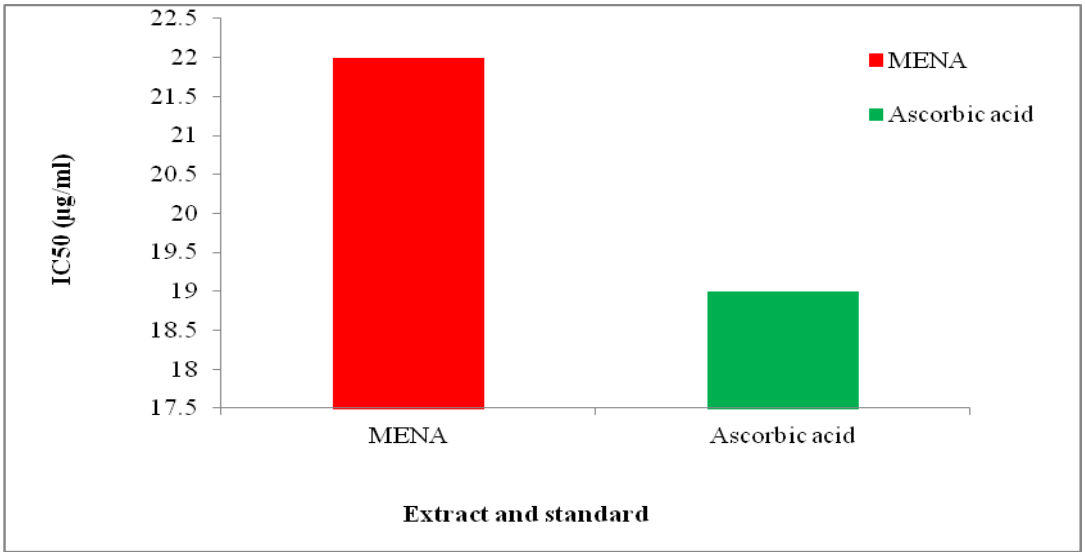


Fig 2: Hydrogen peroxide radical scavenging assay for methanolic extract of *Nyctanthes arbor-tristis* leaves.

Results of *In vivo* models

Figure 1, 2 and 3 shows the results increase in serum TC, TG, LDL, VLDL and significant decrease in HDL as compare to normal control (a = p < 0.01, b = p < 0.05). Methanolic extract of *Nyctanthes arbor-tristis* at the dose 200 mg/kg bd.wt & 400 mg/kg bd.wt showed significane decrease in cholesterol level when results were compared with normal (a = p < 0.01 and b = p < 0.05). Treatment with MENA at a doses of 200 mg/kg & 400 mg/kg reduced the serum cholesterol levels significantly (p<0.01) and standard group atorvastatin at a dose of 10 mg/kg showed significant (p<0.01) reduction in total cholesterol, triglycerides, LDL, and VLDL levels when compared to that of hyperlipidemic rats. Whereas HDL showed significant increase in total HDL levels when compared to that of hyperlipidemia rats (* = p < 0.01, ** = p < 0.05)

Cholesterol diet induced hyperlipidemic rat model

Table 3: Anti-hyperlipidemic activity for methanolic extract *Nyctanthes arbor-tristis* leaves on cholesterol diet induced hyperlipidemic rats.

Treatment	Lipid Profile (mg/dL)				
	Total Cholesterol	Triglyceride	HDL	LDL	VLDL
Normal control	91.2±1.640	70.14±0.128	46.58±0.941	43.13±2.140	14.08±0.366
Hyperlipidemic control	178.6±2.081^{b}	135.50±1.271^{b}	26.66±0.981^{b}	124.9±1.199^{b}	27.4±1.149^{ns}
MEAB (200 mg/kg)	128.5±1.061$^{a, *, B}$	101.15±0.701$^{a, ns, B}$	33.51±0.730$^{b,**, A}$	79.70±1.019^{a,*,B}	18.02±0.122$^{b,*, A}$
MEAB (400 mg/kg)	110.66±0.762$^{b, ns, A}$	89.16±0.560$^{a, **, A}$	39.41±0.623$^{a, *, B}$	55.43±0.807$^{b, **, A}$	16.83±0.169$^{a,*, B}$
Atorvastatin (10 mg/kg)	98.06±0.821^{a,**}	79.01±0.549$^{a, ns}$	42.5±0.599$^{b, **}$	42.83±0.530^{a,*}	15.08±0.079^{b,**}

