Pharma Science Monitor 6(2), Apr-Jun 2015

PHARMA SCIENCE MONITOR
AN INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES
Journal home page: http://www.pharmasm.com

# STUDY OF THE ANTIHYPERLIPIDEMIC AND ANTIOXIDATIVE ACTIVITY OF *SOLANUM TORVUM* SW. IN RABBITS RECEIVING HIGH FAT DIET

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## ABSTRACT

The aim of the study was to investigate the possible antihyperlipidemic and antioxidative effects of the ethanolic extract of *Solanum torvum* SW. leaves in diet induced hyperlipidemia in rabbits. Twenty rabbits of either sex were taken and divided into four groups of five each as-Normal control - received normal diet, Experimental control - received high fat diet, Standard drug group - received high fat diet plus Atorvastatin 2.1mg/kg/day orally and Test drug group – received high fat diet plus ethanolic extract of *Solanum torvum* leaves for 12 weeks after which blood samples were collected and lipid profile, catalase and malondialdehyde estimations were done. Data were analyzed by using one way ANOVA followed by Bonferroni's multiple comparison test. Values with $p<0.05$ were considered significant. *Solanum torvum* significantly prevented the increase in the levels of total cholesterol, triglycerides and LDL while significantly increasing the levels of HDL. It also significantly increased catalase and decreased malondialdehyde levels. A reduction in the weight of the animals was also seen. *Solanum torvum* has significant antihyperlipidemic and antioxidative properties making it a potential for a new hypolipidemic drug development.

**KEYWORDS:** antihyperlipidemic, antioxidative, *Solanum torvum*, rabbits.

## INTRODUCTION

Cardiovascular diseases have been found to be the most common cause of death worldwide, the most established and best understood risk factors for which are abnormalities in plasma lipoprotein and derangement in lipid metabolism.[1] Hyperlipidemia is a secondary metabolic dysregulation associated with diabetes characterized by, elevated serum level of triglycerides (TG), cholesterol and low density lipoproteins (LDL) which are the major risk factors for the premature development of cardiovascular diseases like atherosclerosis, hypertension, coronary heart disease etc.[2] It can also cause intermittent claudication and gangrene and can jeopardize limb viability.[3]

Pharmacological agents with the ability to decrease LDL, very low density lipoproteins (VLDL) or total cholesterol levels, increase HDL cholesterol or lower TG have beneficial effects in preventing cardiovascular diseases.[4]

Oxidative stress and inflammation contribute to the pathogenesis of cardiovascular diseases, including atherosclerosis, cardiac hypertrophy, heart failure and hypertension. Thus, agents with antioxidant activity may prove to be beneficial in treating cardiovascular diseases.[4]

*Solanum torvum* is a common plant found throughout the Indian subcontinent and West Indies, Bermuda, Indonesia, Malaya, China, Philippines and tropical America.[5] It is an erect spiny shrub that is usually 2 or 3m in height and 2cm in basal diameter. It grows on all types of moist, fertile soil at elevations from near sea level to almost 1,000m. It grows best in full sunlight and does well in light shade or shade for part of the day, but cannot survive under a closed forest canopy.[6]

Many valuable phytoconstituents of therapeutic importance such as steroidal alkaloids, chlorogenone, neochlorogenone, isoflavanoid sulfate, steroidal glycosides, 2, 2 o-spirostannol (Torvonin-A), Solasonine, sterolin (Sitesterol-D-glucoside), protein, fat and minerals have been earlier reported to be isolated.[5]

It is intensively used worldwide in the traditional medicine as poison antidote and for the treatment of fever, wounds, tooth decay, reproductive problems and arterial hypertension. Its antidiabetic, antihypertensive, nephroprotective, antipyretic, antiinflammatory effects have been determined scientifically.[6]

Many hypolipidemic agents are currently in use but due to their side effects and/or lack of cost-effectiveness, there is now growing interest in herbal medicines all over the world which can prove to be a useful source for the development of new oral hypolipidemic agents or simple dietary adjuvant to existing therapies. The hypolipidemic activity of a number of plants/plant products has been evaluated and confirmed in animal models, as well as in human beings.[7]

Literature reviews indicated that no study has been done to evaluate the lipid lowering property of *Solanum torvum*. Considering this, the present study has been undertaken.

