



**POTENTIAL OF *ASCIDIA SYDNEIENSIS* AGAINST TRITON INDUCED
HYPERLIPIDEMIA**

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ABSTRACT

Hyperlipidemia is a disorder of lipid metabolism manifested by elevation of plasma concentrations of various lipid and lipoprotein fractions. It has been ranked as one of the greatest risk factors contributing to the prevalence and severity of coronary heart diseases. Hyperlipidemia is identified as dyslipidemia and it can also be described as elevated total cholesterol (TC) or triglycerides (TG), or low levels of high density lipoprotein cholesterol (HDL). People with high cholesterol have about twice the risk of coronary heart disease. Several risk factors of hyperlipidemia have been reported - age, sex, obesity, wrong diet, blood pressure, blood sugar, stress, smoking and environment. The present study was designed to explore the potential of the ethanol extract of *Ascidia sydneiensis* against Triton X-100 induced hyperlipidemic rats. Body weight, serum biochemical parameters - protein, albumin, globulin, SGPT, SGOT, ALP, serum lipid profile - Total cholesterol (TC), Triglycerides (TG), high density lipoprotein - cholesterol (HDL-C), very low density lipoprotein - cholesterol (VLDL-C), low density lipoprotein - cholesterol (LDL-C) and phospho lipids were analysed following standard procedures. Histopathological examinations of liver sections were also done. Administration of the extract at a dose of 50, 100 and 150 mg/kg b.w was compared with Triton induced hyperlipidemic control and standard drug Atorvastatin (100 mg/kg b.w). A significant decrease in body weight, serum enzymes - SGPT, SGOT, ALP, plasma lipid profile - TC, TG, VLDL-C, LDL-C, PL, faecal lipid profile - TL, TC, TG and an elevation in serum biochemical parameters - protein, albumin, globulin and plasma lipid profile - HDL-C were noted in the extract treated groups when compared to hyperlipidemic control. In the group treated with the highest dose of the extract all the biochemical parameters were brought back to normal level. The effective suppression Triton induced hyperlipidemia in rats on treatment with the extract suggests the potential protective role in coronary heart disease.

KEYWORD: *Ascidia sydneiensis*, Triton X-100, atorvastatin, hyperlipidemia, plasma lipid profile.

INTRODUCTION

Hyperlipidemia, a disorder of lipid metabolism characterized by elevated levels of lipids circulating in the blood, has now become a global concern. It is considered as one of the five leading causes of death in the world.^[1] Coronary heart disease resulting from progressive atherosclerosis remains the most common cause of morbidity and mortality.^[2] Hyperlipidemia (mainly increased level of cholesterol or low density lipoprotein (LDL-C) is a major cause for atherosclerosis an important risk factor in the initiation and associated conditions of atherosclerotic lesions like coronary heart disease, stroke, ischemic cerebrovascular and peripheral vascular diseases.^[3,4,5,6] Feeding animals with cholesterol-rich diet is commonly used as a model for induction of hyperlipidemia to study the etiology of hyperlipidemia-related metabolic disorders and the efficiency of potential anti-hyperlipidemic agents.^[7,8] Generally the therapeutic purpose of using

hypolipidemic drugs is to reduce the elevated levels of plasma lipids, notably cholesterol.^[9] A patient with elevated low-density lipoprotein (LDL) and decreased high density lipoprotein (HDL) will have a high risk of experiencing cardiovascular disorder.^[5,10] Currently available drugs have been associated with number of side effects like hyperuricemia, diarrhoea, nausea, myositis, gastric irritation, flushing, dry skin and abnormal liver function.^[11] Lipid lowering effect of a number of plant extracts have been reported but they are being exploited for various reasons. Marine organisms have natural defence mechanism by way of producing bioactive compounds. *Ascidia sydneiensis* is a simple ascidian occurring predominantly in the coastal regions of Tuticorin. In recent years taxonomy,^[12] ecology, distribution, seasonal variation in the occurrence, breeding biology, recruitment and succession in the fouling community, role as bioindicators, food value,^[13] association with coral reef,^[14] antibacterial, antimicrobial

