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ORIGINAL RESEARCH ARTICLE

Hypolipidemic Activity on Ethanolic Extract of Leaves of *Ziziphus oenoplia* (L) Mill. Gard.

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ABSTRACT

Ziziphus oenoplia belongs to the family Rhamnaceae, a native plant of India and Southeast Asia commonly known as Surai izlanthai in Tamil. It is used traditionally for various properties and hence the present study was aimed to evaluate the Hypolipidemic activity of Ethanolic extract of *Ziziphus oenoplia* leaf. Hypolipidemic activity is screened by inducing Hyperlipidemia with the help of atherogenic diet which containing 2% cholesterol, 1% cholic acid and 2% coconut oil, along with the standard rat chow diet given to the Wistar albino rats for thirty days. On day 30, blood samples from the rats were collected for the estimation of serum levels of various biochemical parameters such as total cholesterol, triglycerides, LDL, VLDL, Atherogenic index(AI), LDL-C/HDL-C ratio and HDL cholesterol. Atherogenic index is used to determine the atherogenic potential of the drugs. Ethanolic extract treated groups at two dose levels (250mg/kg and 500mg/kg) were showed significant hypolipidemic activity ($p<0.01$) by reduction in the level of serum cholesterol, triglyceride, LDL, VLDL, AI, LDL-C/HDL-C ratio and increase in HDL level as compared to cholesterol control groups. These biochemical observations were confirmed by Histopathological examinations of Thoracic aorta. 250mg/kg and 500mg/kg dose of Ethanolic extract of *Ziziphus oenoplia* showed decreased atheromatous plaque size and inflammatory changes as compared to cholesterol control groups. Hypolipidemic and antiatherosclerotic property of *Ziziphus oenoplia* is may be due the presence of Alkaloids, Flavonoids and Phenolic compounds.

Key words: *Ziziphus oenoplia*, Male Wistar albino rats, Atherogenic diet, Lipid profile, Histopathology of Aorta.

INTRODUCTION

Hyperlipidemia or Hyperlipoproteinemia is the condition of abnormally elevated level of any or all lipids or lipoproteins in the blood, such as Cholesterol, Cholesterol esters, Phospolipids and Triglycerides.

Hyperlipidemia is a major cause of Atherosclerosis and Atherosclerosis associated conditions, such as Coronory heart disease (CHD), Ischemic cerebrovascular disease and Peripheral vascular disease. Atherosclerosis is a generic term for thickening and loss of elasticity of arterial wall. It is characterized by intimal lesion called atheromas or atheromatous or fibrofatty plaques, which protrude and obstruct the vascular lumens. These conditions accounts for the most morbidity and mortality among middle aged and older adults.

In atherosclerotic condition the elevated level of serum cholesterol are sufficient to stimulate lesion development, even if the other risk factors are absent. LDL-C which has an important physiologic role in delivering of cholesterol to the peripheral tissues is called bad Cholesterol. In contrast, role of HDL-C is to mobilize cholesterol from developing and existing atheromas and transporting it to liver for excretion in the bile, so it is called good cholesterol. Hence the aim of the present study was to evaluate whether the plant extract had the capacity to decrease the serum total cholesterol, triglycerides, LDL, VLDL, Atherogenic index, LDL-C/HDL-C ratio and increase the HDL cholesterol level [1].

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Cardiovascular disease (CVD) is the leading cause of death in India, accounting for 28% of mortality. Deaths from CVD have increased from 2,266,000 in 1990 to 2,669,100 in 2004, which is a 17.8% increase in less than two decades. The average age of onset of CVD is younger (below 55 years) among Indians than other populations around the world. The fatality rate for coronary heart disease and other CVDs is higher in India than the other developed countries. These statistics illustrate the importance of identifying and managing risk factors of CHD [2].

Herbal drugs have been an effective treatment since ancient times for treatment of various diseases. Nature provides a wide variety of plants that contain medicinal properties The World Health Organization estimates that more than 80% of the world population relies on herbal drugs as their primary medicinal source used for the treatment of common illness [3].

Ziziphus oenoplia is a spreading, climbing, thorny shrub growing up to 1.5m in height. It is distributed from Indian subcontinent through southern China and South east Asia to northern Australia. [4] It grows along road side forests and thickets. *Ziziphus oenoplia* plant is widely used in Ayurveda for the treatment of various diseases, such as ulcer, Stomach ache, obesity, asthma and it has an astringent, digestive, antiseptic, Hepatoprotective, wound healing and diuretic property [5, 6, 7].

