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EVALUATION FOR ANTI HYPERLIPIDEMIC ACTIVITY OF *CASERIA SYLVESTRIS* EXTRACTS

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ABSTRACT

The aim of the present study is to evaluate the effect of Casearia sylvestris Methanolic Extract and Phenolic Extracts on plasma lipid levels in Triton -X-100 induced hyperlipidemic rats. Major complication of hyperlipidemia are atherosclerotic heart disease, heart attack and heart stroke, but atherosclerosis is primary cause of death. Developing countries are reliant on medicinal plants as their main source of treatment for diseases. As Casearia sylvestris is of native habitat, it is locally available, cost effective with no side effects. The results concluded that PECS (500 mg/kg) have definite antihyperlipidemic activity in Triton X-100 induced hyperlipidemic model which is as equipotent as Atorvastatin treated groups. Further studies on this extract may lead to identify the possible mechanism of action and isolation of active principle from the same.

KEY WORDS

Casearia sylvestris, antihyperlipidemic, Atorvastatin.

INTRODUCTION

Lipid is the scientific term for fats in the blood. At Normal levels, lipids perform important functions in your body, but can cause health problems if they are present in excess. The term hyperlipidemia means high lipid levels. Hyperlipidemia includes several conditions, but it usually means that you have high cholesterol and high triglyceride levels.[1]

Lipid and lipoprotein abnormalities are common in the general population and are regarded as a modifiable risk factor for cardiovascular disease due to their influence on atherosclerosis. In addition, some forms may predispose to acute pancreatitis.[2]

Since blood and other body fluids are contain water, fats need a special transport system to travel around the body. They are carried mixed with protein particles, called lipoproteins. Four major lipoproteins exist, each with a different function: chylomicrons, very–low-density lipoproteins (VLDLs), intermediate-density lipoproteins (IDLs), and LDLs. The protein components of the lipoprotein are known as apolipoproteins or apoproteins. The different apolipoproteins serve as cofactors for enzymes, and ligands for receptors. Defects in apolipoprotein metabolism lead to abnormalities in lipid handling. [3-5]

Major complication of hyperlipidemia is atherosclerotic heart disease, heart attack and heart stroke, but atherosclerosis is primary cause of death. Developing countries are reliant on medicinal plants as their main source of treatment for diseases. As *Casearia sylvestris have* the native habitat the production is more so it is locally available cost effective with no side effects.As *Casearia sylvestris* is cost effective and beneficiary in metabolism of cholesterol, so it has been taken in to consideration in order "To evaluate Anti-hyperlipidemic activity of Methanolic Extract and Phenolic Extracts of *Casearia sylvestris* in triton X -100 induced hyperlipidemic rats".[5]

The effect of *Casearia sylvestris* Methanolic Extract and Phenolic Extracts on plasma lipid levels in Triton -X-100 induced hyperlipidemic rats.

Casearia grows as a shrub or small tree usually 2 or 3 meters tall, but sometimes grows up to 10 meters in undisturbed areas of the Amazon. In the clay soils of the Amazon, the plant has adapted for nutrient absorption and support by forming extensive lateral roots that are white, stiff, and covered with a corky bark. The tree produces small white, cream, or greenish flowers (which smell like a mixture of honey and urine) crowded on short stalks on the leaf axils. After flowering it produces small fruits, 3-4 mm in diameter, which split open to reveal three brown seeds covered with a red-to-orange aril. Guacatonga grows wild throughout the tropics, adapting to both forests and plains. It is native to Cuba, Jamaica, Hispaniola, Puerto Rico, the Caribbean, Central America, and South America (including Brazil, Peru, Argentina, Uruguay, and Bolivia). The chemical makeup of guacatonga is quite complex. Scientists conducting the antivenin research discovered that the leaves and twigs of the plant contain a phytochemical called *lapachol*. This is the well known and studied anticancerous/antifungal compound from which another rainforest plant, pau d'arco (*Tabebuia impetiginosa*), gained much renown. While other researchers have been studying the anticancerous and antitumorous properties of guacatonga, a completely different set of phytochemicals has fueled their interest. These compounds, called*clerodane diterpenes*, are found abundantly in guacatonga and some have been patented as antisarcomic agents. Clerodane diterpenes have been documented with a wide range of biological activities ranging from insect antifeedants, to antitumorous, anticancerous, and antibiotic agents, to HIV replication inhibitors. Some of the clerodane diterpenes documented in guacatonga are novel chemicals which scientists have named casearins (A thru S). Other chemicals in guacatonga include caprionic acid, casearia clerodane I thru VI, casearvestrin A thru C, hesperitin, lapachol, and vicenin. [6-8]