activity against human pathogens^[15,16], chemical investigations,^[17-31] antipyretic and analgesic,^[32,33] larvicidal,^[34] antimicrobial,^[35,36] antidiabetic,^[37,38] antiproliferative,^[39] toxicity,^[40-43] antitumour and immunomodulatory,^[44-49] anaesthetic,^[50-52] wound healing,^[53-55] CNS depressant,^[56] antifertility,^[57] hepatoprotective,^[58,59] antiinflammatory,^[60-62] immunostimulating,^[63] cardio protective,^[64] anti-hyperlipidemic activity^[65] of a large number of ascidians have been studied. Chemical investigations,^[66-68] toxicity^[69], antidiabetic^[70], antifertility^[71] effect of the selected ascidian *Ascidia sydneiensis* is available. As anti-hyperlipidemic potential of *Ascidia sydneiensis* had not been elucidated in an exclusive Triton X-100 induced animal model, the present study has been designed.

MATERIALS AND METHODS

Animal material

Samples of *Ascidia sydneiensis* were collected from Tuticorin coast and identified using key to identification of Indian ascidians.^[72] A voucher specimen AS 2252 has been deposited in the National Collections of Ascidians in the Museum of the Department of Zoology, A.P.C. Mahalaxmi College for Women, Tuticorin-628002.

Taxonomic status

Ascidia sydneiensis is a simple ascidian belonging to the Phylum: Chordata, Subphylum: Urochordata, Class: Ascidiacea, Order: Enterogona, Suborder: Phlebobranchia, Family: Ascidiidae, Genus: *Ascidia*, Species: *sydneiensis*.

Preparation of extract

For antihyperlipidemia studies, 100 gram powder was extracted with ethanol using Soxhlet apparatus, cooled to room temperature, evaporated in a rotary evaporator under reduced pressure to obtain a brown residue.

Experimental animals

Normal healthy adult male Wistar albino rats (180-200 g) were obtained from Central Animal House, Annamalai University, Chidambaram, Tamil Nadu, India. They were maintained under standard environmental conditions of temperature - 24±1°C, 12 h dark-light cycle, humidity (60-70%), free access to drinking water and standard pellet diet. Rats were deprived of food except water 16-18 hour prior to the experiments. The rules and regulations of Animal Ethical Committee, Government of India were followed.

Anti-hyperlipidemic studies - Induction of hyperlipidemia

Hyperlipidemia was induced in Wistar albino rats by intraperitoneal injection of Triton X-100 (100 mg/kg). Experimental animals were divided into six groups of 6 rats each. First group was given saline and treated as normal control. Second group was administered with a single dose of Triton and considered as hyperlipidemia induced control. Third, fourth, fifth and sixth group of hyperlipidemic rats were given the extract of *Ascidia*

sydneiensis at various concentrations of 50, 100 and 150 and the standard drug atorvastatin 100 mg/kg b.w respectively. The experiment was carried out for 14 consecutive days and all drugs were given using intra gastric catheter. Blood samples were collected by cardiac puncture.

Weight of Body

The body weight of adult rat was monitored throughout the treatment period. After 14 days, the final body weight was recorded.

