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Original Article

Hepatoprotective Activity of Ethonolic Extract of *Allophylus Serratus* Root

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Abstract

In the present study we selected a plant namely *allophylus serratus belonging* to the family (sapindaceae) commonly known as Triputa (in Sanskrit). It is a large shrub or small tree grows up to 10 meters in height. It contains beta-sitosterol, phenacetamide, two flavonoid glycosides like luteolin-7-o-[beta]-D-glucopyranoside and apigenin-4'-o[beta]-D-glucoside, quercetin, pinitol, rutin etc. It is useful in bone fractures, dislocations, Inflammations, ulcers, wounds, dyspepsia, anorexia and diarrhoea. The fruits are sweet cooling and nourishing tonic. Roots of widely grown plant *Allophylus serratus* reported to possess a very high amount of polyphenols and gallic acid which are well known potent antioxidants. Evaluation of the hepatoprotective activity was done by estimating the biochemical marker like SGPT, SGOT, ALP, LDH, total bilirubin, total protein in serum. EEAS significantly reversed the above parameters to near normal levels and the activity of EEAS was comparable to that of standard silymarin. The present studies indicated that the EEAS possess potent hepatoprotective activity comparable to standard LIV 52. The possible hepatoprotective mechanism of roots of *Allophylus serratus* may be via its antioxidant property.

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Introduction

The liver is the major site of xenobiotic metabolism and excretion. Liver injury caused by xenobiotics such as toxic chemicals, drugs, herbal products, environmental chemicals and virus infiltration from ingestion or infection represents the leading cause of acute liver failure. Thus liver diseases remain one of the serious health problems. Drugs have been estimated to cause around 15-20% of all cases of fulminant and 10% of all cases of sub-fulminant in Western countries and 10% of all cases in Japan. Traditionally, various plants are being used to treat hepatic patients. It is believed that herbal medicine has little side effects as well, as it requires no cost in few cases. So, the herbal medicine can solve the economic problem for the poor. *Allophylus serratus* has been claimed to be contain flavanoids useful in hepatotoxicity. CCl_4, Paracetamol and Anti-TB drugs cause ROS mediated cellular damage especially in liver where these drugs are metabolized. Polyphenolic compounds like flavonoids, tannins are useful as antioxidants and organ protectants. The liver is the key organ which regulates homeostasis in the body. It is involved with almost all the biochemical pathways related to growth, fight against disease, nutrient supply, energy provision and reproduction [1]. The liver is expected not only to perform physiological functions but also to protect against the hazards of harmful drugs and chemicals [2]. Liver diseases posses a serious health problem. In the absence of reliable liver protective drugs in allopathic medical practices, herbs play a major role

in the management of various liver disorders. Numerous medicinal plants and their formulations are used for liver disorders in ethnomedical practices and in traditional system of medicine in India. However, we do not have satisfactory remedy for serious liver disease; most of the herbal drugs speed up the natural healing process of liver. So the search for effective hepatoprotective drug continues [3].

Many diseases of the liver are accompanied by jaundice caused by increased levels of bilirubin in the system. The bilirubin results from the breakup of the heamoglobin of dead red blood cells; normally, the liver removes bilirubin from the blood and excretes it through bile. The carbon tetrachloride(CCl4) induces hepatotoxicity by metabolic activation; therefore it selectively causes toxicity in liver cells maintaining semi-normal metabolic function. Liver diseases remain one of the serious health problems. In the absences of reliable liver protective drugs in allopathic medical practices. Herbs play important role in the management of various liver disorders . However, in ayurveda many indigenous plants have been used as hepatoprotective agents.

In the present study we selected a plant namely *allophylus serratus* belonging to the family (sapindaceae) commonly known as Triputa (in Sanskrit). It is a large shrub or small tree grows up to 10 meters in height. It contains beta-sitosterol, phenacetamide, two flavonoid glycosides like luteolin-7-o-[beta]-D-glucopyranoside and apigenin-4'-o[beta]-D-glucoside, Quercetin, Pinitol, Rutin. The plant is astringent, bitter, sweet, analgesic, anti-inflammatory, vulnerary, digestive, Carminative and constipating. It is useful in bone fractures, dislocations, Inflammations, ulcers, wounds, dyspepsia, anorexia and diarrhoea. The fruits are sweet cooling and nourishing tonic. However, no scientific report has been carried out on the leaves of *allophylus serratus* to prove the hepatoprotective activity. So the aim of present study is to evaluate hepatoprotective activity of allophylus serratus against CCL4 induced hepatotoxicity in experimental rats.

