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INVESTIGATION FOR ANTI HYPERLIPIDEMIC ACTIVITY PROPERTIES OF SESBANIA GRANDIFLORA

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Abstract:
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 Sesbania grandiflora have the native habitat the production is more so it is locally available cost effective with no side effects. As Sesbania grandiflora 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 Extracts of Sesbania grandiflora in triton X -100 induced hyperlipidemic rats of the respective extracts. And the final results showed that this plant shows the hyperlipidemic activity and also shows minimal side effects towards the liver and cardiac muscle tissues.

Key words: *Sesbania grandiflora, hyperlipidemic activity etc.*

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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.

Special blood tests are carried out to identify the specific disorder when lipid levels in the blood are very high. Specific disorders may include several hereditary disorders, producing different lipid abnormalities and have different risks.

Most blood tests measure levels of LDL (sometimes called "bad") cholesterol, HDL (sometimes called "good") cholesterol, total cholesterol (LDL plus HDL), and triglycerides. To have a low risk of heart disease, desirable lipid levels are:

- LDL less than 130 mg/dL
- HDL greater than 40 mg/dL (men) or 50 mg/dL (women)
- Total cholesterol less than 200 mg/dL
- Triglycerides less than 200 mg/dL

Currently available hypolipidemic drugs have been associated with Number of side effect[11]. The consumption of Synthetic drugs leads to hyperuricemia, diarrhoea, nausea, myositis Gastric irritation flusing, dry skin and abnormal liver function[5]. An herbal treatment for hyper cholesterolemia has almost no side effects and is relatively cheap, locally available. They are effective in reducing the lipid level in the system [12].Medicinal plants play a major role in Antihyperlipidemic Activity [5].

Some of the natural medicine used as anti hyperlipidemic agents are Camellia sinensis (L.) Kuntze (Theaceae), Chlorella pyrenoidosa Chick. (Oocystaceae), Citrus aurantium L. (Rutaceae), Garcinia cambogia L. (Clusiaceae), Lagerstroemia speciosa (L.) Pers. (Lythraceaea), Panax ginseng C.A. Meyer (Araliaceae), Salix matsudana Koidzumi (Salicaceae), Nelumbo nucifera Gaertn. (Nymphaeaceae) and Stellaria media and Clerodendrum phlomites. [13]

The medicinal plants are potential sources of drugs as they are rich in secondary metabolites and essential oils of therapeutic importance.1 Uses of medicinal plants in various ailments are due to being economical, effective, their ease availability and due to their safety.2 Because of these advantages the use of medicinal plants has been widely increased by the traditional medical practitioners in their day to day practice.3 Foods are used commonly to meet our nutritional needs. However, foods obtained by plants contain a wide range of non- nutrient phytochemicals that are synthesized by plants for their own defence and for other biological functions. When we ingest these plant foods to meet our nutritional needs, we also ingest a wide variety of these non-nutrient phytochemicals. These phytochemicals have the potential for preventing chronic diseases and also non-toxic.

Agastya is fast growing and soft wooded tree grows up to 3-8mt in height. Leaves are regular, deciduous, and abruptly pinnate and rounded about 15-30cm long and has 10-20 lea ets. According to different species this plant bears red and white owers. Flowers are long, has 2-4 ower racemes, shallowly 2 lipped and are boat shaped. Plant bears owers in month of September and October. Fruit pods re slender, falcate about 30cm long contains 15-30 seeds inside.

The flowers of the plant have a bitter taste and an astringent action on the body. It can be used in intermittent fever, night blindness, rhinitis, running nose, abdominal pain and all kinds of liver and spleen disorders. It can be used to detoxify the body, if there is chronic toxicity due to improper dietary patterns over a period of time.

Leaves of Agastya plant have a non – slimy, bitter nature with a hot potency. These are heavy to digest and can effectively balance kapha dosha. Leaves prove to be helpful in worm infestations and bleeding disorders like menorrhagia and ulcerative colitis. These also possess tonic properties for the body.

Agastya fruits are very helpful in pacifying pitta dosha. It aids in weight loss and helps to manage abdominal tumours. These also help in improving memory and intelligence. Bark helps to manage IBS symptoms and low digestive power and constipation while the flowers help in treating diarrhoea. Roots and bark of the plant are also used for external application over the body. Juicy extract of the leaves can be used as nasya to relieve kapha predominant disorders of the upper respiratory tract and for epilepsy also. Leaf paste can be applied to treat oral and throat problems. Root bark is applied externally to reduce pain and inflammation in arthritis and gout. Paste of red flower

agastya is used in rheumatism. Leaf juice extract is used in nasya for epileptic sufferers.

