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EVALUATION OF ANTI HYPERLIPIDEMIC ACTIVITY OF MEDICINAL PLANT, *ARGYREIA NERVOSA BURN.F* IN HYPERLIPIDEMIC RATS

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ABSTRACT

The global prevalence of obesity is increasing rapidly and high dietary fat intake is major risk factor for the development of obesity. The present study was taken undertaken to evaluate the effect of ***Argyreia Nervosa Burn.F*** leaf ethanol extract on serum lipid profile in Wistar male albino rat fed with high fat diet and to compare it with a standard hyperlipidemic drug Sibutramine (10mg/kg). Fifty four health Wistar albino male rats were randomized in to 9 groups of 6 animals each. The groups were followed as follows Group I: Sham operated Normal (Normal Diet), Group II: Control (High fat diet), Group III: Sibutramine 10 mg/kg + HFD, Group IV: EEAN (100mg/kg) + HFD, Group V: EEAN (200mg/kg) +HFD, Group VI: EEAN (400mg/kg) + HFD, Remaining groups have received different types of extracts at various doses. Lipid profile in serum with high triglyceride (TG) and cholesterol levels were significantly reduced by treatment of 0.5g/day A. nervosa. The A. nervosa markedly lowers the levels of serum cholesterol and VLDL. The present investigation shows that all triton induced rats displayed hyperlipidemia as shown by their elevated levels of serum and liver cholesterol, triglyceride, PL, VLDL, LDL and the reduction in the HDL level. It can be concluded that 0.5g/day of A. nervosa treatment was effective in reduction of cholesterol, PL, TG, VLDL, LDL and HDL in a dose dependant manner.

INTRODUCTION:

From the beginning of the last century, evidences on the cholesterol-lowering properties of medicinal plants have been accumulating. The importance of such investigations, are confirmed in the treatment of obesity, diabetes mellitus, heart failure, and atherosclerosis. Scientists have reported the role of medicinal plants in the control of elevated serum cholesterol, and the reduction of morbidity and mortality due to vascular diseases associated with it [1]. Coronary artery disease (CAD) is one of the most important causes of death all over the world [2]. According to World Health Organization (WHO), by 2010, Asian Indians will represent 50-60% of the world's cardiac patients, which amounts to about 100 million patients [3]. High plasma level of cholesterol along with generation of reactive species (ROS) play's key role in the development of coronary artery disease (CAD) and atherosclerosis[4].Oxidative stress is currently suggested a mechanism underlying hypercholesterolemia. Free radicals are continuously produced in the body as the result of normal metabolic processes and interaction with environmental stimuli [5]. Although statins have been found effective in lowering serum low-density lipid levels they have been found to cause many side effects. As they are basically enzyme inhibitors, so it

is likely that they may be inhibiting other critical enzymes in body. Statins are ingested on a long term basis so there may be a risk of Chronic toxic effects like carcinogenic, teratogenicity and mutagenic over a life time of use [6]. Recent study has shown that medicinal plants intake in rat's results in an increase of antioxidant enzymes activity and HDL cholesterol, and a decrease in malondialdehyde, which may reduce the risk of heart disease [5]. Traditional medicine is still the mainstay of about 75-80% of world population, mainly in the developing countries. India, having a rich tradition of folk medicine from centuries, has provided very simple but effective remedies to various ailments using plants and plants derived compounds. There is no such risk factor to use the plant medicine as compare with the allopathic drugs [7].

MATERIALS AND METHODS:

Plant authentication and extract preparation:

The plant was collected during the march 2014 from Tirumalla forest area of Chitoor district India. The plant was authenticated by Dr. Madhava Chetty, Department of Botany, Sri Venkateshwara University, Tirupati and voucher specimen of the plant were preserved at institute herbarium library. The shade dried and powdered leaves of A. nervosa were extracted with ethanol by using Soxhlet extractor. The extract was filtered and then solvent was evaporated under reduced pressure to a solvent free concentrated mass, which was then stored in air-tight container in a cool and dry condition.