Estimation of Serum Biochemical Parameters

Protein, albumin, globulin, serum glutamate pyruvate transaminase (SGPT), glutamate oxaloacetate transaminase (SGOT) and alkaline phosphatase (ALP) were measured spectrophotometrically.^[73-76]

Estimation of Plasma lipid profile

Standard procedures were followed to determine Total Cholesterol, Triglycerides, Low Density Lipoprotein-Cholesterol, Very Low Density Lipoprotein-Cholesterol, High Density Lipoprotein-Cholesterol and Phospholipids.^[77-82]

Estimation of Faecal lipid profile

Faecal matter was collected during the last three days of the treatment period and used for determination of Total Lipids (TL), Total Cholesterol (TC) and Triglycerides (TG) by standard methods.^[77,78]

Histopathological study

Liver tissue was excised immediately, washed with ice-cold saline and preserved in 10% formalin solution for histological study. They were dehydrated with varying percentage of ethanol. Sections were cleared in xylene and embedded in molten wax. Thin sections were cut (5 µm), stained with hematoxylin, eosin and viewed under the high power of a microscope.

Statistical Analysis

Values are presented as mean ± S.E.M and statistically evaluated by one-way analysis of variance (ANOVA) followed by student's t - test to identify the differences between ^{*}hyperlipidemic control and extract treated groups and ^aStandard drug and extract treated groups.

RESULTS AND DISCUSSION

Hyperlipidemia is associated with heart disease, which is the leading cause of death in the world. The lowering of the levels of harmful lipids to satisfactory values have been confirmed by several experimental animal and interventional studies indicating lowered morbidity and mortality due to coronary heart diseases.^[83] Triton induced hyperlipidemia in rats is an acute model for primary screening of the potential of anti-hyperlipidemic agents. Administration of Triton physically alters very low density lipoprotein cholesterol rendering them refractive to the action of lipolytic enzymes of blood and tissues, preventing or delaying their removal from blood

and tissues.^[84] In the present study, potential of the extract has been evaluated in normal and Triton induced hyperlipidemia rats.

Effect of the extract of *Ascidia sydneiensis* body weight is shown in Table-1. In normal and Triton induced control mean weight gain was observed whereas the extract and standard drug treated groups exhibited a mean weight loss. The percentage difference of weight gain and loss noted were not significant.

A reduction in the level of protein, albumin and globulin was noticed in Triton induced group when compared with normal control and a dose dependent increase in the extract treated groups. In the standard drug and highest dose treated group, the levels were restored to that of the normal (Table-2). Liver plays a major function in protein metabolism and any variation in its activity alters the stages of protein synthesis. The reduction recorded in Triton induced control may be due to defective protein biosynthesis in liver.^[85] Triton intoxication causes disruption and disassociation of polyribosomes on endoplasmic reticulum and thereby reducing the biosynthesis of protein. In the present study treatment with the extract might have protected the polyribosomes and assisted in normal protein synthesis. The similar effect was observed in the atorvastatin drug treated group also. Marker enzymes such as SGPT, SGOT and ALP which are originally present in high concentration in the cytoplasm was measured to assess liver toxicity. A remarkable elevation in the level of SGPT, SGOT and ALP was observed in the Triton X-100 induced hyperlipidemia which may be due to hepato cellular damage causing the release of enzymes into the blood stream.^[86] The level of SGPT, SGOT and ALP decreased in extract and atorvastatin treated groups, the serum enzymes were restored to their respective normal level indicating non-toxic action of the extract on liver and anti-hyperlipidemic effect.

The results revealed a significant elevation in the level of serum TC, TG, VLDL, LDL, PL and a decrease in HDL-C in Triton X-100 treated group compared to that of normal as shown in Table- 3. In extract treated groups, a significant reduction in the TC, TG, VLDL, LDL, PL and increase in HDL-C was observed. It has been well established that nutrition plays an important role in the etiology of hyperlipidimias and atherosclerosis. Bioactive compounds especially phenols and flavonoid intake decreased LDL-C and increased HDL-C in hypercholesterolemic individuals.^[87] A preliminary chemical screening of the ethanolic extract of *Ascidia sydneiensis* has shown the presence of alkaloids, steroids, tannins, flavonoids, quinones, anthraquinones, phenols, aromatic acids, proteins, lipids and carbohydrates.^[67] Reduced levels of LDL and VLDL in the extract treated rats may be possibly due to increase in the catabolism of LDL. This can be attributed to the protective effect of the extract against hyperlipidemia by lowering the TG, decreasing VLDL synthesis and channelizing VLDL

