IMPROVEMENT OF LIPID AND ANTIOXIDANT STATUS IN HYPERLIPIDAEMIC RATS TREATED WITH STEROIDAL SAPONINS OF *Solanum nigrum* AND *Solanum xanthocarpum*

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Summary

The present study was designed to investigate the effects of the steroidal saponins, isolated from *S. nigrum* and *S. xanthocarpum*, on the lipid and antioxidant status of serum in rats fed with high cholesterol diet (HCD). Feeding of animals with HCD for 28 days resulted in elevation of serum total cholesterol (TC), low-density lipoprotein (LDL) and triglyceride (TG) levels with a concomitant decrease in high-density lipoprotein (HDL). Treatment of the hyperlipidemic rats with steroidal saponins, isolated from *S. nigrum* and *S. xanthocarpum* (100 and 200 mg/kg body weight, p.o.), for 28 days, reversed the serum lipid levels of the treated rats by varying extents. To evaluate the mechanism of action, the serum antioxidant profiles in terms of superoxide dismutase (SOD), catalase (CAT) concentrations and malonedialdehyde (MDA) generation, 2,2'-Azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid) (ABTS) radical cation decolorisation assay and Ferric Ion Reducing Antioxidant Power (FRAP) assay were used. Additionally, the determination of serum GOT, GPT, alkaline phosphatase levels and total protein content showed that no hepatotoxicity to the treated animals occurred. Also, antioxidant activities of the steroidal saponins isolated from *S. nigrum* and *S. xanthocarpum*, were evaluated by four recognized *in vitro* antioxidant assay systems, namely, ABTS assay, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical decolorisation assay, FRAP assay and superoxide radical scavenging assay by NBT (nitro blue tetrazolium) –NADH (nicotinamide adenine dinucleotide reduced)-PMS (phenazonium methosulphate) combination. In the study, atorvastatin has been taken as the positive control group. The present study demonstrates that, the steroidal saponins isolated from *S. nigrum* and *S. xanthocarpum*, besides having significant antihyperlipidaemic effects, can improve the biological antioxidant status of treated animals by reducing lipid peroxidation and modulating the activities of antioxidant enzymes in HCD- fed rats without causing any hepatotoxic effect.

Keywords: steroidal saponins, *Solanum nigrum*, *Solanum xanthocarpum*, antihyperlipidaemic, antioxidant.

Introduction

Mortality from cardiovascular disease is the second leading cause of death worldwide [1]. Cardiovascular disease patients have manifested a significant increase in lipid peroxidation and oxidative DNA damage, which is correlated to the severity of the hypercholesterolemia [2,3]. Therefore, in respect of hypercholesterolemia or hyperlipidaemia, recent interest has been focused on strategies that enhance the removal of reactive oxygen species (ROS), either by using antioxidants or drugs that enhance endogeneous antioxidant systems [4]. Currently available hypolipidaemic drugs are associated with number of side effects [5]. Thus, although a number of medicinal plants have been evaluated for their hypolipidaemic activity, only few have been commercially utilized [6].

Steroidal Saponins, found in many species of genus *Solanum* are an important group of natural products having a number of potent beneficent properties [7]. Enzymatic hydrolysis of steroidal saponins e.g. dioscin and tigonin gives sapogenins e.g. diosgenin and tigogenin respectively, which are the aglycones of saponins, and are characterized by the presence of a spiroketal side chain [8].

Steroidal saponins and sapogenins were found to reduce blood cholesterol in several animal species by inhibiting intestinal absorption of cholesterol [9]. Although compensatory syntheses were also reported, which was accompanied by increased secretion of cholesterol in bile, the net effect being lowering of cholesterol and low density lipoproteins (LDL) in serum and elicitation of concomitant antihypercholesterolemic effect [10]. Also estrogenic [11] and antitumor effects of steroidal saponins [12, 13] were documented. *S. nigrum* and *S. xanthocarpum* (whole plant) are used in the Indian traditional systems of medicine for a variety of therapeutic purposes. However, the antihyperlipidaemic and antioxidant activities of the steroidal saponins of *S. nigrum* and *S. xanthocarpum* have not been reported before. Also, the mechanism of their actions has been delineated in the present study.

