Effect and Evaluation of Anti Hyperlipidemic Activity of Methanolic Extract of Sesbania Grandiflora in Triton-X 100 Induced Hyperlipidemic Rats

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Abstract

Atherosclerotic heart disease, heart attacks, and stroke are major complications of hyperlipidemia, but atherosclerosis is the main killer. Medicinal plants are the primary source of sickness treatment in underdeveloped nations. Sesbania grandiflora is produced in its natural environment, making it more readily available, cost-effective, and side-effect-free locally. In order to evaluate the anti-hyperlipidemic activity of methanolic extracts of Sesbania grandiflora in triton X-100 induced hyperlipidemic rats of the respective extracts, it was taken into account that Sesbania grandiflora is beneficial in the metabolism of cholesterol. The results showed that this plant exhibits hyperlipidemic activity and also exhibits minimal side effects towards the liver and cardiac muscle tissues.

Keywords: *Sesbania grandiflora, hyperlipidemic activity.*

INTRODUCTION

Hyperlipidemias are classified according to the Fredrickson classification(9) which is based on the pattern of lipoproteins on electrophoresis or ultracentrifugation. It was later adopted by the World Health Organization (WHO). It does not directly account for HDL, and it does not distinguish among the different genes that may be partially responsible for some of these conditions. It remains a popular system of classification.

Table 1: Fredrickson classification of Hyperlipidemias

Hyperlipo-proteinemi	Synonyms	Defect	Increased lipoprotein	Treatment	Serum appear
Type I (rare)	"Buerger-Gruetz syndrome", "Primary hyperlipoproteinaemia	Decreased lipoprotein lipase (LPL)	Chylomicrons	Diet control	Creamy top layer
Type IIa	"Polygenic hypercholesterolaemia	LDL receptor deficiency	LDL	Bile acid sequestrants, statins, niacin	Clear
Type IIb	"Combined hyperlipidemia"	Decreased LDL receptor increased ApoB	LDL and VLDL	Statins, niacin, fibrate	Clear
Type III (rare)	"Familial dysbetalipoproteinemia"	Defect in Apo E 2 synthesis	IDL	Fibrates, statins	Turbid
Type IV	"Familial hyperlipemia"	Increased VLDL Decreased elimination	VLDL	Fibrate, niacin], statins	Turbid
Type V (rare)	"Endogenous hypertriglyceridemia"	Increased VLDL production and	VLDL and Chylomicrons	Niacin, fibrate	Creamy top layer & turbid bottom

Causes

Although hyperlipidemia is a frequent finding in all demographic groups that follow Western diets, it occurs somewhat more commonly in men. Additional risk factors include:

Treatment

The mainstay of treatment for hyperlipidemia is dietary and lifestyle modification, followed by drug therapy, as necessary. Hyperlipidemia should not be considered refractory to dietary treatment if the therapeutic regimen included animal products or more than minimal amounts of vegetable oils. Such diets do not lower LDL cholesterol concentrations as effectively as high-fiber, low-fat diets that exclude animal products.Regular exercise can improve lipid concentrations. Low to moderate amounts of physical activity such as walking lower triglyceride concentrations by an average of 10 mg/dL, while raising HDL by 5 mg/dL (these

numbers are means drawn from large groups). More strenuous activity may have greater effects.

MATERIALS

Atorvastatin procured from Dr.Reddys Lab,Hyderabad and provided by Suralabs, Dilsuknagar. Normal saline, Chloroform, Diethyl ether, Triton X-100 from Molychem, Mumbai, provided by Suralabs, Dilsuknagar.

Collection and Authentification of Plant Material

The whole plant of Sesbania Grandiflora will be collected and will be authenticated by Dr K. Rao, Department of Botany, SJCPS, Bhubaneswar.

METHODOLOGY

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” madeof 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.

Fig 1: Soxhlet extraction setup

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 grandifolia extract was carried out for the analysis of Alkaloids, Carbohydrates, Tannins, Saponins, Steroids, Phenols, 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.

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 doseof 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 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 0th and 8th day from retro-orbital plexus of rat using micro capillary technique from animals 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.).