Values are expressed as Mean ± SEM, (n=6). All the groups were compared with control, hyperlipidemic control and standard. (By using student t-test). Significant values are expressed as control group (a = p < 0.01, b = p < 0.05), hyperlipidemic control (* = p<0.01, ** = p<0.05) and standard (A = p < 0.01, B = p < 0.05), ns-non significant

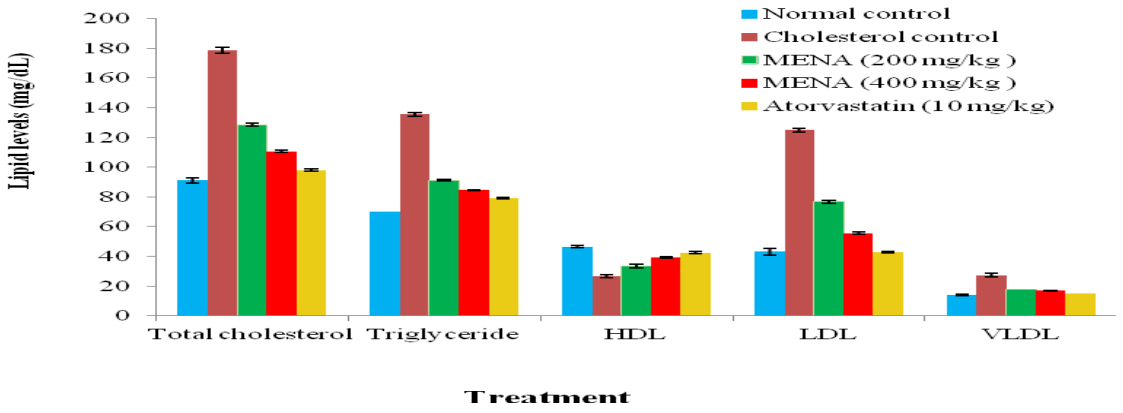


Fig 3: Effect of MENA on lipid levels of cholesterol diet.

b. Triton 100 X induced hyperlipidemic rat model

Table 4: Anti-hyperlipidemic activity for methanolic extract *Nyctanthes arbor-tristis* leaves on triton 100 X induced hyperlipidemic rats.

Treatment	Lipid Profile (mg/dL)				
	Total Cholesterol	Triglyceride	HDL	LDL	VLDL
Normal control	90.0±1.019	75.05±1.049	45.83±1.412	42.51±1.305	15.50±0.295
Hyperlipidemic control	160.6±1.235^{b}	156.1±1.295^{b}	18.42±0.928^{ns}	93.07±0.912^{b}	31.22±0.171^{b}
MEAB (200 mg/kg)	121.06±0.579$^{a, *, B}$	89.3±0.823$^{a, **, A}$	40.12±1.671$^{b,*, A}$	53.02±0.135$^{a, *, ns}$	20.80±0.166$^{ns, **, B}$
MEAB (400 mg/kg)	106.33±1.250$^{a, **, B}$	84.4±1.479$^{b, *, A}$	42.05±1.253$^{b, **, A}$	45.41±0.756$^{a,*, B}$	16.85±0.291$^{a,*, A}$
Atorvastatin (10 mg/kg)	96.01±0.229^{b,**}	78.16±0.765^{a,*}	43.65±0.189^{b,*}	43.51±1.078^{b,*}	15.60±0.171^{ns,*}

Values are expressed as Mean ± SEM, (n=6). All the groups were compared with control, hyperlipidemic control and standard. (By using student t-test). Significant values are expressed as control group (a = p < 0.01, b = p < 0.05), hyperlipidemic control (* = p<0.01, ** = p<0.05) and standard (A = p < 0.01, B = p < 0.05), ns-non significant.