Experimental Animals:

Wistar albino adult male rats weighing 200-250g were obtained from the animal house. The animal were grouped and housed in polyacrylic cages (38x 23x 10 cm) with not more than five animals per cage and maintained under standard laboratory under standard laboratory conditions (temperature 25+2oC) with dark and light cycle (14/10 hour). They were allowed free access to standard dry pellet diet (Hindustan Lever, Kolkata, India) and water ad libitum. The mice were acclimatized to laboratory condition for 10 days before commencement of experiment. The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC) constituted under CPCSEA.

Induction of Hyper lipidemia : Hyperlipidemia was induced in Wistar albino rats by single intraperitoneal injection of freshly prepared solution of Triton-X-100 (100 mg/kg) in physiological saline solution after overnight fasting for 18 h [45] .The animals were divided into four groups of five rats each. The first group was given standard pellet diet, water and orally administered with 5% CMC. The second group was given a single dose of triton administered at a dose of 100mg/kg, i.p. After 72 hours of triton injection, this group received a daily dose of 5% CMC (p.o) for 7 days. The third group was administered a daily dose of 0.5g/day Argyreia nervosa suspended in 5%CMC, p.o., for 7 days, after inducing hyperlipidemia. Fourth group was administered with the standard Fenofibrate 65mg/kg, p.o. for 7 days [46].

Collection of blood: On the 8thday, blood was collected by retero orbital sinus puncture, under mild ether anesthesia. The collected samples were centrifuged for 10 minutes. Then serum samples were collected and used for various biochemical experiments. The animals were then sacrificed and the liver collected [47].

Liver lipid extraction: The liver was homogenized in cold 0.15M KCl and extracted with CHCl3 CH3OH (2% v/v). This lipid extract was used for the estimation of lipid parameters [48].

High Fat diet model Materials: Remi research centrifuge (R-24), Soxhlet extractor, Olampus Research microscope with computer connectivity and image capturing capacity, HPTLC, Shimadzu UV-visible spectrophotometer (UV1800), micrtone. Stat Fax autoanalyser (2000), Afcoset digital balance (ER-180A). Sibutramine was gifted by the Ranbaxy Laboratory Ltd, Devas, MP. 2% Cholesterol was purchased from SRL Pvt. LTD., Mumbai.

Animal selection: 54 (09 x 6) Wistar albino rats (180-250 g) were used in this second model. They were housed six per cage under standard laboratory conditions at a room temperature at 22 ± 2oC with 12h light/dark cycle. The animals were maintained under

standard nutritional and environmental conditions throughout the experiment. The experiment ware carried out between 9:00–16:00 hours at ambient temperature. All the pharmacological experimental protocols were approved by the Institutional Animals Ethics Committee (Ref: CPCSEA/769/2010) of Sigma Institute of Clinical Research and Administration Pvt. Ltd, Hyderabad, India.

Animal grouping: The animals were randomly divided into following 9 groups; each group consists of six animals. Animal grouping and their treatment is as follows:

Group- I: Sham operated Normal (Normal Diet)
Group- II: Control (High fat diet)
Group- III: Sibutramine 10 mg/kg + HFD
Remaining groups have received different types of extracts at various doses as follows.
Group- IV: EEAN (100mg/kg) + HFD
Group- V: EEAN (200mg/kg) +HFD
Group- VI: EEAN (400mg/kg) + HFD

Induction of High fat diet induced obesity:

Animals were divided in to 9 groups each group having 6 animals, first group (lean Rat) had free access to standard pelleted chow which provided 76.8% of energy as carbohydrates, 19.2% as protein, and 4.3% as fat. Remaining 48 rats were fed with a high fat diet providing 60% of energy as fat, 20% as protein and 20% as carbohydrates to the animals. Experimental obesity and other metabolic changes were induced by dietary manipulation (by proving HFD) for 48 days to reaming 9 groups. After 48 days rats were found to be obese, change in body weight during this period was recorded.