Materials and Methods

Chemistry-

Test samples- Authenticated plant materials of *S. nigrum* and *S. xanthocarpum*, cultivated in the Western Himalayas, were obtained from Indian Herbs Ltd, Saharanpur (U.P.). Each specimen, of the two samples, has been preserved in our file as reference.

Chemicals and Reagents- All the chemicals and reagents are of AR grade, 2,2'-Azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ) were obtained from Sigma-Aldrich Chemicals, St. Lewis. Iron (III) chloride, nicotinamide adenine di nucleotide reduced (NADH), nitro blue tetrazolium (NBT), phenazonium methosulphate (PMS), trichloroacetic acid, thiobarbaturic acid, folin-ciocaltaus reagent and pyrogallol were obtained from Merck (India).

Atorvastatin tablets (commercial name Atorva) were obtained from Cadilla Healthcare Ltd. Standard vitamin C was obtained from Merck India.

Extraction of saponins from *S. nigrum* and *S. xanthocarpum*:

Dried and powdered whole plant material of each species was separately extracted with petroleum ether (60º -80º) followed by hot methanol. Solvent was evaporated from the methanol extract under reduced pressure, and the residue was partitioned between *n*-butanol and water.

The *n*-butanol phase was dried and the residue was subjected to column chromatography over silica gel (60-120 mesh) using chloroform-methanol (9:1), followed by chloroform-methanol-water (20:10:1) as eluents. The combined latter fractions were analyzed by HPTLC and HPLC for the steroidal saponins using reference markers.

Apparatus and Techniques:

HPLC of steroidal saponins of *S. nigrum* and *S. xanthocarpum* - WATERS assembly, equipped with a PDA detector (WATERS 2996), pump (WATERS 516), injector (Rheodyne 7725i), Hiber (E. MERCK) [RP C_{18} 250 x 4 mm; 5µm] column were used, acetonitrile-water (1:1) was the mobile phase with a flow rate of 0.4 ml/min.

HPTLC of steroidal saponins of *S. nigrum* and *S. xanthocarpum* - CAMAG winCATS HPTLC assembly with Silica gel $60F_{254}$/Merck plates was used with chloroform:acetic acid:methanol:water (64:32:12:8) as mobile phase followed by densitometric determination at 700 nm after derivatisation with anisaldehyde sulphuric acid spray reagent (10 ml glacial acetic acid is added to 0.4 ml anisaldehyde, followed by addition of 85 ml methanol and 5 ml concentrated H_2SO_4. This solution was prepared 2 hr before use).

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Antioxidant Assays:

2,2-Diphenyl-1-picrylhydrazyl radical (DPPH) scavenging capacity assay [14-16]

DPPH is one of a few stable and commercially available organic nitrogen radicals and has a UV-vis absorption maximum at λ 517 nm. Upon reduction, the solution color fades. The DPPH assay is typically run by the following procedure. DPPH solution (3 mg in 25 ml ethanol) was mixed with sample solution (0.1 mL). The absorbance of the mixture during the reaction progress was monitored at λ 517 nm for 20 min or until the absorbance was stable. Upon reduction, the colour of the solution fades. The concentration that causes a decrease in the initial DPPH concentration by 50% is defined as IC_{50}.

ABTS cation radical decolourisation assay [14,17,18]

ABTS-, the oxidant, was generated by persulfate oxidation of 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^{2-}$). This solution was diluted with phosphate buffer (pH 7.4) until the absorbance reached 0.7 to 0.8 at λ 734 nm. One ml of the resulting solution was mixed with the sample. The absorbance was read at 30 °C, 4 min after mixing at 30 °C. The difference of the absorbance reading was plotted versus the antioxidant concentrations to give a straight line. The concentration that caused a decrease in the initial ABTS concentration by 50% is defined as IC_{50}.