## MATERIALS AND METHODS

Plant material:

Leaves of *Solanum torvum* were collected from areas in and around Dibrugarh, Assam and hilly areas of Naharlagun district of the state of Arunachal Pradesh. They were identified by Prof. L. C. Saikia of Department of Botany, Dibrugarh University. A herbarium specimen bearing

voucher number DULSc.2540 was also preserved in the same college. The collected leaves were air dried and powdered.

Plant extract:

The powder was soaked in 90% ethanol in a percolator and sufficient quantity of 90% ethanol was added to saturate the powder and leave a stratum above it. This was allowed to macerate for 72 hours, after which it was allowed to percolate slowly and then the extract was collected in clean petridishes. Alcohol was evaporated to leave a soft extract at room temperature and it was then transferred to a vacuum dessicator and finally collected and stored in airtight containers at 2-8˚C.[8]A final yield of 13.53% w/w was obtained with respect to the original air dried powder.

Phytochemical screening of the extract[9]:

The extract was subjected to qualitative phytochemical analysis for alkaloids, flavonoids, tannins, saponins, diterpenes, triterpenes and phenols as per the standard methods and all of these were found to be positive.

Drugs and Chemicals:

Atorvastatin was obtained from Lupin Ltd., Kartholi, Jammu. The kits for estimation of HDL, Total Cholesterol and Triglyceride were obtained from Crest Biosystems, Goa, India. Potassium Phosphate Buffer, Hydrogen Peroxide Solution and Tricarboxylic acid were obtained from Sigma Private Limited, Bangalore, India. Thiobarbituric acid was obtained from HiMedia Laboratories Private Limited, Mumbai, India.

High fat diet:

This was prepared by mixing coconut oil with vanaspati ghee in the ratio of 2:3 (v/v). It was given to the rabbits at a dose of 10ml/kg body weight mixed with food.[10]

Animal:

Healthy New Zealand white rabbit (*Oryctolagus cuniculus*) of either sex weighing 1.5-2.5kg were taken and approval was taken from Institutional Animal Ethical Committee(IAEC) of Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh, Assam (Reg. No.: 1576/GO/a/11/CPCSEA dated 17/02/2012) vide approval number IAEC/DU/77. The animals were housed under standard conditions as approved by the CPCSEA. They were fed with normal diet, high fat diet (according to their group) and water ad libitum.

Acute oral toxicity studies:

Acute oral toxicity test for the ethanolic extract of *Solanum torvum* SW. was carried out as per Organization for Economic Cooperation and Development OECD Guidelines 425.[11] An

arbitrary dose of 200mg/kg was selected for the study as the extract was found safe even at doses more than 2000mg/kg without any sign of toxicity or mortality.

Experimental design:

Twenty rabbits of either sex were taken and divided into four groups of five rabbits each:

Normal control: received normal diet and water ad libitum.

Hyperlipidemic control: received normal diet plus high fat diet and water ad libitum

Standard drug: received normal diet plus high fat diet plus Atorvastatin in the dose of 2.1mg/kg body weight plus water ad libitum. [12]

Test drug: received normal diet plus high fat diet plus ethanolic extract of *Solanum torvum* SW. at a dose of 200mg/kg body weight plus water ad libitum. [11]

All the animals used for the experiment were kept under observation for daily food intake. The drugs were administered to the animals in the doses given above orally, once daily, for 12 weeks by means of intragastric feeding tube. Weights of all the animals were taken before and after the experiment (as shown in Fig.3).

At the end of the 12 weeks, all the animals were kept fasting for 18 hours.

5ml of blood was collected from each animal via marginal ear vein. [13]

Biochemical estimations:

After collecting blood from the animals they were stored in separate vials for biochemical estimation.