In the present work, the Ethanolic extracts of *Ziziphus oenoplia* (EEZO) leaves were evaluated for Hypolipidemic and antiatherosclerotic property at the dose level of 250 mg/kg and 500mg/kg., b.w, using Wistar albino rats.

MATERIALS AND METHODS

Plant material:

The leaves of the plant of *Ziziphus oenoplia* (L) Mill. Gard were collected from Muthulapuram village, Theni District, Tamilnadu, during August 2012. The collected plant materials were authenticated by Botanist Dr. P.Jayaraman, Director, Institute of Herbal Botany, Plant Anatomy Research Centre, Tambaram, Chennai (Specimen No. PARC/2012/1306). The specimen voucher was deposited in the Department of Pharmacognosy, College of Pharmacy, Madras Medical College, Chennai, Tamil nadu, India. The shade dried leaves were coarsely powdered and used for further studies.

Preparation of Ethanolic extract:

The shade dried, coarsely powdered leaf material of *Ziziphus oenoplia* was extracted with Ethanol by using Soxhlet apparatus for about 48 h. The solvent was completely recovered, collected and concentrated by rotary vacuum evaporator to get the semi solid mass. The weight of extract obtained was 10.85%w/w. The concentrated extract was used for further studies.

Qualitative Phytochemical Analysis:

Freshly prepared extracts were subjected to phytochemical evaluation for the detection of various constituents using conventional protocol [8-10].

Pharmacological studies:

Adult male Wistar albino rats 150- 170g were obtained from animal house of Madras Medical College, Chennai, Tamil Nadu, India. The animals were kept individually in clean and dry standard metallic cages and maintained in a well ventilated animal house at a temperature of 22 ± 2 C and a 12 h light–dark cycle. The animals were fed with standard pellet diet and water *ad libitum*. The entire process was approved by the Institutional Animal Ethical Committee which is certified by the Committee for the purpose of Control and Supervision of Experiments on Animals, India (CPCSEA). **Approval number: 5/243 CPCSEA dated 23.08.2012.**

MATERIALS:

- Ethanolic extract of leaves of *Ziziphus oenoplia*.
- Cholesterol and Cholic acid was obtained from Kiran light laboratories, Mumbai, India.
- Atorvastatin was obtained from Maithrin Laboratories, Hyderabad, India.

Acute toxicity studies:

Acute toxicity study was performed for the Ethanolic extract of *Ziziphus oenoplia* to ascertain safe dose by acute oral toxic class method as per the Organization of Economic Co-operation and Development (OECD), 423 guidelines using Wistar albino rats. Three animal were administered single dose of 2000mg/kg body weight of Ethanolic extract of *Ziziphus oenoplia* by oral route. The animals were observed for mortality up to 72 h [11].

Atherogenic diet induced Hyperlipidemia in experimental rats:

Preparation of diet: [12]

Hyper caloric diet of Atherogenic diet was prepared by following constituents. The percentage is for 100g diet. The feed was

prepared, dried and administered every day in morning to animals with water *ad libitum.*

The Atherogenic diet was given for 30 days along with standard rat chow diet.

Atherogenic diet formula

1. Cholesterol – 2%
2. Cholic acid – 1%
3. Coconut oil – 2%

Experimental design: [13]

Male Wistar albino rats (150-170g) were divided into five groups containing six animals each.

Group I - Served as normal control which received normal saline 5ml/kg p.o for 30 days.

Group II - Served as Cholesterol control which received Cholesterol diet 400mg/kg p.o for 30days.

Group III - Served as Standard control which received Cholesterol diet 400mg/kg p.o for 30 days with Atorvastatin 1mg/kg p.o daily from 16^{th}- 30^{th} day.

Group IV - Served as low dose treated group, which received Cholesterol diet 400mg/kg p.o for 30 days with 250mg/kg of Ethanolic extract of *Ziziphus oenoplia* p.o daily from 16^{th}- 30^{th} day.

Group V - Served as high dose treated group, which received Cholesterol diet 400mg/kg p.o for 30 days with 500mg/kg of Ethanolic extract of *Ziziphus oenoplia* p.o daily from 16^{th}- 30^{th} day.

Food intake and weight gain in rats of each group were observed for 30days. At the end of 30^{th} day blood was collected by retro orbital puncture method. The collected blood was allowed to clot for 30mins at room temperature. Blood samples were centrifuged at 3000 rpm for 20 minutes. Serum was separated and stored at -20°C for biochemical estimation of lipid profile. Samples of Thoracic aorta were collected from the each group of animals for Histopathological study.

Biochemical analysis:

The serum was analyzed for total cholesterol, triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL) by using standard protocol methods. (Auto Analyzer).