Ferric ion reducing antioxidant power (FRAP) assay [14,15,18]

The oxidant in the FRAP assay was prepared by mixing TPTZ, acetate buffer and $FeCl_3.H_2O$. The conglomerate is referred to as "FRAP reagent". The final solution has Fe(III) of 1.67 mM and TPTZ of 0.83 mM concentration, To measure FRAP value, 300 µL of freshly prepared FRAP reagent was warmed to 37 °C and a reagent blank reading was taken at λ 593 nm, then sample and 30 µL of water are added. Absorbance readings were taken λ 593 nm. The concentration that caused a increase in the initial FRAP concentration by 50% is defined as IC_{50}.

$O_2^{\cdot-}$ scavenging capacity assay [14,15]

This assay was conducted following a published procedure. Briefly, using NADH-phenazine methosulphate-O_2 as the $O_2^{\cdot-}$ generator and nitroblue tatrazolium as the probe. The absorbance was measured at λ 540 nm and IC_{50} value calculated in the usual way.

Pharmacology:

Animals- Albino rats (Sprague Dawley strain) of either sex, 3-4 months old and weighing around 200 to 300 gm, procured from Central Research Institute (Ayurveda), Govt. of India, Salt Lake City, Kolkata, were used. The animals were housed in an animal room with alternating light-dark cycle of 12 hr each. The animals were acclimatized for at least 7 days to the laboratory conditions before conducting experiments. Experiments were carried out between 0900 h and 1700 h. The study was conducted in accordance with Good Laboratory Practice (GLP) Regulations of WHO (WHO Document, 1998). The "Principles of laboratory animal care" (NIH Publication # 85-23, 1985) were also followed in the study. The 'Institutional Animal Ethics Committee' (IAEC) approved the experimental protocol.

The animals were allocated in groups of six per cage and had free access to water and normal or high cholesterol diet (HCD) *ad libitum*. The HCD were made from Bengal gram powder (40%), sugar (15%), milk powder (20%), ground nut oil (10%), goat liver (10%) and butter (5%). After one week of adaptations, the rats were randomly divided into seven groups containing 6 animals in each group (1 to 7).

Group1: received normal diet (NC).

Group 2: received high cholesterol diet (HCD).

Group 3: received HCD + saponins of *S. nigrum* (100 mg/kg body weight p.o.).

Group 4: received HCD + saponins of *S. nigrum* (200 mg/kg body weight p.o.).

Group 5: received HCD + saponins of *S. xanthocarpum* (100 mg/kg body weight p.o.).

Group 6: received HCD + saponins of *S. xanthocarpum* (200 mg/kg body weight p.o.).

Group 7: received HCD + atorvastatin (0.8 mg/kg body weight p.o.).

Blood samples were collected from the animals at 14^{th} day and 28^{th} day one hour after administration of the drugs for the estimation of biochemical changes. Serum samples were taken by centrifuging the blood samples at 3500 rpm for 7 minutes. The results were incorporated in the Tables 3-6.

Calculation of body mass index (BMI) [19]**:**

BMI value was determined by dividing the body weight (in gm) by square of the naso-anal length (in cm^2).

Biochemical analysis:

TG, TC, HDL SGOT, SGPT and alkaline phosphate in serum were determined using enzymatic kits (Span diagnostic Ltd.). LDL was calculated using the following Friedewald's equation:

LDL = TC- TG/5 – HDL.
VLDL was calculated using the following formula:
VLDL= TC – (HDL + LDL).
Atherogenic index (AI) was calculated by logTG/HDL [20].

Measurement of antioxidant parameters- Serum samples were analysed for different antioxidant enzymes by following published procedures. The different antioxidant parameters analysed were: MDA [21], SOD [22], CAT [23], ABTS and FRAP.

Protein estimation: Protein estimations from serum were done by following the method described by Lowry *et al* [24].

Statistical analysis- Statistical analysis was carried out using Prism software ver.4.0 (Graph pad Inc). All the results were expressed as Mean ± standard deviation of the mean (SD). Data were analyzed using one-way ANOVA followed by Tukey's test. In the entire test, the criterion for statistical significance was $p<0.05$.