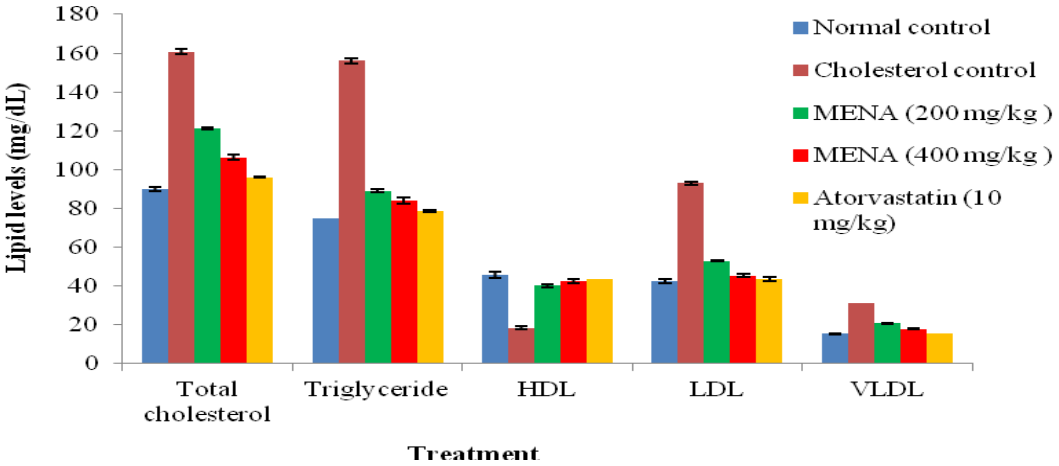


Fig 4: Effect of MENA on lipid levels of triton 100X

c. Fructose induced hyperlipidemic rat model

Table 5: Anti-hyperlipidemic activity for methanolic extract *Nyctanthes arbor-tristis* leaves on fructose induced hyperlipidemic rats.

Treatment	Lipid Profile (mg/dL)				
	Total Cholesterol	Triglyceride	HDL	LDL	VLDL
Normal control	102.3±0.091	70.81±0.09	45.80±0.041	41.15±0.190	14.49±0.025
Hyperlipidemic control	179.60 ±1.809^{b}	140.4±2.013^{b}	27.60±1.501^{ns}	110.35±1.270^{b}	28.80±1.170^{ns}
MEAB (200 mg/kg)	125.75 ±0.591$^{a, *, B}$	86.05±0.059$^{a, **, B}$	34.80±0.219$^{b, *, A}$	67.41±1.070$^{a, **, A}$	18.60±0.653$^{a, *, B}$
MEAB (400 mg/kg)	115.6±0.062$^{b, *, A}$	77.76±0.012$^{a, *, ns}$	38.34±1.053$^{ns, *, A}$	56.80±0.290$^{b, *, B}$	16.40±0.510$^{a,* *, B}$
Atorvastatin (10 mg/kg)	106.3±0.501$^{ns, **}$	75.02±0.172^{b,*}	42.33±0.699^{a,*}	44.75±0.660$^{ns, **}$	15.55±0.122$^{a, *}$

Values are expressed as Mean ± SEM, (n=6). All the groups were compared with control, hyperlipidemic control and standard. (By using student t-test). Significant values are expressed as control group (a = $p < 0.01$, b = $p < 0.05$), hyperlipidemic control (* = $p<0.01$, ** = $p<0.05$) and standard (A = $p < 0.01$, B = $p < 0.05$), ns-non significant.