Materials and Method

Chemicals and Reagents

CCL_4 was obtained from Sigma-Aldrich, Bangalore. LIV 52 was obtained from local medical hall. And all other reagents used were of analytical grade. SGPT, SGOT, alkaline phosphatase, total cholesterol and HDL, total bilirubin and Total protein estimation kits were procured from S.V Biological agency, Kadapa.

Collection of Plant Material

Allophylus serratus was obtained from local area of kadapa & authentified by Sri madhava chetty taxonomist S.V University Tirupathi.

Preparation of Extract

The collected plant material *Allophylus serratus* was washed thoroughly in water, cut into small pieces and air dried for two weeks at 35-40^{0}C temperature. Extraction was done by using soxhlet apparatus with 70% ethanol (hydro alcoholic) as solvent. The extracts were concentrated under reduced pressure dried and stored at 4^{0}c temp in air tight containers for further studies.

Experimental Animals and Ethical Clearance

Experimental animals of either sex weighing 150-170 g were obtained from Raghavendra enterprises (Bangalore). The animals were housed in stainless steel cages at a controlled room temperature of 24^{0}C, under a 12 h light and 12 h dark cycle. After one week of acclimatization, the experimental animals were divided randomly in to 5 groups (n=6). The experimental protocol was approved by the Institutional Animal Ethical Committee of R R S college of Pharmacy UP.

Experimental Design

Treatment schedule for assessing the hepatoprotective activity of Ethanolic extract of *Allophylus serratus* (EEAS) (Table 1)

Assessment of Hepatoprotective Activity

All the animals were killed on day 21 under light ether anaesthesia. The blood samples were collected separately by carotid bleeding into sterilized dry centrifuge tubes and allowed to coagulate for 30 min at 370 C. The clear serum was separated at 2500 rpm for 10 min and biochemical investigations were carried out to assess liver function viz., total bilirubin, total protein, serum transaminases and serum alkaline phosphatase .

Measurement of Biochemical Parameters

The parameters *viz.* Estimation of SGPT (Serum glutamyl pyruvate transaminase) *(Modified IFCC UV-*

kinetic method), Estimation of SGOT (Serum glutamyl oxalacetic acid transaminase) *(Modified IFCC UV-Kinetic method),* Estimation of Alkaline phosphatase *(Kind and King's Method)*, Estimation of Total cholesterol, *(Chod-Pod/ Phosphotungstate Method),* Estimation of HDL, Estimation of Bilirubin *(Modified Jendrassik & Grof's Method)* were determined for experimental rats.

Collection of Blood Sample

The blood samples were withdrawn on 0^{th}, 7^{th}, 14^{th}, and 21^{st} day from the retrorbital venous plexus of rats without any coagulant for the separation of serum. These were then allowed to clot at room temperature for half an hour and centrifuged at 4000 r/min for 15 min using a WIFUNG centrifuge LABOR-50M. The clear straw colored serum was then collected from the upper part of the tubes in vials with a Pasteur pipette.

Histopathology

After draining the blood, liver samples were excised, washed with normal saline and processed separately for histological observations. Initially, the materials were fixed in 10% buffered neutral formalin for 48 h and then with bovine solution for 6 h. Paraffin sections were taken at 5mm thickness, processed in alcohol- xylene series and were stained with alum hematoxylin and eosin.The sections were examined microscopically for histopathological changes.

Statistical Analysis

All the data was expressed as mean ± S.E.M. Statistical significance between more than two groups was tested using one way ANOVA followed by the Tukey test using computer based fitting program (Prism graph pad.). Statistical significance was taken as $P< 0.05$.

Results

Rats treated with carbon tetrachloride showed a significant hepatic damage as observed from elevated serum level of hepatospecific enzymes as well as severe alteration in different liver parameters.

Morphological Observation

Morphological observations showed an increased size and enlargement of the liver in CCl_4 treated control group. These changes were reversed by treatment with LIV-52 and also EEAS at the two different doses in tested groups.(Table 2, Figure 1)

Biochemical Estimations

Biochemical estimations was studied on 14^{th} day and the results were presented (Table 3 & Figure 2-8)

Table 1: Dosing pattern

S. No.	Groups	No of animals	Treatment	Purpose
I	Normal	6	OLIVE OIL	To serve as negative control
II	Control	6	CCL4(1ml/kg)+ OLIVE OIL (1:1)	To serve as positive control
III	Standard	6	LIV-52 Syrup (4 mL/kg, p.o.)	To serve as standard
IV	Treatment-1	6	EEAS (250 mg/kg, p.o.)+ CCL4(1ml/kg)	To assess the hepatoprotective activity of EEAS. (250mg/kg,p.o).
V	Treatment -2	6	EEAS (500 mg/kg, p.o.)+ CCL4(1ml/kg)	To assess the hepatoprotective activity of EEAS. (500mg/kg,p.o.)