Results

Identities of Saponins:

The sapogenins, obtained after acidic hydrolysis of the steroidal saponins isolated from the two plant extracts were identified as diosgenin, sarasapogenin and tigogenin (in decreasing order of abundance). Among the monosaccharides, isolated from the aqueous acidic hydrolysates, glucose, rhamnose and galactose were identified. The yield % (based on total extractives weight) of steroidal saponins was higher in *S. xanthocarpum* than *S. nigrum*.

In vitro antioxidant activity of steroidal saponins of *S. nigrum* and *S. xanthocarpum*:

Saponins of *S. nigrum* and *S. xanthocarpum* were tested for antioxidant activity. Four accepted *in vitro* hydrogen atom transfer (HAT) and electron transfer (ET) assay systems were employed. These are, 2,2'-Azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid) (ABTS) radical cation decolorisation assay, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical decolorisation assay, Ferric Ion Reducing Antioxidant Power (FRAP) assay and superoxide radical scavenging assay by NBT (nitro blue tetrazolium) –NADH (nicotinamide adenine di nucleotide reduced)-PMS (phenazonium methosulphate) composite method. A well-known antioxidant, vitamin C, was used for comparison. The IC_{50} values are incorporated in Table 1.

Table 1. Antioxidant assays of steroidal saponins of *S. nigrum* and *S. xanthocarpum*.

Samples	**Assay methods**			
	DPPH assay	**ABTS assay**	**FRAP assay**	**Superoxide assay**
	IC_{50} values in µg/ml			
Saponins of *S. nigrum*	457.23±9.27	52.99±1.24	142.76±2.17	383.40±8.54
Saponins of *S. xanthocarpum*	187.05±7.64	48.08±1.04	60.91±2.07	279.12±13.37
Vitamin C	3.82±0.29	4.1±0.18	1.62±0.37	33.58±0.76

Data represented as Mean ± SD; n=4.

Note: In view of the significant difference in the molar ratio of vitamin C/steroidal saponins = 176/~880, and the fact that the antioxidant pharmacophore is seemingly restricted to the spiro-ketal function (backed by the hydrophobic cyclopentano perhydro phenanthrene ring system), the IC_{50} values of the steroidal saponins appear to be quite significant.

Effect on body weight and body mass index of rats due to HCD:

Consumption of HCD for 28 days increases the body weight and BMI of the animals in the groups 2-7 in varying extent. In group-2, the extent of increase is maximum. In group 7 the increase is also quite prominent. But in groups 3-6, the gaining of body weight and BMI is lower than both groups 2 and 7. The results were incorporated in Table 2.

Table 2. Effect steroidal saponins of *S. nigrum* and *S. xanthocarpum* on body weight and body mass index of rats.

Body Weight and Body Mass Index (BMI) at 0 day							
Parameters	**Groups**						
	Gr-1	**Gr-2**	**Gr-3**	**Gr-4**	**Gr-5**	**Gr-6**	**Gr-7**
Body Weight (gm)	260.5±33.86	279.63±38.12	272.17±47.33	269.33±44.29	272.67±26.69	269.67±20.95	287.67±24.18
BMI (g/cm^2)	0.71	0.73	0.71	0.68	0.69	0.71	0.72
Body Weight and BMI after 14 days							
	Gr-1	**Gr-2**	**Gr-3**	**Gr-4**	**Gr-5**	**Gr-6**	**Gr-7**
Body Weight (gm)	260.83±34.01	310.00±61.46	285.83±44.49	279.67±44.31	284.50±27.32	278.83±9.34	312.50±27.80
BMI (g/cm^2)	0.72	0.81	0.74	0.70	0.72	0.74	0.78
Body Weight and BMI after 28 days							
	Gr-1	**Gr-2**	**Gr-3**	**Gr-4**	**Gr-5**	**Gr-6**	**Gr-7**
Body Weight (gm)	261.00±10.53	319.00±64.47	284.83±38.66	280.33±47.11	285.50±27.11	283.00±27.89	318.17±28.19
BMI (g/cm^2)	0.72	0.83	0.74	0.71	0.72	0.75	0.79

Data represented as Mean ± SD; for 6 rats.