Lipid Profile Estimation:

Total cholesterol was estimated by CHOD/PAP method[14], Triglycerides were measured by GPO/PAP method[15], HDL cholesterol by PEG precipitation method[16] by the colorimetric method and LDL cholesterol was calculated using Freidewald's formula.[17]

Catalase estimation:

Catalase was measured in blood using Continuous Spectrophotometric Rate determination using Beers and Sizers method[18]. Phosphate buffer (2.5ml pH 7.8) was added to the supernatant (obtained after centrifugation of the plasma) and incubated at 25˚C for 30 minutes. After transferring into a cuvette, the absorbance was measured at 240nm spectrophotometrically. Hydrogen peroxide (650µl) was added and change in absorbance was measured for 3 minutes. Values were expressed as µmol/min/mg of protein.

Malondialdehyde(MDA) estimation:

MDA was estimated by Satoh K. method[19]. 75mg of thiobarbituric acid(TBA) was dissolved in 15% Trichloroacetic acid (TCA) to which 2.08ml of 0.2N HCl was added and the volume was

made upto 100ml using 15% TCA. 3.0ml of this reagent was added to 0.75ml of serum. The test tubes were kept in boiling water bath for 15 minutes and were then cooled and centrifuged for 10min at 10000rpm. Absorbance of the supernatant was read against the blank at 535nm and the results were expressed as nmol/ml of serum.

Statistical Analysis:

The results of the study were expressed as mean± S.E.M. Data was analyzed by using one way analysis of variance test (ANOVA) followed by Bonferroni's multiple comparison test. Values with $p < 0.05$ were considered as significant.

**RESULTS**

Acute toxicity test:

There was no mortality among animals even at the dose of 2000mg/kg so $LD_{50}$ was considered to be more than 2000mg/kg body weight.

Lipid profile estimation (Table 1):

Table 1

EFFECT OF EELST ON SERUM LIPIDS

AT THE END OF THE 12TH WEEK OF EXPERIMENT

| **GROUPS** | **Serum Total Cholesterol (mg/100ml)** | **Serum Triglycerides (mg/100ml)** | **Serum High Density Lipoproteins (mg/100ml)** | **Serum Low Density Lipoproteins (mg/100ml)** |
|---|---|---|---|---|
| **Normal Control** | 56.29±1.813 | 75.80±1.800 | 24.93±0.034 | 16.20±1.972 |
| **Hyperlipidemic Control** | 106.70±5.025[a] | 206.4±2.993[a] | 13.33±2.040[a] | 52.05±7.136[a] |
| **Standard Drug** | 74.07±3.314[b] | 115.2±1.960[b] | 28.33±3.333[b] | 22.70±3.698[b] |
| **Test Drug** | 87.40±2.770[b] | 107.2±1.960[b] | 29.99±3.334[b] | 35.97±4.830[b] |
| F | 38.37 | 634.1 | 8.556 | 10.95 |
| ANOVA df | 3, 16 | 3, 16 | 3, 16 | 3, 16 |
| P | <0.05 | <0.05 | <0.05 | <0.05 |

*Values are expressed as MEAN ± SEM (n=5).*

*One Way ANOVA followed by Bonferroni's Multiple Comparison test is done.*

*[a]p <0.05, when compared to the Normal control Group.*

*[b]p <0.05, when compared to the Hyperlipidemic Control Group.*

There was a significant decrease ($p<0.05$) in total cholesterol, triglycerides and LDL levels in the standard drug group and test drug group as compared to the hyperlipidemic control group which showed a significant ($p<0.05$) increase in these levels as compared to the normal control group and a significant increase ($p<0.05$) in the levels of HDL in the standard drug and test drug groups as compared to the hyperlipidemic control group which showed a significant ($p<0.05$) decrease in HDL in comparison with the normal control group.

Similar rise in lipid parameters was noted by Murty D *et al*[20] who induced hyperlipidemia in rabbits by using 1% w/w cholesterol and 10% v/w groundnut oil. However, the fall in HDL levels noted by them in their study, in the hyperlipidemic control group was not significant as compared with the normal group.