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

The AI was calculated by the following formula.

- $\mathbf{AI} = \frac{\mathbf{Total\ cholesterol - HDL - C}}{\mathbf{HDL - C}}$
- $\%\ \boldsymbol{Protection} = \frac{\mathbf{AI\ (control) - AI\ (Treated)}}{\mathbf{AI\ (control)}} \times \mathbf{100}$
- $\frac{\mathbf{LDL - C}}{\boldsymbol{HDL - C}}$ **Ratio was calculated as the ratio of plasma LDL-C to HDL-C levels.**

Statistical analysis:

Results were expressed as mean ± SEM. The data was analyzed by one way ANOVA followed by post hoc Dunnette test for variance using IBM SPSS statistics, version 21. P values <0.01 were considered as statistically significant.

RESULTS

Qualitative Phytochemical Analysis

The results of qualitative phytochemical analysis are given in the (**Table 1**). It revealed the presence of carbohydrates, amino acids, alkaloids, flavonoids, phenolic compounds present in the Ethanolic extract.

Acute toxicity study:

LD50 cut off value of Ethanolic extract was found to be safe up to the dose of 5000mg/kg b.w. The extract belongs to the category 5 of the Globally Harmonized Classification System (GHS). Therefore $1/10^{th}$ (500mg/kg) and $1/20^{th}$ (250mg/kg) were selected for further studies.

Food intake and weight gain:

The food intake and weight was increased in high cholesterol fed diet rats as compared to normal control. Treatment with Ethanolic extract of *Ziziphus oenoplia* 250mg/kg and 500mg/kg groups showed no change in food intake but there was significant decrease in weight gain as compared to high cholesterol fed diet rats. 500mg/kg treated groups showed more effect in decrease the weight gain when compare to 250mg/kg of Ethanolic extract treated groups. The results are given in (**Table 2**).

Effect on serum lipid profile:

High Cholesterol diet (HCD) fed rats produced significant increase ($p<0.01$) in serum cholesterol, triglyceride, VLDL-C, LDL-C, atherogenic index but significant decrease ($p<0.01$) in HDL-C level as compared to normal control rats. Treatment with Ethanolic extract of 250mg/kg and 500mg/kg showed significant reduction ($p<0.01$) in serum cholesterol, triglyceride, VLDL-C, LDL-C, atherogenic index and significant increase ($p<0.01$) in HDL-C as compared to high cholesterol diet fed rats. The results are given in (**Table 3**).

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

Atherogenic index was statistically found to be increased in cholesterol control group 87% compared with the values found in their normal control groups. Atherogenic index of both 250mg/kg and 500mg/kg of Ethanolic extract of *Ziziphus oenoplia* treated groups decreased the AI

by 59 and 72% when compared to the cholesterol control groups.

LDL-C/HDL-C ratio is a productive indicator of cardio vascular diseases incidence. The cholesterol control groups showed increase in the value when compared to normal control and both 250mg/kg and 500mg/kg of Ethanolic extract of *Ziziphus oenoplia* treated groups showed decrease in the value when compared to cholesterol control groups. These results are given in (Table 3).

Histopathological studies of Thoracic aorta:

In Histopathological study the rat aorta of cholesterol control showed cholesterol deposition and atheromatous plaque size when compared to normal control.

Ethanolic extract of *Ziziphus oenoplia* treated group showed decrease in the level of cholesterol deposition and atheromatous plaque size and inflammatory changes as compared to cholesterol control groups. The results are given in (**Fig 1**).

Table 1: Qualitative Phytochemical Analysis

S. No	Phytochemical tests	Ethanol extract
1	Alkaloids	+
2	Carbohydrates	+
3	Glycosides	-
4	Phytosterol	-
5	Fixed oil and Fats	-
6	Resins	-
7	Phenolic compounds	+
8	Tannins	+
9	Protein and Amino acids	+
10	Flavonoids	+
11	Terpenoids	-
12	Saponins	-
13	Gum and Mucilage	-

+ =Presence; - = Absence

Table 2: Changes in Body Weight

Groups	Change in body weight (g)
Group I - Normal control	23.33±15.27
Group II - Cholesterol control	44.66 ± 5.77^{b}
Group III - Standard control	16.66 ± 5.77^{a}
Group IV - EEZO 250mg/kg	23.33 ± 5.77^{a}
Group V - EEZO 500mg/kg	18.33 ± 10.40^{a}

Values are mean± SEM of 6 animals in each group.