Effect of steroidal saponins of *S. nigrum* and *S. xanthocarpum* on serum lipid parameters:

Consumption of HCD resulted in a significant increase in TC, TG, LDL and VLDL and a concomitant decrease in HDL level of rat serum among the rats of Gr-2 compared to the rats fed with normal diet (p<0.001) even in 14 days. The LDL/HDL ratio also found to be increased significantly (p<0.001). Administration of steroidal saponins of S. *nigrum* and *S. xanthocarpum* for 28 consecutive days improved the abovementioned lipid status in a dose dependent manner, compared to those of Gr-2. The results are also comparable to standard atorvastatin drug in 28 days. Results are incorporated in Tables 3 and 4.

Table 3. Effect of steroidal saponins of *S.nigrum* and *S. xanthocarpum* on serum lipid parameters after treatment for 14 days.

Lipid parameters after 14 days							
Parameters	**Groups**						
	Gr-1	**Gr-2**	**Gr-3**	**Gr-4**	**Gr-5**	**Gr-6**	**Gr-7**
TC (mg/dl)	108.33±10.53	157.33±13.75$^{\#\#\#}$	146.36±9.09	138.01±7.46*	143.95±6.53	141.01±9.34	125.73±5.45***
TG (mg/dl)	68.02±7.48	123.17±13.30$^{\#\#\#}$	110.96±7.20	104.33±5.93*	98.79±8.58**	89.51±13.87***	78.23±4.98***
HDL (mg/dl)	48.16±5.85	27.45±3.33$^{\#\#\#}$	34.03±3.52	35.33±5.14*	32.23±3.50	36.97±4.80**	37.69±2.17**
LDL (mg/dl)	45.57±13.33	105.25±10.06$^{\#\#\#}$	90.14±6.90	81.81±6.42**	91.96±3.39	86.14±12.96*	72.39±6.97***
VLDL (mg/dl)	13.60±1.50	24.63±2.66$^{\#\#\#}$	22.19±1.44	20.87±1.19*	19.76±1.72**	17.90±2.77***	15.65±0.99***
LDL/HDL	1.0±0.36	3.87±0.44$^{\#\#\#}$	2.67±0.33***	2.37±0.49***	2.87±0.24**	2.40±0.70***	1.93±0.30***
AI	0.15±0.09	0.65±0.02$^{\#\#\#}$	0.51±0.06**	0.47±0.06***	0.49±0.06**	0.38±0.08***	0.32±0.04***

Data represented as Mean ± SD; for 6 rats.
$^{\#}p$<0.05; $^{\#\#}p$ <0.01; $^{\#\#\#}p$ *<0.001;* in comparison to Group 1 (normal control) rats treated with vehicle and fed with normal diet.
*p<0.05; ** p <0.01; *** p *<0.001;* in comparison to Group 2 (negative control) rats treated with vehicle and fed with HCD.

Table 4. Effect of steroidal saponins of *S.nigrum* and *S. xanthocarpum* on serum lipid parameters after treatment for 28 days.

Lipid parameters after 28 days							
Parameters	**Groups**						
	Gr-1	**Gr-2**	**Gr-3**	**Gr-4**	**Gr-5**	**Gr-6**	**Gr-7**
TC	105.90±9.48	171.25±11.14$^{\#\#\#}$	148.35±9.06**	120.67±8.45***	149.06±8.41**	130.51±4.97***	109.46±11.01***
TG	67.99±8.22	134.05±14.49$^{\#\#\#}$	113.29±9.46*	89.39±6.76***	114.01±11.65*	79.28±8.21***	72.60±7.38***
HDL	46.77±7.39	23.80±3.25$^{\#\#\#}$	35.27±4.51**	37.97±3.87***	34.28±4.46**	40.88±4.21***	42.44±2.78***
LDL	45.53±15.56	120.64±10.78$^{\#\#\#}$	90.43±11.59**	64.82±11.08***	91.98±7.49**	73.77±8.50***	52.50±14.30***
VLDL	13.60±1.64	26.81±2.90$^{\#\#\#}$	22.66±1.89*	17.88±1.35***	22.80±2.33*	15.86±1.64***	14.52±1.47***
LDL/HDL	1.03±0.50	5.16±0.92$^{\#\#\#}$	2.62±0.62***	1.74±0.45***	2.72±0.38***	1.84±0.39***	1.25±0.39***
AI	0.16±0.09	0.75±0.05$^{\#\#\#}$	0.51±0.04***	0.37±0.06***	0.52±0.08***	0.29±0.04***	0.023±0.04***