through pathways other than to LDL or by elevating the activity of the enzyme lipoprotein lipase.^[88] The increase in TC and TG due to Triton X-100 injection results mostly from an increase of VLDL secretion by the liver accompanied by a strong reduction of VLDL and LDL catabolism.^[89] The reduction of TC by the extract of *Ascidia sydneiensis* was associated with a decrease of its LDL fraction, which is the target of several hypolipidemic drugs. This result suggests that cholesterol lowering activity of the extract may be by the rapid catabolism of LDL cholesterol through its hepatic receptors.^[90] HDL-C levels have a protective role in coronary heart diseases.^[91] The increased level of HDL-C and decreased cholesterol level along with its LDL fraction evident on treatment with the extract could be due to decreased cholesterol absorption through gastro intestinal tract and an increased cholesterol excretion.

Atorvastatin which was used as positive control in this study is a 3-hydroxy-3methyl glutaryl coenzyme-A reductase (HMG-COA reductase) inhibitor. It plays a beneficial role in competitive inhibition of HMG-COA reductase which blocks the cholesterol biosynthesis and stimulates the synthesis of LDL receptors thus lowering the plasma LDL cholesterol concentration.^[92] Another pathway involved in the lowering of TC might be the reduction in the biosynthesis of cholesterol by inhibiting the activity of HMG-COA reductase, which is the key enzyme in cholesterol synthesis.^[93]

The possible mechanism of increase of HDL-C on treatment with *Ascidia sydneiensis* may be attributed to the mobilization of cholesterol from peripheral cells to the liver by the action of lecithin-cholesterol acyl transferase (LCAT) as reported on studies with plant extract of *Rhinacanthus nasutus*.^[86] The increased HDL-C facilitates the transport of TG or cholesterol from serum to liver by a pathway termed 'reverse cholesterol transport' where it is catabolised and excreted out of the body. Elevation of the activity of an enzyme LCAT which plays an important role in the incorporation of free cholesterol into HDL may be suggested. Presence of certain compounds such as tetradecanoic acid, Bis-(2-methylpropyl) ester of 1,2-benzenedicarboxylic acid, n-Hexadecanoic acid, Diisooctyl ester of 1,2-benzene dicarboxylic acid, cyclopropyl methyl ester of cylopropaneoctanoic acid in the ethanolic extract of *Ascidia sydneiensis* are also known to have anti-hyperlipidemic activity.^[66]

Treatment with *Ascidia sydneiensis* extract (150 mg/kg) showed marked reduction in TG level as compared to control. This effect might be due to increase in activity of the endothelium bound lipoprotein lipase which hydrolyses the triglyceride into fatty acid or may be due to inhibition of lipolysis so that fatty acids do not get converted into triglyceride.^[94] The extract may have stimulation of lipoprotein lipase activities resulting in decrease of plasma triglyceride and might increase the

uptake of triglyceride from plasma by skeletal muscle and adipose tissues.^[95]

The faecal lipid profile indicated an increase in Triton induced group whereas in the extract treated group a dose dependent significant decrease was noticed. Standard drug administered group brought back TL, TC and TG near to that of normal as given in Table 4. Consumption of animal sterol and their esters has been reported to not only lower intestinal cholesterol absorption but decreased blood levels of the atherogenic LDL-C as well.^[96,97] This may be either due to the steroids present in the extract reduce the absorption of cholesterol or inefficient hepatic catabolism of cholesterol lowers its synthesis.^[98]