MATERIALS

Atorvastatin is bought from Dr.Reddys Lab, Hyderabad. Normal saline is bought from Claris life sciences.Ltd., Ahmedabad, India. Chloroform is bought from Molychem, Mumbai. Diethyl ether is bought from Finar Ltd, Ahmedabad, India. Triton X-100 is bought from Unisource Chemical Pvt, Ltd.

Materials and Method

Collection and Authentification of Plant Material

The Aerial Parts of *Caesaria sylvestris* for the study were procured and authenticated

Extraction of Plant Material

The plant is grinded in to a coarse powder with the help of suitable grinder.

Cold Extraction (Methanol Extraction)

In this work the cold extraction process was done with the help of methanol. About 200gms of powdered material was taken in a clean, flat bottomed glass container and soaked in 750 ml of methanol. The container with its contents were sealed and kept for period of 7 days accompanied by continuous shaking with the shaker. The whole mixture then went under a coarse filtration by a piece of a clean, white cotton wool.[9]

Evaporation of Solvent

The filtrates (methanol extract) obtained were evaporated using Rotary evaporator in a porcelain dish. They rendered a gummy concentrate of greenish black. The extract was kept in vaccum dissecator for 7 days.

Preliminary Phytochemical Screening

Preliminary phytochemical screening of the *Caesaria sylvestris* extract was carried out for the analysis of Alkaloids, Carbohydrates, Tannins, Saponins, Steroids, Phenols, and Flavonoids as per the standard methods [10]

Animals

Healthy Adult Male mice of 5 weeks old with Average weight in the range of 30-40gms were selected. Animals are housed 4 per cage in temperature controlled (27 ^{0}C ±3 ^{0}c) room with light/dark cycle in a ratio of 12:12 hrs is to be maintained. The Animals are allowed to acclimatize to the environment for seven days and are supplied with a standard diet and water *ad libitum*. The guidelines of committee for the purpose of control and supervision of experiments on Animals (CPCSEA), Govt of India were followed, and prior permission was sought from the Institutional Animal Ethics Committee (IAEC) for conducting the study.

Acute toxicity studies

The Acute Toxicity Studies was performed using female rats as per OECD Guideline No.423 (Short term toxicity). Male mice were selected of weight around 50 ±10 gm for main test. Single animals are dosed in sequence

usually at 48 h intervals. A Dose Progression Factor of 3.2 is used. Using the default dose progression factor, doses would be selected from the sequence (1.75, 5.5, 17.5, 55, 175, 550, 1750, and 5000). However, the time intervals between dosing are determined by the onset, duration, and severity of toxic signs. Treatment of an animal at the next dose should be delayed until one is confident of survival of the previously dosed animal. If the animal survives, the second animal receives a higher dose. If the first animal dies or appears moribund, the second animal receives a lower dose. The toxicological effects were observed in terms of mortality expressed as LD50. The number of animals dying or surviving during a period was noted. [11-13]

Method of Induction

The systemic administration of the surfactant Triton X-100 to mice results in a biphasic elevation of plasma cholesterol and triglycerides. 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.[14]

Experimental Animal Protocol

Experimental rats, straved for 18 hr, were provided water *ad libitum*. The rats were divided in to six groups containing four animals in each group.

Group – I : Normal Control.(Normal saline 10ml/kg orally for 7 days)

Group – II : Hyperlipedemic control, (Triton x 100.)

Group – III : Hyperlipedemic rats treated with MEBM at dose of 500mg/kg. for 7days

Group – IV : Hyperlipedemic rats treated with PEBM at dose of 400mg/kg for 7days.

Group – V : Hyperlipedemic rats treated with PEBM at dose of 500mg/kg. for 7days.