Table 2: Hepatoprotective activities of EEAS on Liver weights

S. No.	Groups	Treatment	Liver Weights
I	Normal	olive oil	5.783 ± 0.13^{ns}
II	Control	CCl_4 (1ml/kg)+ olive oil (1:1)	$8.167 \pm 0.19^{###}$
III	Standard	Liv 52 syrup (4 ml/kg, p.o.)	$5.717 \pm 0.09^{***}$
IV	Treatmaent 1	EEAS (250 mg/kg, p.o.)+ CCl_4 (1ml/kg)	$7.117 \pm 0.19^{***}$
V	Treatment 2	EEAS (500 mg/kg, p.o.)+ CCl_4 (1ml/kg)	$6.217 \pm 0.14^{***}$

Table 3: Hepatoprotective activities of EEAS on all biochemical parameters

Group	SGOT	SGPT	ALP	Total Protein	Total Bilirubin	Cholesterol	HDL Cholesterol
Group – I (Control)	116.7±4.24	125.3±3.42	288.7±8.96	5.08±0.76	6.74±0.42	165±21.51	0.66±0.03
Group – II (Normal)	45.22±0.62	43.17±2.7	173.7±4.35	7.81±0.44	1.66±0.8	75.33±4.98	0.36±0.02
Group – III (Standard)	56.18±2.00	47.67±1.3	189.8±4.68	8.01±0.51	2.04±12.6	78.67±4.31	0.37±0.02
Group – IV (EEAS 250 mg/kg)	80.33±3.19	86.00±9.8	230.7±22.86	6.76±0.46	3.06±0.26	86.17±3.40	0.38±0.02
Group – V (EEAS 500 mg/kg)	56.83±2.55	45.17±3.0	191.7±2.76	7.43±0.39	2.03±0.22	76.01±5.24	0.42±0.03

All values are shown as mean ± SEM and n=6.
indicate $p<0.001$ when compared to normal group.
*** indicate $p<0.001$ when compared to control group.