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)

Clinical Significance

Determination of serum Triglycerides concentration is used to assess the possible presence of Increased blood and Serum levels of triglycerides.

Principle

Triglycerides are hydrolysed by lipase to glycerol and free fatty Acids. Glycerol is phosphorylated by ATP in the presence of glycerolkinase (GK) to glycerol– 3 –phosphate

(G-3-P). which is oxidized by the enzyme Glycerol–3–phosphoxidase (G-P-O) producing hydrogen peroxide. Hydrogen peroxide so formed reacts with 4-Amino-Hydrogen peroxidave (POP), to produce a brown colour complex. The intensity of the colour developed is proportional to the triglyceride concentrate.

Procedure

Wave length : 546 (Green Filter) Temperature : 37º C

Reaction type : End point with standard. Pipette in to clean dry tube labelled Blank (B), Standard (S) and Test (T) and then add f:ollowing :

Table 2: Reagent for Estimation of Triglycerides

	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 370 C. Read absorbance of standard and test against blank.

Calculations

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

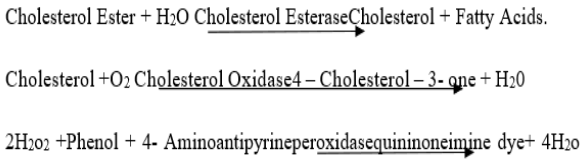
Estimation of cholesterol (Total cholesterol). CHOD/POD Method.

Clinical Significance

Heart disease is often the result of cholesterol deposits on the arteries. While not the only factor for heart disease, serum cholesterol levels are often checked to determine the risk of heart disease on patient.

Principle

Enzymatic determinations of total cholesterol according to the following reactions:-



Procedure

Wave Length: 500nm (green filter)

Temperature: 370C.

Reaction type: End point with standard.

Table 3: Reagent for Estimation of Cholesterol

	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 (370c). The final color is stable for at least 1 hour.

Calculations

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

Estimation of HDL cholesterol.

Procedure: It includes two steps.

Step:1 – Precipitation

Table 4: Reagent for HDL Precipitating agent

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. Incubation for 5 min at 370c and read the optical density at 500nm against blank.

Table 5: Reagent for Estimation HDL Cholesterol

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

Calculations

HDL CALCULATION

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 CALCULATION

VLDL = Triglycerides (mg/dl) / 5

According to these guidelines, the normal range of lipid profile

Table 6: 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, Phenol in Methanolic Extract of Sesbania grandiflora.

Table 7: Results of 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 LD50 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.

Table 8: Results of Acute toxicity study

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***
MESG500mg/kg.	122.74 ± 7.74*	108.93 ± 6.67*	32.04 ± 4.32**	68.11 ± 10.51***	22.58 ± 1.33***

MESG750mg/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.IITC: Total Cholesterol ; TG: Triglycerides ; HDL-C : High Density Lipoprotein cholesterol; LDL-C : Low DensityLipoprotein- cholesterol ; VLDL-C : Very Low Density Lipoprotein ;MESG: Methanolic Extract of Sesbania grandiflora .;MESG:Methanolic Extract of Sesbania grandiflora Effect of Sesbania grandiflora Extractson 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, MEAG750 mg/kg, MESG 400 mg/kg, MESG750 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&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 134.19 ± 3.5, and group treated with MESG at dose of 400mg/kg &750mg/kg were 121.74 ± 7.74 and 112.97 ± 5.25 respectively. And lowering of cholesterol was dose dependent manner in MEAG. In Standard (Atorvastatin) group, the total cholesterol was reduced to 92.29 ± 5.63.

Effect of Sesbania grandiflora Extractson Serum Triglyceride levels

In the Normal rats the Triglycerides levels were to found be 82.66 ± 2.46on 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, MESG750 mg/kg, MESG 400 mg/kg, MESG750 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 750mg/kgwere found to be 117.57 ± 5.25and MESG at dose of 400mg/kg and 750mg/kgwere 107.93 ± 6.67 and 103.55 ± 4.2. Administration of various doses of the MESG was able to produce a dose dependant decrease in the triglyceride levels and lowering of triglycerides was dose dependent manner in MESG. In Standard (Atorvastatin) group the triglycerides was reduced to 102.26 ± 7.68.