Histopathological observation

Histopathology of the liver of the wistar albino rats treated with *Ascidia sydneiensis* extract is shown in Plate 1. Control rats' liver shows hepatic cords, normal hepatocytes with vesicular nucleus, blood sinusoids lined

with endothelium and von Kupffer cells. Fatty changes in centrilobular portions of the liver with abnormal lobular structure, deformed hepatocytes with obvious small and large fat granules were noted in the rats that received Triton X (100 mg/kg b.w). 50 mg/kg b.w of extract treated group exhibited mild recovery which was well evidenced by the reduction of fat vacuoles in hepatocytes. 50% of the hepatocytes look normal radiating from the central vein. Liver of the group treated with 100 mg/kg b.w indicates moderate recovery of hepatocytes which was authenticated by the reduction of fat vacuoles. Nearly 80% of the hepatocytes look normal radiating from the central vein. Treatment with 150 mg/kg b.w of the extract resulted almost complete recovery of hepatocytes which was well defined in the reduction of fat vacuoles in hepatocytes. 90% of the hepatocytes look normal radiating from the central vein. Liver of rat treated with standard drug exhibited normal hepatocytes with vesicular nucleus and very mild fat vacuoles.

Table 1. Effect of the extract of *Ascidia sydneiensis* on body weight

Groups & Treatment	Initial Body Weight IU mg ⁻¹	Final Body Weight (Gm)	Mean Weight Gain (G↑) / loss(L↓) (Gm)	% Difference
I – Control	194.65±5.60	209.56±4.10	14.91↑	7.65%
II - Triton X - 100 mg/kg	201.50±4.15	214.82±5.80	13.32↑	6.61%
III - AS 50 mg/kg	198.60±6.30	180.50±4.36	18.10↓	9.11%
IV- AS 100 mg/kg	206.75±5.90	189.35±6.90*	17.40↓	8.41%
V- AS 150 mg/kg	198.34±4.30	179.28±5.60*	19.06↓	9.60%
VI – Atorvastatin	205.10±6.50	188.90±4.30	16.20↓	7.89%

Data represented as mean ± SEM, (N=6). Significance between hyperlipidemic control and extract treated group *P>0.05.

Table 2. Effect of the extract of *Ascidia sydneiensis* on protein, albumin, globulin, SGPT, SGOT and ALP

Groups & Treatment	Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	SGPT (u/l)	SGOT (u/l)	ALP (u/l)
I- Control	8.11±0.36	4.51±0.31	3.60±0.13	12.26±0.11	16.34±0.92	169.54±6.36
II - Triton X - 100 mg/kg	7.12±0.16	4.12±0.26	3.00±0.34	26.84±0.27	24.88±0.85	204.65±4.18
III - AS 50 mg/kg	7.18±0.26 ^{*a}	4.24±0.16 ^{*a}	2.94±0.11 ^{*a}	26.15±0.36 ^{*a}	20.16±0.18 ^{*a}	180.16±5.84 ^{*a}
IV- AS 100 mg/kg	7.84±0.12 ^{**aa}	4.38±0.74 ^{**aa}	3.46±0.27 ^{**aa}	24.75±0.15 ^{**aa}	18.24±0.39 ^{*a}	173.80±4.16 ^{**aa}
V- AS 150 mg/kg	8.18±0.26 ^{***aaa}	4.49±0.12 ^{***aaa}	3.69±0.14 ^{***aaa}	13.14±0.36 ^{***aaa}	15.34±0.18 ^{*aaa}	164.84±6.76 ^{***aaa}
VI – Atorvastatin	8.12±0.13	4.56±0.15	3.56±0.23	13.26±0.17	14.16±0.35	173.16±5.18

Data represented as mean ± SEM, (N=5). Significance between *Hyperlipidemic control and extract treated group. * p <0.05, ** p <0.01, *** p <0.001, ^aStandard drug and extract treated^a <0.05, ^{aa}<0.01, ^{aaa}<0.001.