MATERIALS:

Atorvastatin (Dr.Reddys Lab, Hyderabad.), Normal saline (Claris life sciences .Ltd., Ahmedabad, India.), Chloroform, Diethyl ether (Finar Ltd, Ahmedabad, India.) Triton X-100 (Unisource Chemical Pvt, Ltd).

METHODS:

Collection and Authentification of Plant Material:
The whole plant of *Sesbania grandiflora* collected and authenticated by Dr. K madhava chetty, department of botany, Sri Venkateswara University, tirupathy.

Animal Ethics permission:
the housing of the animals were carried out in the animal house of the Teja College of pharmacy-kodad, the treatment and sample collection, analysis of samples carried out in VYAS LABS, Medchal, Malkajgiri with approved CPCSEA registration number-2085/PO/RCBIBT/S/19/CPCSEA

Extraction of Plant Material:
The plant is grinded in to a coarse powder with the help of suitable grinder

Hot Continuous Extraction (Soxhlet):
In this method, the finely ground crude drug is placed in a porous bag or "thimble" made of strong filter paper, which is placed in chamber E of the Soxhlet apparatus. The extracting solvent in flask A is heated, and its vapors condense in condenser D. The condensed extractant drips into the thimble containing the crude drug, and extracts it by contact. When the level of liquid in chamber E rises to the top of siphon tube C, the liquid contents of chamber E siphon into flask A. This process is continuous and is carried out until a drop of solvent from the siphon tube does not leave residue when evaporated. The advantage of this method, compared to previously described methods, is that large amounts of drug can be extracted with a much smaller quantity of solvent. This effects tremendous economy in terms of time, energy and consequently financial inputs. At small scale, it is employed as a batch process only, but it becomes much more economical and viable when converted into a continuous extraction procedure on medium or large scale.

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 vacuum desiccator for 7 days.

Preliminary Phytochemical Screening:
Preliminary phytochemical screening of the Sesbania grandiflora extract was carried out for the analysis of Alkaloids, Carbohydrates, Tannins, Saponins, Steroids, Phenols, and Flavonoids. As per the standard methods.

Acute toxicity studies:
Objective of performing Acute Toxicity Studies:
The aim of performing acute toxicity studies is for establishing the therapeutic Index (TI) of a particular drug and to ensure the safety in vivo. Acute toxicity study is generally carried out for the determination of LD50 value in experimental animals.

Requirements:
Animal: wistar rats (male) 150-200gm
Drugs/extracts: extracts of Sesbania grandiflora.
Procedure

- The overnight fasted rats were weighed and selected.
- The extracts were dosed in a stepwise procedure, with the initial dose being selected as the dose expected to produce some signs of toxicity and were observed for a period of two weeks.
- The toxic doses were selected based on the Guideline 423.
- The wistar rats of single sex, weighing between 150 to 200 g were selected and divided in to 5 groups each consisting of 5 animals. They were maintained under standard conditions (room temperature at 22±3 ˚C, 12 hr light/dark) and allowed free access to water along with standard pelleted diet for one week before the experiment. The animals were subjected for acute toxicity study using each extract at a dose of 2000 mg/kg orally in 12 groups and observed at regular intervals of 1, 2, 4, 8, 12 and 24 hours for skin changes, morbidity, aggressiveness, increase oral secretion, sensitivity to the sound and pain as well as respiratory movements and mortality.

Method of Induction:
The systemic administration of the surfactant Triton X-100 to rats results in a biphasic elevation of plasma cholesterol and triglycerides. Hyperlipidemia was induced in Wister 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.[13]

Experimental Animal Protocol:

Experimental rats, starved 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 : Hyperlipidemic control, (Triton x 100.)

Group – III : Hyperlipidemic rats treated with MESG at dose of 250mg/kg. For 7days

Group – IV : Hyperlipidemic rats treated with MESG at dose of 500mg/kg for 7days.

Group – V : Hyperlipidemic rats treated with MESG at dose of 750mg/kg. For 7days.

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

All the groups receives single i.p. injection of Triton 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 MESG, at daily dose of 250mg/kg orally for 7 days and Group – IV, Group – V receives MESG at daily dose of 500mg/kg and 750mg/kg orally for 7 days

Blood Sample Collection and Analysis:

The animals are anesthetized by ether and then Blood samples were collected on 0^{th} and 8^{th} day[13] from retro-orbital plexus of rat using micro capillary technique from animals of all the groups[43], 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.) [44].