D. Mchedlishvili *et al* in their study showed that flavonoids isolated from *Satureja hortensis* L. resulted in a significant attenuation of rise in serum cholesterol value after 8 weeks, when compared to the cholesterol alone group. [21]

In 2011, Garcia MD *et al*, in their study, showed that diet supplementation with “triguero” asparagus was able to prevent atherogenic risk markers as well as the oxidative hepatic damage in hypercholesterolemic conditions and for this they hypothesized that the functional components present in this asparagus variety-flavonoids and steroidal saponins, could be responsible, at least in part, for this protective effect. [22] Vazquez-Castilla S *et al* in their study showed that rats fed a diet rich in cholesterol exhibited increased plasma triglycerides, Total Cholesterol(TC) and LDL and decreased circulation of HDL and an overweight state. However, “triguero” asparagus and its major bioactive fractions, namely flavonoids and fibers, showed strong hypotriglyceridemic and hypocholesterolemic effects, reducing plasma triglycerides, TC and LDL contents in these rats; the mechanism of which could be down-regulation of serum cholesterol content by inhibition of cholesterol synthesis and increased expression of LDL receptors. [23]

Work done by Nichols LA *et al* showed that citrus flavonoids regulated the transcription of the low-density lipoprotein receptor (LDLR) gene in HepG2 cells leading to their hypocholesterolemic effects. [24]

Also, studies done by Lee *et al*[25] and Afrose *et al*[26] suggest the important role of saponins in maintaining favorable lipid levels by inhibiting intestinal reabsorption which may be one of the underlying mechanisms by which *S. torvum* exerts its antihyperlipidemic action.

Catalase (Fig. 1) and MDA estimation (Fig. 2):

FIG. 1

EFFECT OF EELST ON CATALASE

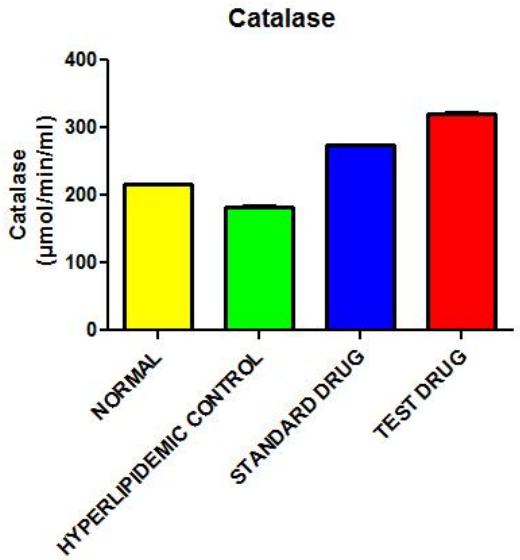
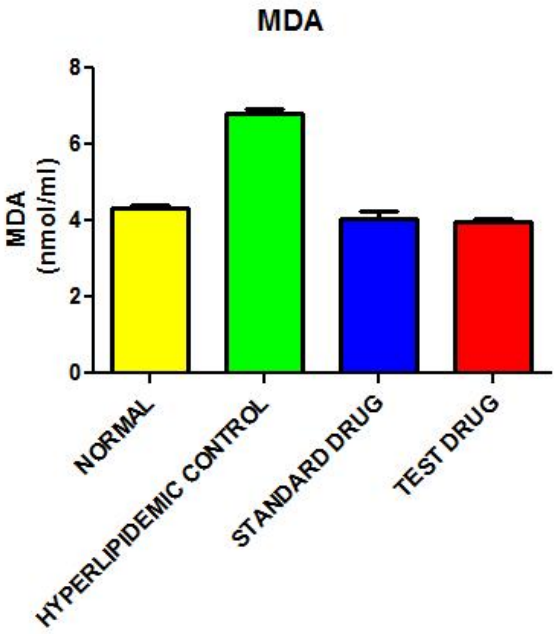



FIG. 2

EFFECT OF EELST ON MDA




There was a significant decrease ($p<0.05$) in MDA levels in the standard drug group and test drug group as compared to the hyperlipidemic control group and a significant increase ($p<0.05$)

in Catalase in the standard drug and test drug groups as compared to the hyperlipidemic control group which showed a significant ($p<0.05$) increase in MDA levels and a significant ($p<0.05$) decrease in Catalase in comparison to the normal control group.