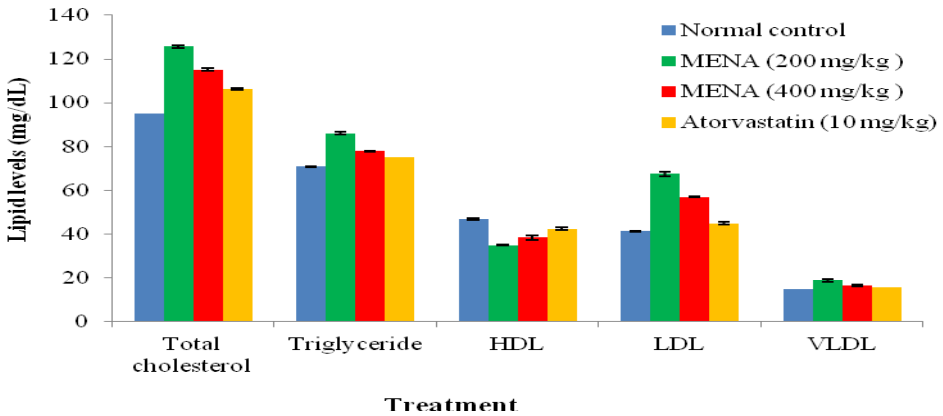


Fig 5: Effect of MENA on lipid levels.

Effect of MENA on body weights of rats in grams

Table 6,7 and 8 and figure 6,7 and 8 shows treatment with MENA at a doses of 200 mg/kg & 400 mg/kg decreased the body weights significantly (p<0.01) and standard group atorvastatin at a dose of 10 mg/kg showed significant increase in total HDL levels when compared to that of hyperlipidemia rats.

Table 6: Effect for methanolic extract of *Nyctanthes arbor-tristis* leaves on body weight by cholesterol diet induced hyperlipidemic rats.

Groups	Treatment	Body weights of rats
I	Normal control	185.4±2.051
II	Cholesterol control	230.5±2.651[b]
III	MENA (200mg/kg)	212.06±1.078[b, *, ns]
IV	MENA (400 mg/kg)	209.17±1.049 [a, **, A]
V	Atorvastatin(10mg/kg)	192.16±0.078 [a, **]

Values are expressed as Mean ± SEM, (n=6). All the groups were compared with control, hyperlipidemic control and standard. (By using student t-test). Significant values are expressed as control group (a = p < 0.01, b = p < 0.05), hyperlipidemic control (* = p<0.01, ** = p<0.05) and standard (A = p < 0.01), ns- non significant.

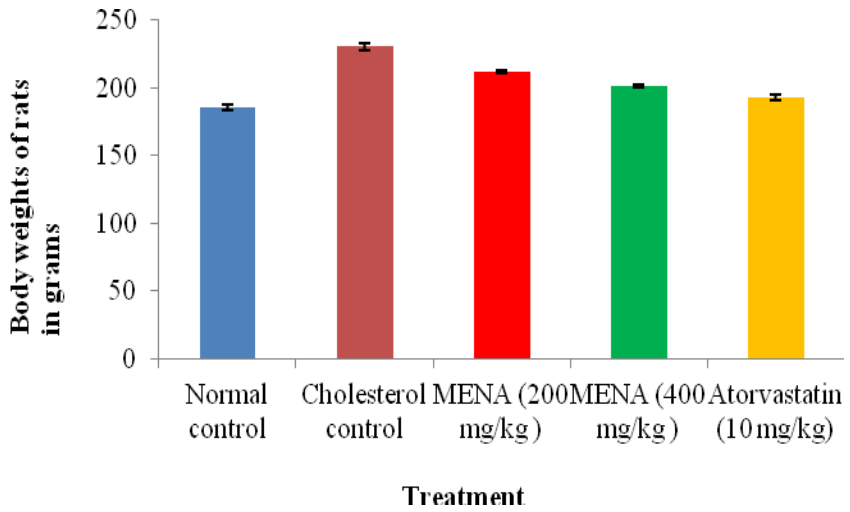


Fig 6: Shows the effect of MENA on body weights of rats in grams by cholesterol diet induced hyperlipidemia.

Table 7: Effect for methanolic extract of *Nyctanthes arbor-tristis* leaves on body weight by triton 100 X induced hyperlipidemic rats.

Groups	Treatment	Body weights of rats
I	Normal control	180.04±2.336
II	Cholesterol control	229.17±2.556 [b]
III	MENA (200mg/kg)	211.08±1.059 [a, *, B]
IV	MENA (400 mg/kg)	202.4±0.176 [b,**,B]
V	Atorvastatin(10mg/kg)	196.5±0.765 [b,*]

Values are expressed as Mean ± SEM, (n=6). All the groups were compared with control, hyperlipidemic control and standard. (By using student t-test). Significant values are expressed as control group (a = p < 0.01, b = p < 0.05), hyperlipidemic control (* = p<0.01, ** = p<0.05) and standard (B = p < 0.05), ns- non significant.