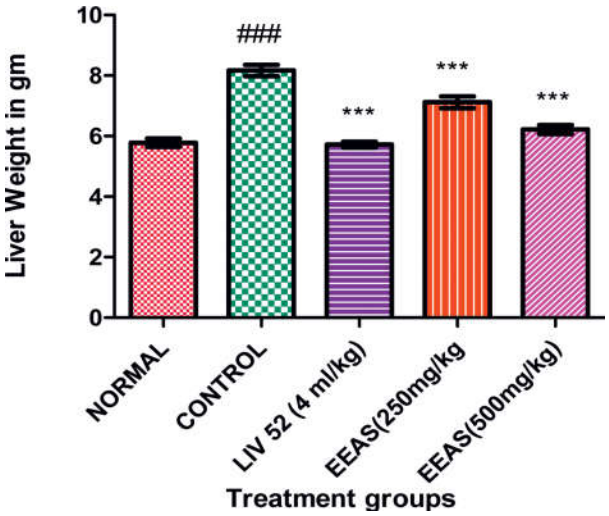

Fig. 1: Effect of EEAS on Liver weights

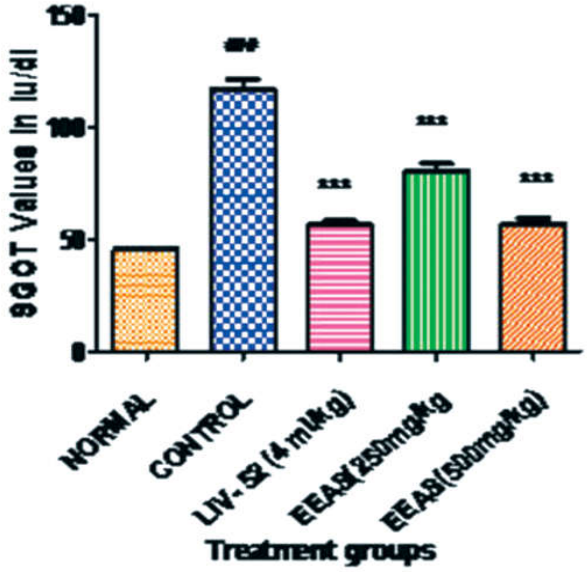

Fig. 2: Effect of EEAS on SGOT levels

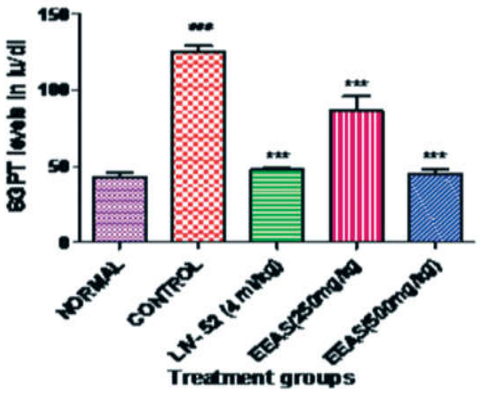

Fig. 3: Effect of EEAS on SGPT levels

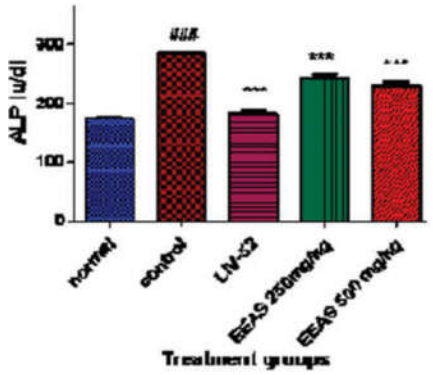

Fig. 4: Effect of EEAS on ALP levels

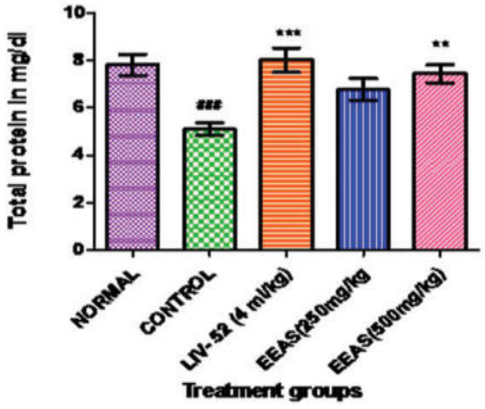

Fig. 5: Effect of EEAS on total protien levels

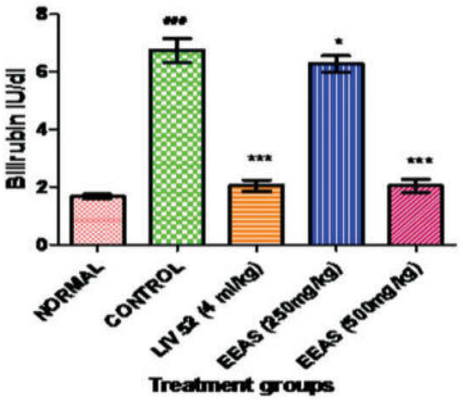

Fig. 6: Effect of EEAS on total bilirubin

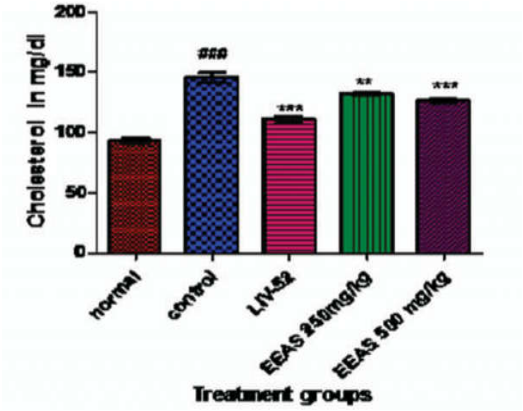

Fig. 7: Effect of EEAS on cholesterol

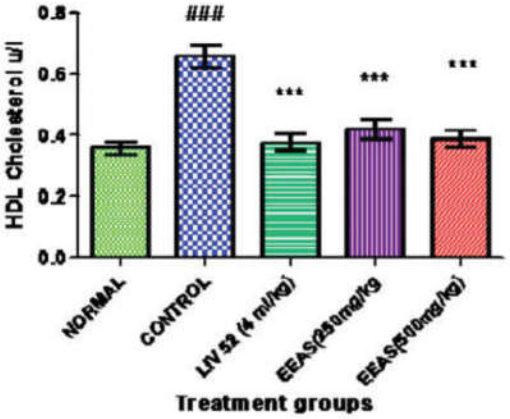

Fig. 8: Effect of EEAS on HDL cholesterol

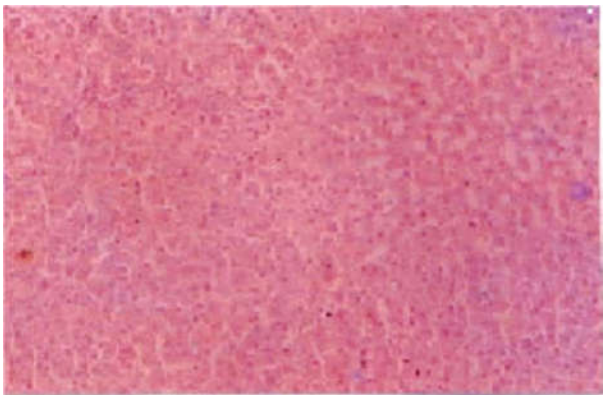

Fig. 9(a): Normal group hepatic cell