RESULTS AND DISCUSSION:

A. nervosa has also been shown to indirectly modify the total cholesterol and high density lipoprotein cholesterol values. The ethanolic extract of A. nervosa was found to be non–toxic up to the dose of 2 g/kg and did not cause any death of the tested animals. The Phytochemical tests with the ethanol extract of A. nervosa indicated the presence of carbohydrates, glycosides, terpenes, saponins, proteins and amino acids. Hyperlipidemia is associated with heart disease, which is the leading cause of death in the world. The lowering of the levels of harmful lipids to satisfactory values has been confirmed by several experimental animal and interventional studies indicating lowered morbidity and mortality in coronary heart diseases. The results are discussed under the lipid profile in serum. Lipid profile in serum with high triglyceride (TG) and cholesterol levels were significantly reduced by treatment of 0.5g/day A. nervosa. LDL and VLDL levels were significantly increased in triton-injected animals to control rats. The results are shown in Tables 1 and 3. The A. nervosa markedly lowers the levels of serum cholesterol and VLDL. The decrease in cholesterol may indicate increased oxidation of mobilized fatty acids of inhibition or lipolysis. The present investigation shows that all triton induced rats displayed hyperlipidemia as shown by their elevated levels of serum and liver cholesterol, triglyceride, PL, VLDL, LDL and the reduction in the HDL level. It can be concluded that 0.5g/day of A. nervosa treatment was effective in reduction of cholesterol, PL, TG, VLDL, LDL and HDL in a dose dependant manner. Histopathological studies revelaed that control group showing normal architecture; (b) hyperlipidemic group showing fatty infiltration and granular degeneration; (c) Fenofibrate group showing negligible cytoplasmic fatty infiltration and granular degeneration; (d) group treated with 200mg/kg extract showing mild cytoplasmic fatty infiltration and mild granular degeneration; (e) group treated with 400mg/kg extract showing mild cytoplasmic fatty infiltration and mild to moderate granular degeneration.

CONCLUSION:

Hyperlipidemia is associated with heart disease, which is the leading cause of death in the world. The lowering of the levels of harmful lipids to satisfactory values has been confirmed by several experimental animal and interventional studies indicating lowered morbidity and mortality in coronary heart diseases. The results are discussed under the lipid profile in serum. Lipid profile in serum with high triglyceride (TG) and cholesterol levels were significantly reduced by treatment of 200mg/kg extract and 400 mg/kg extract A. nervosa.

Table 1: Effect of Ethanolic extract of Argyreia nervosa on body weight in Triton-X induced Hyperlipidemia in rats.EEAN- Ethanolic Extract of Argyreia nervosa

Treatment Groups	1st Day	7th Day	14th Day	21st day	48th Day
Sham operated Normal	157.5±2.81	162.5±2.14	164.3±2.36	169.8±1.662	174.5±1.258
Triton-X -100 Control	157.5±2.81	162.5±2.14	164.3±2.36	169.8±1.662	174.5±1.258
Standard - Sibutramine10mg/kg	155.0±1.82	165.2±3.13	168.5±3.05*	169.5±1.83***	170.2±2.19***
EEAN (100mg/kg)	153.3±3.07	168.2±0.79	168.8±2.77*	171.0±4.25***	192.0±4.50**
EEAN (200mg/kg)	153.3±3.07	169.0±1.52	167.5±0.84*	175.0±0.81***	183.2±2.72**
EEAN (400mg/kg)	155.2±2.89	161.3±1.56	166.8±1.77*	172.8±1.68***	185.3±5.10**

Table 2: Effect of Ethanolic extract of Argyreia nervosa on Glucose, SGOT and SGPT levels on Triton-X induced hyperlipidemia in rats.

Treatment Groups	Glucose mg/dl	SGOT mg/dl	SGPT mg/dl
Sham operated Normal	77.83±4.42	12.84±0.82	34.12±2.35
Triton-X -100 Control	87.5±2.81	25.50±2.14	44.31 ±2.36
Standard (Sibutramine10mg/kg)	64.00±1.39***	12.70±0.87***	28.97±1.45***
EEAN (100mg/kg)	85.17±1.24***	23.44±1.12ns	59.53±3.50**
EEAN (200mg/kg)	92.17±4.28***	21.02±1.29ns	45.35±4.37***
EEAN (400mg/kg)	63.83±1.49***	13.59±1.35***	38.98±4.26***

Table 3: Effect of Ethanolic extract of Argyreia nervosa on Lipid profile levels onTriton-X induced hyperlipidemia in rats.