Table 3. Effect of the extract of *Ascidia sydneiensis* on the plasma lipid profile

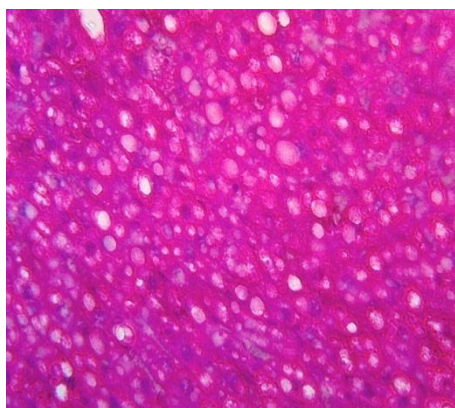
Groups & Treatment	TC (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	VLDL (mg/dl)	LDL (mg/dl)	PL (mg/dl)
I – Control	124.88±2.16	148.36±4.18	42.65±1.65	29.67±1.03	52.56±1.34	179.14±2.34
II - Triton X - 100 mg/kg	249.54±4.34	268.15±5.90	29.54±1.18	53.63±1.37	166.37±2.59	290.09±2.65
III - AS 50 mg/kg	206.16±3.19 ^{*a}	246.54±4.86	34.16±1.56 ^{*a}	49.31±1.53 ^a	122.69±2.13	251.48±2.48
IV- AS 100 mg/kg	178.36±5.14 ^{*a}	201.34±4.80	37.80±2.04 ^{**aa}	40.27±1.84 ^{aa}	100.29±1.84	226.74±3.63
V- AS 150 mg/kg	141.54±4.92 ^{**aa}	173.16±4.15 ^{*a}	39.11±1.92 ^{**aaa}	34.63±1.22 ^{*aaa}	67.80±1.33 ^{*a}	193.97±2.88 ^{*a}
VI – Atorvastatin	132.86±3.16	165.39±3.60	38.36±1.65	33.08±1.93	61.42±1.37	186.24±2.19

Data represented as mean ± SEM, (N=5). Significance between *Hyperlipidemic control and extract treated group. * p <0.05, ** p <0.01, ^aStandard drug and extract treated^a <0.05, ^{aa}<0.01, ^{aaa}<0.001.

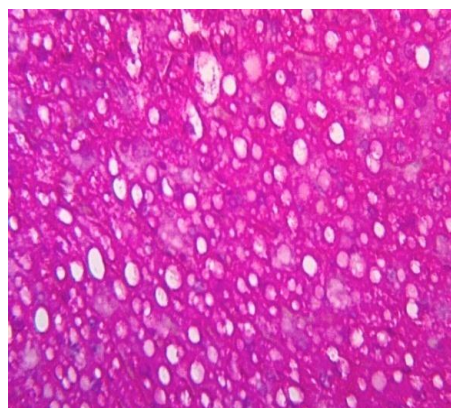
Table 4. Effect of the extract of *Ascidia sydneiensis* on the Faecal Lipid profile

Groups & Treatment	TL (mg/dl)	TC (mg/dl)	TG (mg/dl)
I – Control	61.36±1.36	16.35±0.15	15.16±0.43
II - Triton X - 100 mg/kg	92.15±2.18	29.16±0.54	21.54±0.26
III - AS 50 mg/kg	84.26±1.15	24.81±0.15 ^{*aa}	21.34±0.37 ^a
IV- AS 100 mg/kg	73.11±1.36 ^{*a}	21.54±0.29 ^{**aaa}	19.92±0.18 ^{*aa}
V- AS 150 mg/kg	60.34±1.94 ^{***aa}	20.36±0.18 ^{**aaa}	19.05±0.34 ^{*aa}
VI – Atorvastatin	63.91±1.65	21.68±0.56	16.84±0.26ns