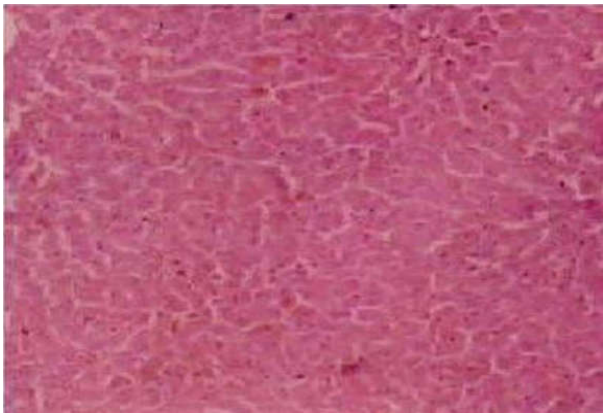

Fig. 9(b): Low dose group hepatic cell

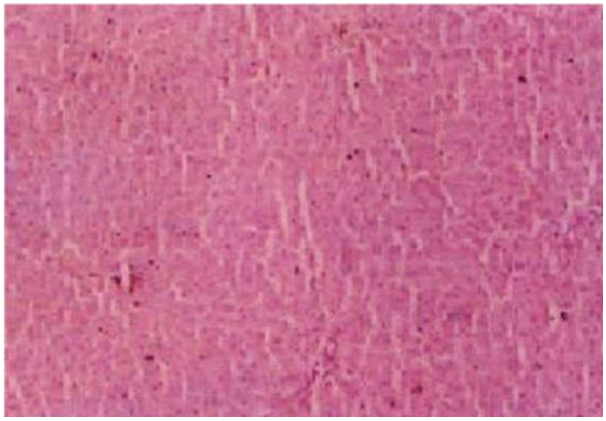

Fig. 9(c): High dose group hepatic cell

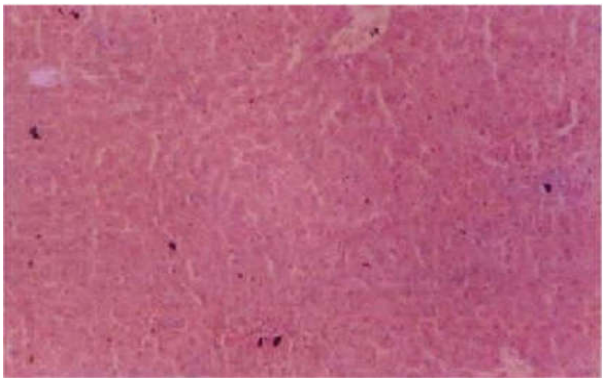

Fig. 9(d): Control group hepatic cell

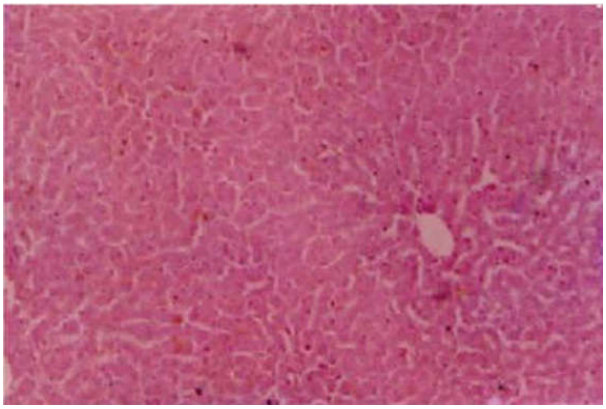

Fig. 9(e): Standard group hepatic cell

Histopathological Observations

Histopathological Findings in the Liver

It was found from the histopathological studies that CCl4-intoxication caused centrilobular hepatocyte necrosis, fatty changes, vacuolization and inflammatory changes. Treatment EEAS either reversed or prevented the changes in these histopathological parameters of the liver indicating that the fractions showed remarkable hepatoprotective activity