Data represented as Mean ± SD; for 6 rats.
$^{\#}p<0.05$; $^{\#\#}p<0.01$; $^{\#\#\#}p<0.001$; in comparison to Group 1 (normal control) rats treated with vehicle and fed with normal diet.
$*p<0.05$; $**p<0.01$; $***p<0.001$; in comparison to Group 2 (negative control) rats treated with vehicle and fed with HCD.

Effect of steroidal saponins of *S. nigrum* and *S. xanthocarpum* on serum antioxidants:

The effects of the saponins of *S. nigrum* and *S. xanthocarpum* on the serum antioxidant status of hyperlipidaemic rats, lipid peroxidation and antioxidant defense system capabilities were evaluated. Significant improvement of serum antioxidant status was observed in the rats treated with saponins of *S. nigrum* and *S. xanthocarpum*. Results were incorporated in Table 5.

Table 5. Effect of steroidal saponins of *S. nigrum* and *S. xanthocarpum* on serum antioxidants after treatment for 28 days.

Antioxidant parameters after 28 days							
Parameters	**Groups**						
	Gr-1	**Gr-2**	**Gr-3**	**Gr-4**	**Gr-5**	**Gr-6**	**Gr-7**
ABTS IC_{50} in µg/ml serum	1.18±0.09	1.77±0.22$^{\#\#\#}$	1.68±0.08	1.53±0.07*	1.63±0.07	1.50±0.10**	1.59±0.08
FRAP IC_{50} in µg/ml serum	1.43±0.08	2.18±0.29$^{\#\#\#}$	2.05±0.10	1.85±0.13**	1.98±0.10	1.83±0.06**	1.91±0.11*
SOD in serum U/ml	20.88±2.48	14.25±2.65$^{\#\#}$	16.29±2.13	20.20±3.45*	16.16±2.17	20.68±4.14*	20.53±3.56*
CAT in serum U/ml	13.29±1.40	10.53±0.57$^{\#\#\#}$	10.54±1.28	12.51±0.77*	10.21±1.12	12.52±0.68*	12.38±1.04*
MDA (µ mole/l serum)	2.66±0.23	3.80±0.32$^{\#\#\#}$	3.44±0.41	3.01±0.35**	3.47±0.32	3.01±0.28**	3.11±0.22**

Data represented as Mean ± SD; for 6 rats.

$^{\#}p<0.05$; $^{\#\#}p<0.01$; $^{\#\#\#}p<0.001$; in comparison to Group 1 (normal control) rats treated with vehicle and fed with normal diet.

$*p<0.05$; $**p<0.01$; $***p<0.001$; in comparison to Group 2 (negative control) rats treated with vehicle and fed with HCD.

Effect of steroidal saponins of *S. nigrum* and *S. xanthocarpum* on serum GOT, GPT, alkaline phosphatase and total protein content:

To evaluate the hepatic status of rats treated with HCD and also any alteration due to oral administration of steroidal saponins of *S. nigrum* and *S. xanthocarpum* for 28 consecutive days, SGOT, SGPT, alkaline phosphatase and total protein of the rat serum were measured which indicated mild hepatoprotective potential of saponins of *S. nigrum* and *S. xanthocarpum*. The results were incorporated in Table 6.

Table 6. Effect of steroidal saponins of *S. nigrum* and *S. xanthocarpum* on serum GOT, GPT, alkaline phosphatase and total protein content after treatment for 28 days.