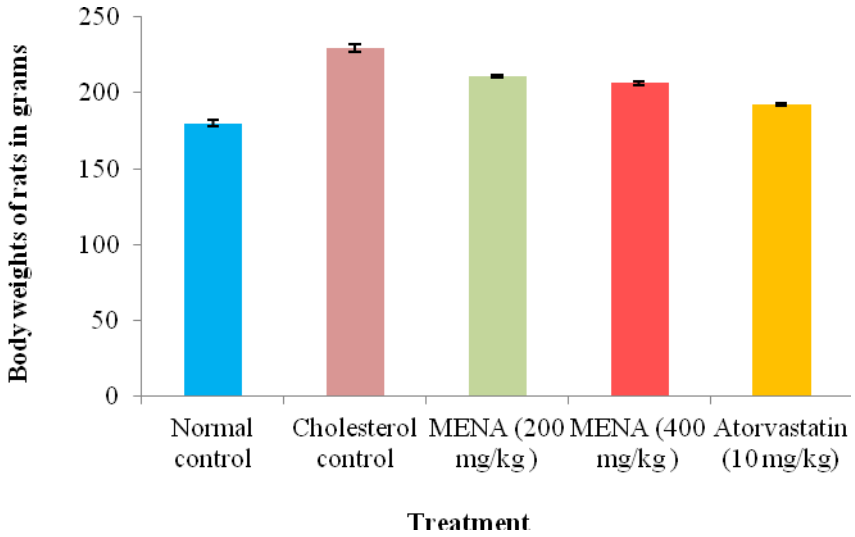


Fig 7: Shows the effect of MENA on body weights of rats in grams by triton 100 X induced hyperlipidemia.

Table 8: Effect for methanolic extract of *Nyctanthes arbor-tristis* leaves on body weight by fructose hyperlipidemic rats.

Groups	Treatment	Body weights of rats
I	Normal control	188.02±2.543
II	Cholesterol control	220.5±2.669^{ns}
III	MENA (200mg/kg)	217.07±0.679$^{a, *, A}$
IV	MENA (400 mg/kg)	201.15±1.799$^{b, **, B}$
V	Atorvastatin(10mg/kg)	190.78±0.567$^{b, **}$

Values are expressed as Mean ± SEM, (n=6). All the groups were compared with control, hyperlipidemic control and standard. (By using student t-test). Significant values are expressed as control group (a = $p < 0.01$, b = $p < 0.05$), hyperlipidemic control (* = $p<0.01$, ** = $p<0.05$) and standard (A = $p < 0.01$, B = $p < 0.05$), ns-non significant.