Group – VI : Hyperlipedemic rats treated with Atorvostatin at 10 mg/kg for 7days.

All the groups recives single i.p. injection of Trition X-100 at dose of 100mg/kg, simultaneously with Group- II, Group – III, Group – IV, Group – V, Group – VI , expect Group – I (Normal control). After 72 hours of Triton X-100 injection. The Group – VI receives Atorvostatin at dose of 10 mg/kg, was prepared by suspending bulk Atorvastatin in aqueous 0.5% methyl cellulose[42] for 7 days. The Group– III, receive MEBM, at daily dose of 500mg/kg orally for 7 days and Group – IV, Group – V receives PEBM at daily dose of 400mg/kg and 500mg/kg orally for 7 days [15]

Blood Sample Collection and Analysis

The rats are anesthetised by ether and then Blood samples were collected on 0^{th} and 8^{th} day from retro-orbital plexus of rat using micro capillary technique from rats of all the groups, and centrifuged at 3000 rpm for 15 min so as to get serum. The serum is analyzed for total cholesterol, triglycerides and HDL levels using biochemical kits (diagnostic kit.).[16]

VLDL, and LDL- Cholesterol were calculated by the below formula

Serum LDL- Cholesterol concentration was calculated According to the equation of Fried and wald.

LDL-Cholesterol=Total Cholesterol – (HDL- Cholesterol +TG/5)

VLDL-C = TG/5

Bio Chemical Assays for lipids

Estimation Procedures: Plasma Lipid Profile Estimation

Total cholesterol LDL cholesterol, HDL Cholesterol, VLDL cholesterol, Triglycerides levels were measured using commercial kits.

Estimation of Triglycerides. (GPO/PAP Method)

Procedure

Wave length: 546 (Green Filter)

Temperature: 37 ^{o}C

Reaction type: End point with standard.

Pipettle in to clean dry tube labelled Blank (B), Standard (S) and Test (T) and then add following:

Table No 1

	Blank	Standard	Test
Enzyme reagent	1.0 ml	1.0 ml	1.0 ml
Standard	-	0.01ml	-
Serum / Plasma	-	-	0.01 ml

Mix well and incubate for 10 minutes at 37^{0} C. Read absorbance of standard and test against blank.[17]

Calculations

Triglyceride concentration in mg% = Absorbance of test /Absorbance of Standard X 200

Estimation of cholesterol (Total cholesterol). CHOD/POD Method.

Procedure

Wave Length: 500 nm (green filter)
Temperature: 37^{0}C.
Reaction type: End point with standard.

Table No 2

	Blank	Standard	Test
Enzyme Reagent	1 ml	1ml	1 ml
Deionized Water	0.01 ml	-	-
Standard	-	0.01 ml	-
Serum / Plasma	-	-	0.01 ml

Pipette in a clean dry test tube labelled as Blank (B), Standard (S), Test (T). Mix and read the optical density (OD) at 500nm against blank after 5min incubation (37^{0}c). The final colour is stable for atleast 1 hour.[18]

Calculations

Cholesterol concentration in mg% = Absorbances of Test/Absorbances of Standard×200 (Standard).

Estimation of HDL cholesterol.

Procedure: It includes two steps.

Step:1- precipitation

Table No 3

Serum	0.2 ml
HDL precipitating reagent	0.3 ml

Step :2 – colour development

Take 3 clean glass tubes labelled as blank (B), standard (S), and test (T).

Mix well and stand at room temperaturefor 10 min, centrifuge at 3000 rpm for 10 min.

Table No 4

	Blank	Standard	Test
Enzyme reagent	1 ml	1ml	1 ml
Cholesterol (Standard)	-	0.01 ml	-
Supernatant serum Step-1	-	-	0.1 ml
Distilled water	0.1 ml	0.1 ml	-

Incubation for 5 min at 37^{0}C and read the optical density at 500nm against blank.

Calculations

HDL cholesterol =Absorbance of test /Absorbance of standard × 50(Standard concentration) .

LDL CALCULATION.

It is calculated using formula: LDL = TC-HDL-TG/5.0 (mg/dl).