Serum lipid parameters after 28 days							
Parameters	**Groups**						
	Gr-1	**Gr-2**	**Gr-3**	**Gr-4**	**Gr-5**	**Gr-6**	**Gr-7**
SGOT in IU/L	71.67±7.15	82.62±4.92	78.12±6.48	75.97±6.44	79.23±6.45	76.25±6.37	79.78±6.25
SGPT in IU/L	62.05±5.15	73.06±8.02	71.61±6.18	66.99±5.65	72.14±5.19	67.45±7.23	71.53±7.50
Alkaline phosphatase in IU/L	19.51±2.36	25.80±4.31$^{\#}$	24.10±3.26	20.45±2.24*	20.25±1.92	20.36±2.40*	25.12±3.35
Total Protein in mg/dl	7.43±1.15	5.57±1.49	5.78±1.30	6.02±1.19	5.37±0.84	6.37±1.09	5.91±1.01

Data represented as Mean ± SD; for 6 rats.
$^{\#}p<0.05$; $^{\#\#}p<0.01$; $^{\#\#\#}p<0.001$; in comparison to Group 1 (normal control) rats treated with vehicle and fed with normal diet.
$*p<0.05$; $**p<0.01$; $***p<0.001$; in comparison to Group 2 (negative control) rats treated with vehicle and fed with HCD.

Discussion

Increased serum concentration of TC and TG is a major lipid abnormality syndrome as they are often associated with cardio vascular disorders (CVD) and many other pathological syndromes [25]. Both LDL and VLDL have a positive role in atherogenesis [26]. AI is used as marker to assess the susceptibility of atherogenesis and marked increase in AI value indicated various pathological disorders [6]. On the other hand, HDL, which is considered as a beneficial lipoprotein, has an inhibitory effect in the pathogenesis of atherosclerosis [6]. In the present study, total lipid contents of serum of rats fed with HCD (Group-2) were significantly elevated compared to the normal control group (Group-1). Treatment of steroidal saponins of *S. nigrum* and *S. xanthocarpum* reduced TC, TG, LDL, VLDL, LDL/HDL ratio and AI significantly with a concomitant increase in HDL level. The effect was observed in 14 days, but became more pronounced in 28 days. Both the study samples were found to have the effects comparable that of standard drug Atorvastatin after 28 days of treatment. The mechanism of action of the beneficial effects of the steroidal saponins was also delineated.

Hypercholesterolemia leads to increased production of reactive oxygen species (ROS), which leads to lipid peroxidation. ROS initiates a series of chain reactions *in vivo* and ultimately damages tissue and DNA [27]. The MDA content is a marker of the extent of lipid peroxidation [28]. In addition, SOD and CAT are the two major enzymes that play an important role in the elimination of ROS derived oxidative damages [29]. Due to consumption of HCD, an increase in MDA content and decrease in SOD and CAT levels occurred. Treatment of albino rats with steroidal saponins of *S. nigrum* and *S. xanthocarpum* despite feeding with HCD (Groups 3-6) improved the antioxidant status of the serum of treated animals considerably by lowering MDA content and augmenting SOD and CAT contents. The same trend was observed in the ABTS and FRAP assays.

To evaluate the effects of consumption of HCD and steroidal saponins of *S. nigrum* and *S. xanthocarpum* on the hepatic status of animals, serum GOT, GPT, alkaline phosphatase and total protein content were measured. It was observed that, steroidal saponins of *S. nigrum* and *S. xanthocarpum* had no hepatotoxic effect. Besides, they tended to act as hepatoprotective agents as they were able to normalize the levels of the serum GOT, GPT, alkaline phosphatase and total protein content of experimental albino rats.

Based on the above results it is proposed that, steroidal saponins of *S. nigrum* and *S. xanthocarpum*, if properly formulated, can be used as an antihyperlipidaemic agent with beneficial to the antioxidant and hepatoprotective functions.

Acknowledgement

AKG and PG thank M/S Natreon Inc. Kolkata, for providing instrumental facilities and for permitting to carryout independent research investigation under the supervision of the senior authors (UKM and SG).

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