Treatment Groups	TC (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)
Sham operated Normal	125.7±1.45	66.84±0.48	40.05±0.66	72.33±1.53	13.37±0.09
Triton-X-100 (control)	184.6±2.55	76.84±0.48	68.15±0.66	92.33±1.53	18.37±0.09
Standard (Sibutramine10mg/kg)	142.2±2.10***	68.00±1.77***	47.89±1.20***	80.40±3.28***	13.89±0.22***
EEAN(100mg/kg)	185.7±2.96***	72.17±1.88***	39.34±1.67***	131.9±3.35ns	14.43±0.37***
EEAN (200mg/kg)	172.1±1.23***	70.24±1.58***	54.15±1.08***	118.4±0.80ns	14.04±0.31***
EEAN (400mg/kg	162.7±1.03***	63.70±1.08***	56.31±1.52***	103.9±2.11*	12.73±0.21***

All the values are mean ± SEM, n=6, ns=Not significant, One way Analysis of Variance (ANOVA) followed by multiple comparison Dunnett's test, ** $p<0.01$, *** $p<0.001$ vs. control group, and [a] $p<0.001$

Table 4: Effect of Ethanolic extract of Argyreia nervosaon body weight in HFD induced hyperlipidemia and obesity in rats.EEAN- Ethanolic Extract of Argyreia nervosa

Treatment Groups	**1st Day**	7th Day	14th Day	21st day	48th Day
Sham operated Normal	157.5±2.81	162.5±2.14	164.3±2.36	169.8±1.662	174.5±1.258
HFD (control)	157.5±2.81	172.5±2.14	184.3±2.36	189.8±1.662	194.5±1.258
Standard (Sibutramine10mg/kg)	155.0±1.82	165.2±3.13	168.5±3.05*	169.5±1.83***	170.2±2.19***
EEAN (100mg/kg)	153.3±3.07	168.2±0.79	168.8±2.77*	171.0±4.25***	192.0±4.50***
EEAN (200mg/kg)	153.3±3.07	169.0±1.52	167.5±0.84**	175.0±0.81***	183.2±2.72***
EEAN (400mg/kg)	155.2±2.89	161.3±1.56	166.8±1.77**	172.8±1.68***	185.3±5.10***

Table 5: Effect of Ethanolic extract of Argyreia nervosa on Glucose, SGOT and SGPT levels on HFD induced hyperlipidemia in rats

Treatment Groups	**Glucose mg/dl**	**SGOT mg/dl**	**SGPT mg/dl**
Sham operated Normal	77.83±4.42	12.84±0.82	34.12±2.35
HFD (control)	88.5±2.81	25.50±2.14	44.31 ±2.36
Standard (Sibutramine10mg/kg)	64.00±1.39***	12.70±0.87***	28.97±1.45***
EEAN (100mg/kg)	75.17±1.24***	23.44±1.12ns	56.53±3.50**
EEAN (200mg/kg)	72.17±4.28***	21.02±1.29ns	45.35±4.37***
EEAN (400mg/kg)	63.83±1.49***	13.59±1.35***	38.98±4.26***

All the values are mean ± SEM ,n=6, ns=Not significant, One way Analysis of Variance (ANOVA) followed by multiple comparison Dunnett's test, ** $p<0.01$, *** $p<0.001$ vs. control group ,and $^{a}p<0.001$

Table 6: Effect of Ethanolic extract of Argyreia nervosa on Lipid profile levels on HFD induced hyperlipidemia in rats

Treatment Groups	TC (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)
Sham operated Normal	125.7±1.45	66.84±0.48	40.05±0.66	72.33±1.53	13.37±0.09
HFD (control)	199.6±2.55	86.84±0.48	78.15±0.66	82.33±1.53	20.37±0.09
Standard (Sibutramine10mg/kg)	142.2±2.10***	68.00±1.77***	47.89±1.20***	80.40±3.28***	13.89±0.22***
EEAN(100mg/kg)	185.7±2.96***	72.17±1.88***	39.34±1.67***	131.9±3.35ns	14.43±0.37***
EEAN (200mg/kg)	172.1±1.23***	70.24±1.58***	54.15±1.08***	118.4±0.80ns	14.04±0.31***
EEAN (400mg/kg)	162.7±1.03***	63.70±1.08***	56.31±1.52***	103.9±2.11*	12.73±0.21***

All the values are mean ± SEM, n=6, ns=Not significant, One way Analysis of Variance (ANOVA) followed by multiple comparison Dunnett's test, ** $p<0.01$, *** $p<0.001$ vs. control group ,and $^{a}p<0.001$