The values were calculated by using ONE WAY ANOVA followed by Post hoc Dunnette test.

a – Standard group has a highly significant activity when compared to the cholesterol control group ($p<0.0001$).

b- Extract treated group has a significant activity when compared to the cholesterol control group ($p<0.01$).

Table 3: Effects on Ethanolic Extract of *Ziziphus Oenoplia* on Lipid Profile

Groups	Total Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	AI	LDL/HDL
Group I (Normal)	105.18±0.55	96.93 ±0.48	55.68±0.13	31.12±1.39	19.38 ±0.09	0.88 ±0.01	0.55±0.02
Group II (Cholesterol)	$254.30^{b}\pm1.17$	$198.62^{b}\pm0.12$	$33.00^{b}\pm0.25$	$181.58^{b}\pm1.34$	$39.72^{b}\pm0.02$	$6.71^{b}\pm0.08$	$5.50^{b}\pm0.07$
Group III (Standard)	$112.28^{a}\pm0.62$	$102.16^{a}\pm0.06$	$49.21^{b}\pm0.05$	$42.63^{b}\pm0.58$	$20.43^{a}\pm0.01$	$1.28^{a}\pm0.01$	$0.86^{a}\pm0.01$
Group IV (250mg/kg)	$163.36^{b}\pm0.48$	$148.00^{b}\pm0.08$	$43.42^{b}\pm0.18$	$90.34^{b}\pm0.50$	$29.59^{b}\pm0.02$	$2.76^{b}\pm0.02$	$2.08^{b}\pm0.02$
Group V (500mg/kg)	$132.16^{b}\pm0.34$	$125.40^{b}\pm0.05$	$46.38^{b}\pm0.11$	$60.20^{b}\pm0.59$	$25.07^{b}\pm0.002$	$1.87^{b}\pm0.03$	$1.29^{b}\pm0.01$

Values are mean± SEM of 6 animals in each group.

The values were calculated by using ONE WAY ANOVA followed by Post hoc Dunnette test.

a – Standard group has a highly significant activity when compared to the cholesterol control group ($p<0.0001$).

b- Extract treated group has a significant activity when compared to the cholesterol control group ($p<0.01$).