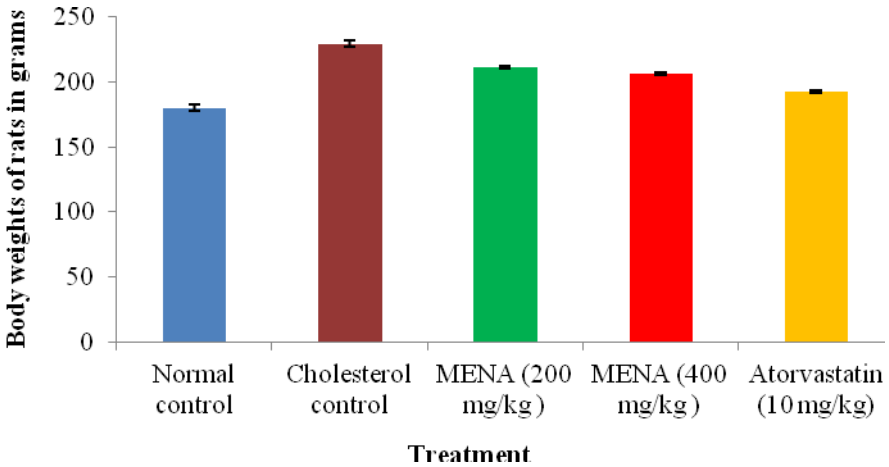
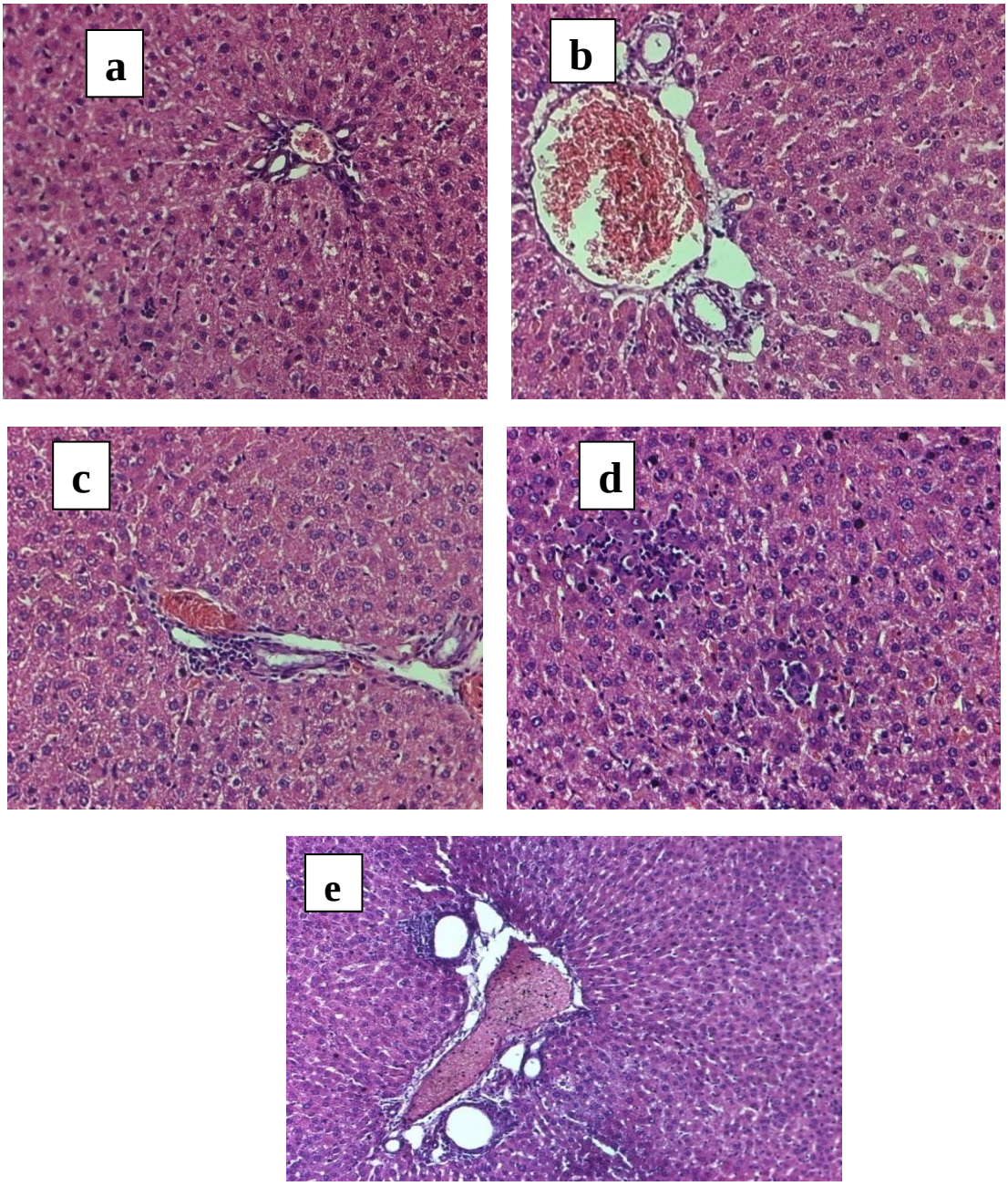


Fig 8: Shows the effect of MENA on body weights of rats in grams by fructose Induced hyperlipidemia.