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

Serum LDL- Cholesterol concentration was calculated According to the equation of Fried and wald[45].

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) [46]:

Procedure

Wave length: 546 (Green Filter)

Temperature: 37 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:

	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 minute at 37^0 C. Read absorbance of standard and test against blank.

Calculations:

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

Estimation of cholesterol (Total cholesterol). CHOD/POD Method [47].

Procedure:

Wave Length : 500 nm (green filter)

Temperature : 37^0C.

Reaction type : End point with standard.

	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 (37^0c). The final colour is stable for at least 1 hour.

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

Estimation of HDL cholesterol [47].
Procedure : It includes two steps.
Step:1- precipitation

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 temperature for 10 min, centrifuge at 3000 rpm for 10 min.

	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 37^0c and read the optical density at 500nm against blank.

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

LDL CALCULATION [47]:
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

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 *Sesbania grandiflora* was found to be **34.75**

Preliminary Phytochemical Screening:
Investigation revealed the presence of Alkaloid, Tannin, Saponin, and Phenol in Methanolic Extract of *Sesbania grandiflora*.

Table.no.2. 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 *Sesbania grandiflora* 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 750 mg/kg) of the lethal dose for the purpose of antihyperlipidemic investigations.

RESULTS:

Table.no:3 Lipids Levels Obtained on 8th Day (After Treatment).

GROUPS	TC	TG	HDL	LDL	VLDL
Normal Control	63.03 ± 1.45	83.66 ± 2.46	39.91 ± 2.33	9.45 ± 3.43	17.53 ± 0.49
Hyperlipidemic Control	193.47 ± 5.05	167.9±5.28	22.86±2.74	137.82±7.00	34.79±1.05
MESG 250mg/kg	135.19 ± 3.5*	118.57 ± 5.25*	28.1 ± 2.99***	84.58 ± 5.26*	24.51 ± 1.05***
MESG 500mg/kg.	122.74 ± 7.74*	108.93 ± 6.67*	32.04 ± 4.32**	68.11 ± 10.51***	22.58 ± 1.33***
MESG 750mg/kg.	113.97 ± 5.25*	104.55 ± 4.2*	34.15 ± 2.51**	58.1 ± 6.89*	21.71 ± 0.84***
Standard Atrovastatin10mg/kg	91.29 ± 5.63*	101.26 ± 7.68*	38.18 ± 3.14**	31.91 ± 7.61*	21.44 ± 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 ; **MESG:** Methanolic Extract of *Sesbania grandiflora* .; **MESG:** Methanolic Extract of *Sesbania grandiflora*

Effect of *Sesbania grandiflora* Extracts on Serum Total Cholesterol levels.

In the Normal rats the Total Cholesterol levels were found to be 64.03 ± 1.45 on 0th day respectively. Treatment with Triton-X-100 caused a significant rise in the levels of Total Cholesterol in Group- II, Group- III Group- IV Group- V Group- VI (i.e. Hyperlipidemic Control, MESG 250 mg/kg, MESG 500 mg/kg, MESG 750 mg/kg, & Standard (Atorvostatin) 10mg/kg) and the levels were found to be 192.47 ± 5.05, 175.28 ± 4.43, 180.97 ± 5.21, 187.86 ± 9.66, and 180.79 ±9.1, respectively.

Administration of various doses of the MESG after the Induction with Triton-X-100 resulted in the decreasing of Cholesterol levels. The total cholesterol levels of groups treated with MESG at dose of 750mg/kg were 135.19 ± 3.5*, and group treated with MESG at dose of 500mg/kg & 750mg/kg were 122.74 ± 7.74* and 113.97 ± 5.25* respectively. And lowering of cholesterol was dose dependent manner in MESG. In Standard (Atorvastatin) group, the total cholesterol was reduced to 91.29 ± 5.63*.

Effect of *Sesbania grandiflora* Extracts on Serum Triglyceride levels.

In the Normal rats the Triglycerides levels were to found be 82.66 ± 2.46 on 0th day respectively. Induction of hyperlipidemia resulted in significantly raised in Triglyceride levels in Group-II, Group- III Group- IV Group- V Group- VI (i.e Hyperlipidemic Control, MESG 750 mg/kg, MESG 400 mg/kg, MESG 750 mg/kg, & Standard Atorvostatin 10mg/kg). and the levels were found to be 168.9 ± 5.28, 136.43 ± 7.74, 138.46 ± 1.61, 144.11 ± 7.12, and 148.78 ± 10.23, respectively.