HISTOPATHOLOGICAL STUDIES OF RAT AORTA

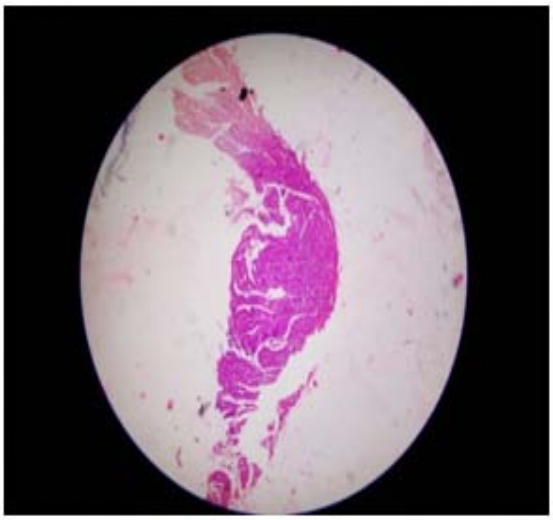

Fig 1: Normal control

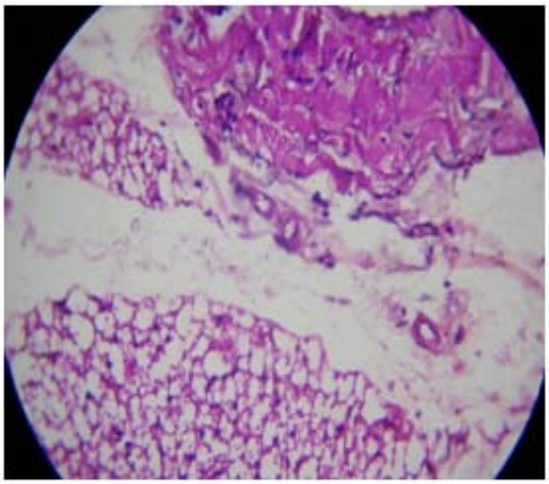

Fig 2: Cholesterol control

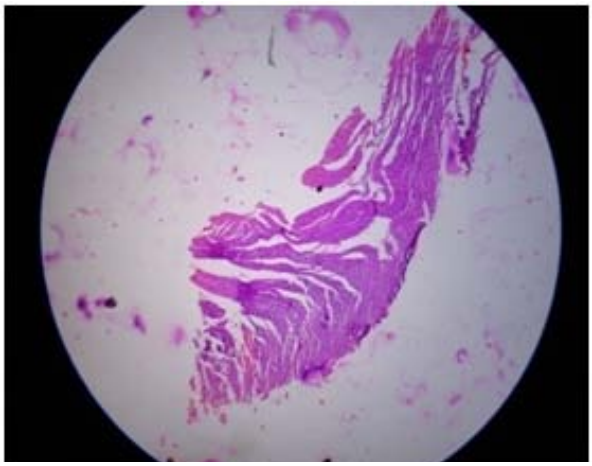

Fig 3: Standard (Atorvastatin) control

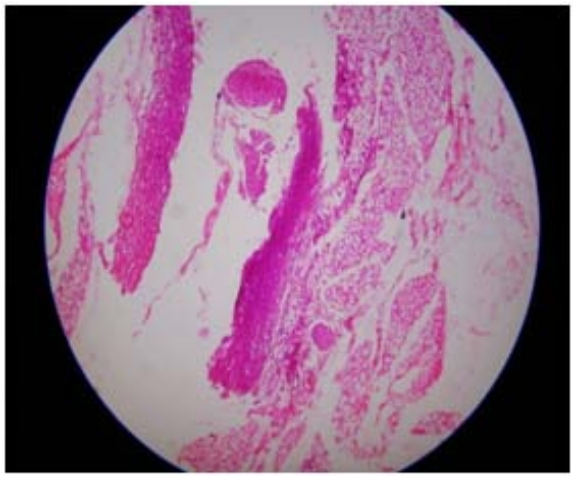

Fig 4: 250mg/kg Ethanolic extract of *Ziziphus oenoplia*

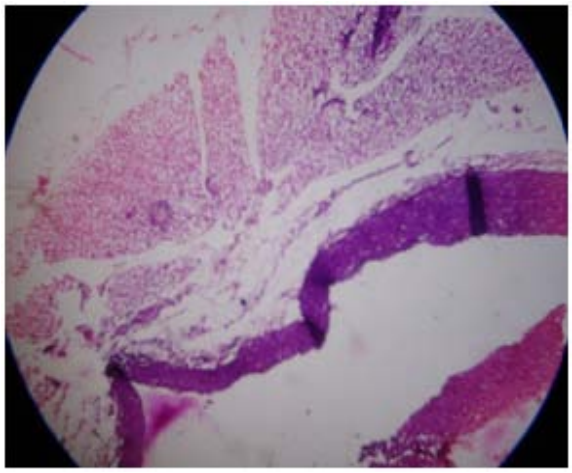

Fig 5: 500mg/kg Ethanolic extract of *Ziziphus oenoplia*

DISCUSSION

Hyperlipidemia is one of the major risk factors for premature atherosclerosis and essentially the cholesterol in atherosclerotic plaque is derived from that of circulatory cholesterol. Hence treatment with *Ziziphus oenoplia* showed significantly decreases the Cholesterol, Triglyceride, VLDL-C, LDL-C, Atherogenic index and a significantly increase in HDL-C in serum. These results were further substantiated with Histopathological results. Atherogenic index indicates the deposition of foam cells or plaque or fatty infilteration or lipids in aorta. The higher the atherogenic index, the higher is the risk of CHD in cholesterol control groups showed increase the AI and LDL-C/HDL-C ratio since there is a positive correlation between an increased LDL-C/HDL-C ratio and the development of atherosclerosis. More over the administration of Ethanolic extract of *Ziziphus oenoplia* significantly suppressed the elevated levels of AI and LDL-C/HDL-C ratio showing the beneficial effect of this plant in preventing atherosclerosis incidence.

In addition ethanolic extracts of *Ziziphus oenoplia* showed protective action which is reported to have a preventive function against atherogenesis since an independent inverse relationship between blood HDL-C levels and cardio vascular risk incidence. The lipoprotein called "good cholesterol" facilitates the mobilization of triglycerides and cholesterol from plasma to liver where it is catabolised and eliminated in the form of bile acids. The possible mechanism of this activity may result from the enhancement of lecithin cholesteryl acyl transferase (LCAT) and inhibition of Hepatic Triglyceride Lipase (HTL) on HDL which may lead to a rapid catabolism of blood lipids through enterohepatic tissues

In histopathological study we found treatment of ethanolic extract of *Ziziphus oenoplia* significantly decreases the plaque size and inflammatory changes in aorta.

Thus from above results it can be concluded that ethanolic extract of *Ziziphus oenoplia* has significant hypolipidemic activity which may be due to the presence of Alkaloid, Flavonoids and Phenolic compounds. Further studies on isolation of active constituents which is responsible for this activity and *in vivo* hypolipidemic potential of the isolated compound are under investigation.

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