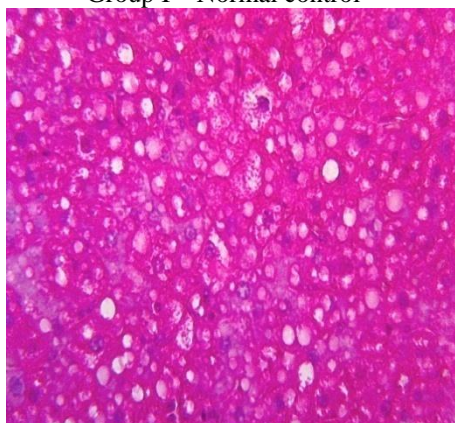
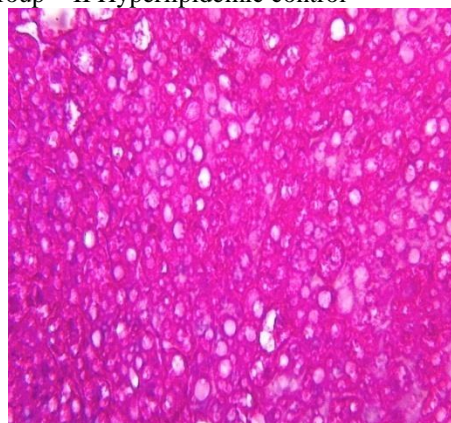
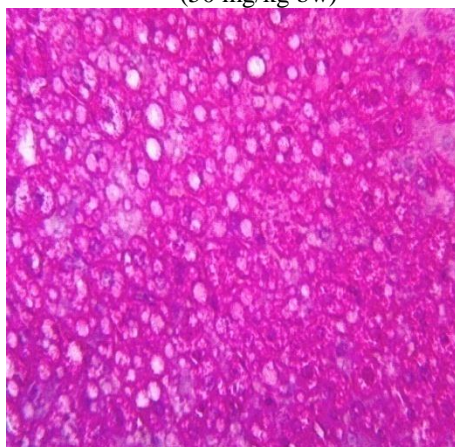
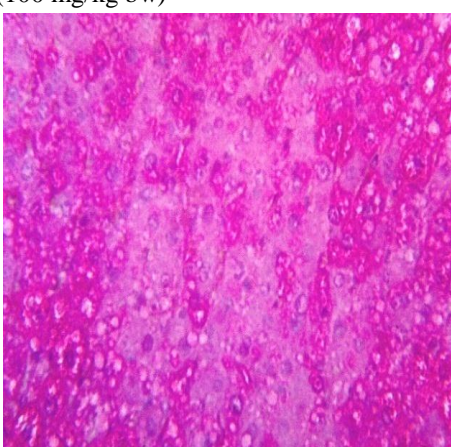
Data represented as mean ± SEM, (N=5). Significance between ^{*}Hepatic control and extract treated group. ^{*}p <0.05, ^{**}p <0.01, ^{***}p <0.001, ^aStandard drug and extract treated ^a<0.05, ^{aa}<0.01, ^{aaa}<0.001.



Group I – Normal control



Group – II Hyperlipidemic control

Group III – Extract of *A. sydneiensis*
(50 mg/kg bw)Group IV – Extract of *A. sydneiensis*
(100 mg/kg bw)Group V – Extract of *A. sydneiensis*
(150 mg/kg bw)Group VI– Atorvastatin
(100 mg/kg bw)**Plate1. Photomicrograph showing histopathological changes in the Liver**

CONCLUSION

The results obtained from the pharmacological screening have led to the conclusions that, ethanolic extract of *Ascidia sydneiensis* has significant anti-hyperlipidemic activity. Hence it can be exploited as an anti-hyperlipidemic therapeutic agent or adjuvant in existing therapy for the treatment of hyperlipidemia. Their hypolipidemic effect may be due to the low activity of cholesterol biosynthesis enzymes and/or low level of lipolysis. This effect could prevent or be helpful in reducing the complications of lipid profile in which hyperglycemia and hypercholesterolemia coexist. Further findings are going on to separate the active compound in *Ascidia sydneiensis* and to elucidate the mechanism of action.

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