Results of Histopathological Studies



(a) Normal rat liver- Bile duct appeared normal, portal triad appeared normal & no inflammation or fibrosis noticed surrounding the portal region of liver. (b) Cholesterol control rat liver- Periportal and peri bilary inflammation and fibrosis noticed in periportal region of liver. (c) rat's liver treated with MENA 200 mg/kg- Mild to moderate sinusoidal space dilatation along with hemorrhages noticed in the sinusoidal space of liver. (d) rat's liver treated with MENA 400mg/kg- Hepatocytes appeared normal, periportal and centrilobular region appeared normal but mild sinusoidal space dilation along with hemorrhage s noticed in the sinusoidal spaces.(e) rat's liver treated with Atorvastatin 10 mg/kg- Hepatocytes appeared normal, periportal and centrilobular region appeared normal but mild sinusoidal space dilatation noticed in the pessri portal region of liver.

DISCUSSION:

Phytochemical screening of *Nyctanthes arbor-tristis* methanolic extract showed presence of different phytoconstituents viz. Triterpenoids, carbohydrates, steroids, saponins, flavonoids, tannins & phenolic compounds. Several phytoconstituents like glycosides, saponins, and flavonoids are known to have antihyperlipidemic properties.

It is reported that phenolic constituents have the ability to strongly inhibit antioxidants & free radical scavengers It is also reported that triterpenoids (friedelane, friedelinol) reduced the total cholesterol and triglycerides. It has been reported that saponins have cholesterol-lowering activity either by inhibiting the absorption of cholesterol from the small intestine or by the reabsorption of bile acids. Triterpenoids, phytosterols, flavonoids, tannins, saponins & phenolic constituents in MENA might be responsible for the hypolipidemic activity.

Antihyperlipidemic activity

The present studies were performed to assess the antihyperlipidemic activity of MENA and to prove its claim in folklore practice against various disorders. Cholesterol is synthesized in all animal tissue. Its important relates to its role in the stabilization of membrane structures because of its rigid planar structure. It also as a precursor for the synthesis of steroid hormones. Increased amount of cholesterol leads to cardiovascular disease particularly coronary heart disease (CHD).

The plasma cholesterol was reduced remarkably on treating the Cholesterol diet, Triton and Fructose treated rats with methanol extract of *Nyctanthes arbor-tristis* (MENA). The lipid lowering effects may be due to the presence of plant sterol. Plant sterol (β-sigmasterol, β-sitosterol) reduces the absorption of cholesterol and thus increases the fecal excretion of steroids that results in decrease of body lipids reduction i.e. 1% cholesterol produces a 2% to 3% reduction in coronary heart disease risk.

Triglycerides are mainly stored in the adipose tissue. The plasma lipoproteins are major sources of fatty acid to synthesize triacylglycerols. The excess of fat diet increased the TG level which is one of the causes of hardening of arteries. In present study, the administration of MENA at a dose of 200 mg/kg & 400 mg/kg significantly lowered triglycerides level in serum in dose dependent manner.

Another risk factor for developing atherosclerosis is the reduced serum level of HDL-C. This effect which is largely attributed to its central role to reserve cholesterol transport, a process whereby excess cell cholesterol is up taken and which is subsequently processed by HDL-C particles for further delivery to the liver for metabolism. Therefore, it is logical that an increase in HDL-C level can contribute to lower the risk of atherosclerosis. The results clearly indicated that methanolic extract of *Nyctanthes arbor-tristis* were capable of increasing level of HDL-C in serum. It is widely accepted that the elevation of plasma LDL-C level is major risk factor for CHD. Direct correlation between LDL-C level and atherosclerosis as well as the reversibility of the related pathological events by lowering the serum level of LDL-C has already been reported [17]

CONCLUSION:

The results obtained from the pharmacological screening let us to the conclude that, Methanolic extract for leaves of *Nyctanthes arbor-tristis* have significant antihyperlipidemic activity. The observed activity might be due to the different phytochemicals present in the extract. Thus it can be reported that methanolic extract of *Nyctanthes arbor-tristis* possess significant anti-hyperlipidemic activity.

CONFLICT OF INTERESTS

There is no conflict of interest.

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