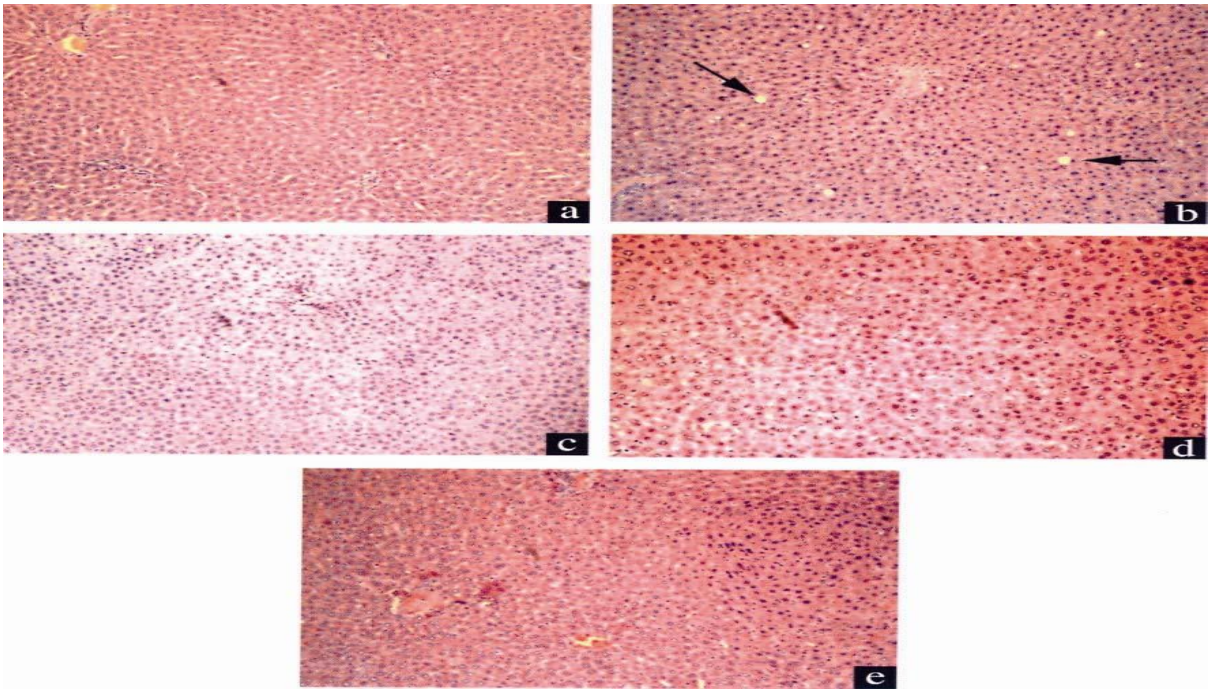

Fig 1: Hepatocytes of rats stained with hematoxylin and eosin (100 × magnification) — (a) control group showing normal architecture; (b) hyperlipidemic group showing fatty infiltration (→) and granular degeneration; (c) Fenofibrate group showing negligible cytoplasmic fatty infiltration and granular degeneration; (d) group treated with 200mg/kg extract showing mild cytoplasmic fatty infiltration and mild granular degeneration; (e) group treated with 400mg/kg extract showing mild cytoplasmic fatty infiltration and mild to moderate granular degeneration.

LDL and VLDL levels were significantly increased in triton-injected animals to control rats. The results are shown in Tables 1 and 3. The A. nervosa markedly lowers the levels of serum cholesterol and VLDL. The decrease in cholesterol may indicate increased oxidation of mobilized fatty acids of inhibition or lipolysis. The present investigation shows that all triton induced rats displayed hyperlipidemia as shown by their elevated levels of serum and liver cholesterol, triglyceride, PL, VLDL, LDL and the reduction in the HDL level. Histopathological studies revelaed that group treated with 200mg/kg extract and 400 mg/kg extract showed mild cytoplasmic fatty infiltration and mild granular degeneration as compared to normal and control groups. It can be concluded that 200mg/kg extract and 400 mg/kg extract of A. nervosa treatment was effective in reduction of cholesterol, PL, TG, VLDL, LDL and HDL in a dose dependant manner.

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