Malondialdehyde a secondary product of lipid peroxidation is a major reactive aldehyde; higher levels of which can lead to peroxidation of biological membranes. The antioxidant enzymes, mainly superoxide dismutase and catalase are the first line defensive enzymes against free radicals. It is well known that flavonoids and polyphenols are natural antioxidants which also significantly increase SOD and catalase activities.[27]

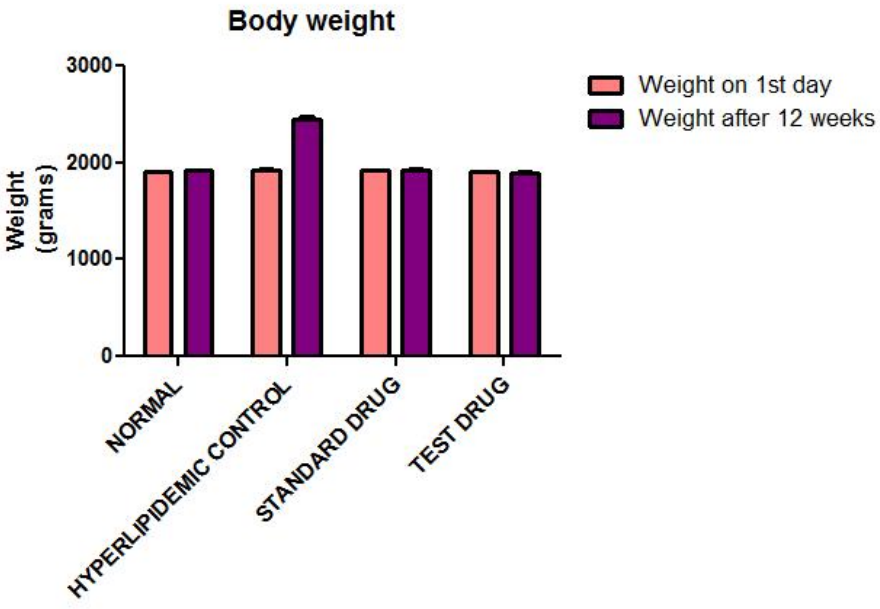
Vázquez-Castilla S *et al* in their study, showed that flavonoids could be the main compounds involved in preventing liver peroxidation and decreasing MDA levels.[23]

The above mechanisms may be responsible for the antihyperlipidemic and antioxidative effects shown by *Solanum torvum* SW. due to the presence of polyphenols and flavonoids in it.

Body Weight estimation (Fig. 3):

FIG. 3

EFFECT OF EELST ON WEIGHT




The weights of the animals in the normal control group, hyperlipidemic control group and standard drug group increased at the end of the experiment ie, after 12 weeks, while that of the test drug group decreased. The increase in weight in the standard drug group and the decrease in

weight in the test drug group was significant ($p<0.05$) as compared to the hyperlipidemic group, the increase in weight of which was significant ($p<0.05$) as compared to the normal control group.

Saponins are known to inhibit growth rate and tannins were reported to be involved in growth regulation. Tannins could potentially inhibit the activity of lipases thereby lowering the body fat content.[28] The weight lowering potential of *S. torvum* could at least partially be attributed to the presence of tannins and saponins found in the plant.

## CONCLUSION

To conclude, ethanolic extract of leaves of *Solanum torvum* SW. has significant antihyperlipidemic and antioxidant activity. However, isolation of the bioactive fractions and their study for the probable mechanism of action needs to be undertaken before it can be fully exploited as a potential for the development of a new antihyperlipidemic agent.

## ACKNOWLEDGEMENT

I am highly grateful to The Department of Biotechnology, Government of India, for funding this study. I am also grateful to Dr. (Mrs.) Swarnamoni Das, Head of the Department, Department of Pharmacology, Assam Medical College, Dibrugarh; for providing me with her valuable guidance and suggestions.

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