VLDL is calculated using formula:

VLDL = Triglycerides (mg/dl) / 5,

According to these guidelines, the normal range of lipid profile

Table No 5

Total cholesterol	< 200 mg/dl
Triglycerides	< 200 mg/dl
HDL	> 40 mg/dl
LDL	< 150 mg/dl
VLDL	5-30 mg/dl

LDL/HDL and TC/HDL ≤ 5 mg/dl are the favourable risk factor.

Statistical Analysis

Results are expressed as Mean ± S. D. all the results were compared with control subject one-way analysis of variance (ANOVA), followed by the dunnet t-test using Graph Pad Prism Software 6 version. P Values < 0.05 were as considered statistically significant.

RESULTS AND DISCUSSION

%Yield of Methanolic Extract from Aerial Parts of *Casearia sylvestris* was found to be **34.75**

% Yield value of Phenolic Extract from Aerial Parts of *Casearia sylvestris* was found to be **8.6**

Preliminary Phytochemical Screening

Investigation revealed the presence of Alkaloid, Tannin, Saponin, Phenol in Methanolic Extract of *Casearia sylvestris* while only Phenol were present in Phenolic Extract of *Casearia sylvestris*

Table.no.6. Preliminary Phytochemical Screening

Phytochemical	Results
Steroid	-
Alkaloid	+
Tannin	+
Carbohydrate	-
Phenol	+
Flavonoid	+
Saponin	+

(+) Present; **(-)** Absent

Acute toxicity studies

As per (OECD) draft guidelines 423 adopted, Female albino rats were administered with *Casearia sylvestris* and doses was be selected in the sequence (1.75- 5000) using the default dose progression factor, for the purpose of toxicity study. Animals are observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours and daily thereafter, for a total of 14 days, In all the cases, no death was observed within 14 days. Additional observations like behavioral changes in skin, fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous systems and somato motor activity and behavior pattern were also found to be normal. Attention was also given to observation of tremors and convulsions, salivation, diarrhoea, lethargy, sleep and coma. Overall results suggested the LD_{50} value as 5000 mg/kg. Hence therapeutic dose was calculated (i.e. 400mg/kg and 500 mg/kg) of the lethal dose for the purpose of antihyperlipidemic [19-20] investigations

RESULTS

Table.no:7 Lipids Levels Obtained on 8th Day (After Treatment).

Sl.NO	GROUPS	TC	TG	HDL	LDL	VLDL
I	Normal Control	64.03 ± 1.45	82.66 ± 2.46	38.91 ± 2.33	8.45 ± 3.43	16.53 ± 0.49
II	Hyperlipidemic Control	192.47 ± 5.05	168.9±5.28	21.86±2.74	136.82±7.00	33.79±1.05
III	MECS 500mg/kg	134.19 ± 3.5*	117.57 ± 5.25*	27.1 ± 2.99***	83.58 ± 5.26*	23.51 ± 1.05***
IV	PECS 400mg/kg.	121.74 ± 7.74*	107.93 ± 6.67*	31.04 ± 4.32**	69.11 ± 10.51***	21.58 ± 1.33***
V	PECS 500mg/kg.	112.97 ± 5.25*	103.55 ± 4.2*	33.15 ± 2.51**	59.1 ± 6.89*	20.71 ± 0.84***
VI	Standard Atorvostatin10mg/kg	92.29 ± 5.63*	102.26 ± 7.68*	39.18 ± 3.14**	32.91 ± 7.61*	20.44 ± 1.53**

All the data are expressed as MEAN ± S.D (n=4), *P = < 0.001, **P = < 0.01, ***P = < 0.05. vs GROUP. II

TC: Total Cholesterol; TG: Triglycerides; HDL-C: High Density Lipoprotein cholesterol;

LDL-C: Low Density Lipoprotein- cholesterol; VLDL-C : Very Low Density Lipoprotein ;

MECS: Methanolic Extract of *Casearia sylvestris* ; PECS : Phenolic Extract of *Casearia sylvestris*

CONCLUSION

The results concluded that PECS (500 mg/kg) have definite antihyperlipidemic activity in Triton X-100 induced hyperlipidemic model which is equipotent activity when compared with Atorvastatin treated groups. Further studies on this extract may lead to identify the possible mechanism of action and isolation of active principle from the same.

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