The triglyceride values of hyperlipidemic rats treated with MESG at dose of 250mg/kg were found to be 118.57 ± 5.25* and MESG at dose of 500mg/kg and 750mg/kg were 108.93 ± 6.67* and 104.55 ± 4.2*. Administration of various doses of the MESG was able to produce a dose dependent decrease in the triglyceride levels and lowering of triglycerides was dose dependent manner in MESG. In Standard (Atorvastatin) group the triglycerides was reduced to 101.26 ± 7.68*.

Effect of *Sesbania grandiflora* Extracts on Serum LDL-C levels.

In the Normal rats the LDL-C levels were to found be 8.45 ± 3.43 on 0th day respectively. Treatment with Triton-X-100 caused a significant rise in the levels of LDL-C in Group-II, Group- III, Group- IV, Group-V, Group- VI (i.e Hyperlipidemic Control, MESG 750 mg/kg, MESG 400 mg/kg, MESG 750 mg/kg, & Standard Atorvostatin 10mg/kg) and the levels were found to be 136.82 ± 7.00, 122.7 ± 10.93, 132.3 ± 5.05, 139.8 ± 3.44, 130.52 ± 7.98.

Administration of various doses of the MESG & MESG after the induction of Titon-X-100 resulted in the decreasing of LDL-C levels. The LDL-C levels of groups treated with MESG at dose of 250mg/kg were 84.58 ± 5.26*, and Groups treated with MESG at dose of 500mg/kg & 750mg/kg were 68.11 ± 10.51*** and 58.1 ± 6.89* respectively. And lowering of LDL-C was dose dependent manner in MESG. In Standard (Atorvastatin) group the LDL-C was reduced to 31.91 ± 7.61*. The reduction in LDL-C level by MESG and MESG was significant at ($p<0.01$).

Effect of *Sesbania grandiflora* Extracts on Serum VLDL-C levels.

The VLDL-C levels in Normal rats at 0th were found to be 16.5 ± 0.5. Administration of Triton-X-100 resulted in a rise in VLDL-C levels. Treatment with Triton-X-100 caused a significant rise in the levels of VLDL-C in Group-II, Group- III, Group- IV, Group-V, Group- VI (i.e Hyperlipidemic Control, MESG 250 mg/kg, MESG 500 mg/kg, MESG 750 mg/kg, & Standard Atorvostatin 10mg/kg) and the levels were found to be 33.79 ± 1.05, 27.28 ± 1.54, 27.69 ± 0.32, 28.97 ± 1.4229.75 ± 2.05.

Administration of various doses of the MESG after the Induction with Titon-X-100 resulted in the decreasing of VLDL-C levels. The VLDL-C levels of groups treated with MESG at dose of 250mg/kg were 24.51 ± 1.05***and group treated with MESG at dose of 500mg/kg & 750mg/kg were 22.58 ± 1.33*** and 21.71 ± 0.84*** respectively. And lowering of VLDL-C was dose dependent manner in PETP. In Standard (Atorvastatin) group the VLDL-C was reduced to 21.44 ± 1.53**. The reduction in cholesterol level by MESG was significant at ($p<0.05$).

Effect of *Sesbania grandiflora* on Serum HDL-C levels.

The HDL-C levels in normal rats at 0th were found to be 38.91 ± 2.33. Treatment with Triton-X-100 caused a significant fall in the levels of HDL-C in Group-II, Group- III, Group- IV, Group- V, and Group- VI (i.e Hyperlipidemic Control, MESG 750 mg/kg, MESG 400 mg/kg, MESG 750 mg/kg, & Standard Atorvostatin 10mg/kg). And the levels were found to be 21.86±2.74, 25.3 ± 4.94, 20.98 ± 0.48, 19.01 ±4.29 and 20.53±0.93.

Where as groups treated with MESG at dose of 250mg/kg were 28.1 ± 2.99*** and groups treated

with MESG at dose of 500mg/kg and 750mg/kg showed a dose dependent increase in the HDL-C levels 32.04 ± 4.32**and 34.15 ± 2.51** respectively. In Atorvastatin group the HDL-C was elevated to 38.18 ± 3.14**.

CONCLUSION:

The results concluded that MESG (500 mg/kg, 750 mg/kg) have definite antihyperlipidemic activity in Triton X-100 induced hyperlipidemic model and which is equipotent activity when compared with Atorvastatin treated groups. And also an observation on body weight, blood pressure and heart rate has been made and it's noted that the increased hyperlipidemia in experimental animal can cause cardiac problems.

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Conflicts of interest:

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