In the Normal rats the LDL-Clevels were to found be 8.45 ± 3.43on 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,

MESG750 mg/kg, MESG 400 mg/kg, MEAG750 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&MESGafter the induction ofTiton-X-100 resulted in the decreasing of LDL-C levels. The LDL-C levels of groups treated with MESG at dose of 750mg/kg were 83.58 ± 5.26 , and Groups treated with MESG at dose of 400mg/kg &750mg/kg were 69.11 ± 10.51 and 59.1 ± 6.89 respectively. and lowering of LDL-C was dose dependent manner in MEAG. In Standard (Atorvastatin) group the LDL-C was reduced to 32.91 ± 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, MESG750 mg/kg, MESG 400 mg/kg, MESG750 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&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 750mg/kg were 23.51 ± 1.05, and group treated with MESG at dose of 400mg/kg &750mg/kg were 21.58 ± 1.33 and 20.71 ± 0.84 respectively. and lowering of vldl-c was dose dependent manner in PETP. In Standard (Atorvastatin) group the VLDL-C was reduced to 20.44 ± 1.53. The reduction in cholesterol level by MESG and MESG was significant at ($p<0.05$).

Effect of Sesbania grandifloraon 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, MESG750 mg/kg, MESG 400 mg/kg, MESG750 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 750mg/kg were 27.1 ± 2.99 and groups treated with MESG at dose of 400mg/kg and 750mg/kg showed a dose dependant increase in the HDL-C levels 31.04 ± 4.32 and 33.15 ± 2.51 respectively. In Atorvastatin group the HDL-C was elevated to 39.18 ± 3.14.

Table 9: Estimation of blood pressure and heart rate

Sl.NO	GROUPS	BODY WEIGHT(gm)	BLOOD PRESSURE(mmHg)		HEART RATE/MIN
			MEAN-BP	SYSTOLIC-BP	
I	Normal Control	168.24±2.15	121±0.68	122±0.38	336±0.84
II	Hyperlipidemic Control	179.32±2.31	148±0.35	152±0.74	276±0.68
III	MESG 750mg/kg	175.64±2.15	143±0.68	142±0.65	283±0.41
IV	MESG 400mg/kg.	174.48±3.10	141±0.68	138±0.29	322±0.64
V	MEAG750mg/kg.	172.16±1.19	131±0.87	131±0.15	326±0.68

Discussion

The present study was designed to investigate the antihyperlipidemic activity of Sesbania grandiflora extract in Triton X-100 induced hyperlipidemic rats.

Phytochemical Investigation revealed the presence of Alkaloid, Tannin, Saponin, Phenol in Methanolic Extract of Sesbania grandiflora %Yield value of Methanolic Extractfrom Aerial Parts of Sesbania grandiflora was found to be 34.75% Administration of Triton-X-100 (100mg/kg) to all the fasted rats caused an elevation of TC,TG, VLDL and LDL and reduction in HDL levels.After 72 hrs of induction of Triton X-100 results in hyperlipidemia which is compared with normal control group.which results in significantly increased serum lipid levels in hyperlipidemic group.

The change in lipid levels in group number III to VI, were comparable with group of Hyperlipidemic control (i.e Triton X-100, Group- II). The Standard group (i.e Atorvostatin group) significantly lowers the serum lipid level ($P<0.001$).

The results of the study clearly indicate that MESG Extract at a dose of 750 mg/kg & 400 mg/kg significantly lowered serum lipid levels ($P<0.01$). MESG Extract at a dose of 750mg/kg significantly lowered serum lipidlevels, ($P<0.001$) i.e. antihyperlipidemic activity which was found to be more effective in higher dose of MESG as compared to lower dose of MESG when administered orally in triton induced hyperlipidemic models.

MESG Extract having very low hyperlipidemic activity. MESG Extracts showed a dose dependent decrease in the levels of cholesterol, Triglyceride, LDL-C and VLDL-C level. Among three groups (i.e. group number III-V), Group number- V reduced the elevated lipid

levels more significantly than the other Groups.($P<0.001$)

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 its noted that the increased hyperlipidemia in experimental animal can cause cardiac problems.

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