- The normal histological liver structure showed in Figure (9a).
- Control group (group II) shows marked inflammatory changes associated with fatty changes are seen in liver sections of CCl_4 treated group II, in Figure 9(e).
- The liver sections of EEAS extract treated group (group IV & Group V) showed periportal lymphocytic and neutrophilic infiltration without any lesions in the hepatocytes. Figure (9b)(9c).
- Lesser degree of inflammation was seen in the LIV-52 treated group when compared with control (Group III). Figure (9d)

All these results indicate a hepatoprotective potential by the ethanol extract of *Allophylus serrates* (EEAS).

Discussion

CCl_4 cause ROS mediated cellular damage especially in liver, the site of metabolism of toxins. During the regular physiological functioning the cells/tissues/organs use oxygen and various nutrients to generate energy. The free radicals are also generated in this process as the reaction intermediates. These free radicals may be very useful because they may promote beneficial oxidative processes. However the higher quantities of such radicals like superoxide anion (O_2^-), NO^- radical, and hydroxyl ion radical (^-OH), NOO^-, etc. may interact with the membrane lipids leading to lipid peroxidation and attack the DNA resulting DNA strand breaks. The lipid peroxidation also damage cell membrane resulting in the leakage of enzymes into the blood stream. Therefore the elevated biochemical levels are treated as biochemical markers of tissue damage. In addition the extent of lipid peroxidation is directly proportional to the tissue damage [4].

There are certain inbuilt protective mechanisms, tissue enzymes GSH, SOD, CAT etc. which are involved in the process of combating free radical induced tissue damage. Over powering the inbuilt protective mechanism due to excessive generation of free radicals may lead to destruction of the tissues/ organs [5].

Antioxidants are the chemical constituents, which are used for inhibiting the tissue damage by countering the free radicals; most of the antioxidants available in the markets are from natural origin e.g. - vit-E, vit-C, tocopherol, quercetine, b-carotene etc. In addition there are reports that polyphenolic compounds like flavonoids are useful as antioxidants and organ protectants. Therefore many researchers are attempting to screen the herbs and herbal preparations containing polyphenolic compounds for organ protective properties.

SGOT is a mitochondrial enzyme released from heart, liver, skeletal muscle and kidney. Liver toxicity elevated the SGOT level in serum due to the damage to the tissues producing acute necrosis, such as severe viral hepatitis & acute cholestasis. Alcoholic liver damage and cirrhosis can also associate with mild to moderate elevation of transaminases [6]. EEAS reversed these enzyme levels indicating stabilization of cell membrane by preventing the damage due to free radicals generated by CCl_4.

SGPT is a cytosolic enzyme primarily present in the liver. The level of SGPT in serum increases due to leakage of this cellular enzyme into plasma by hepatic injury [7]. Serum levels of SGPT can increase due to damage of the tissues producing acute hepatic necrosis, such as viral hepatitis and acute cholestasis. Alcoholic liver damage and cirrhosis also can associate with mild to moderate elevation of transaminase. EEAS effectivey reduced the SGPT levels by preventing the damage of hepatocytes due to free radicals.

In case of liver toxicity, ALP levels are very high, which may be due to defective hepatic excretion or by increased production of ALP by hepatic parenchymal or duct cells [6]. EEAS decreased these enzyme levels indicating stabilization of cell membrane by preventing the damage due to free radicals generated by CCl_4.

A reduction in total serum protein observed in the CCl_4 treated control rats may be associated with the decrease in the number of hepatocytes which in turn might result in decreased hepatic capacity to synthesize protein. when the EEAS was administered along CCl_4 a significant increase in protein content was observed indicating the hepatoprotection of EEAS.

In case of liver toxicity, bilirubin levels are elevated. Hyperbilirubinemia can result from impaired hepatic uptake of unconjugated bilirubin. Such a situation can occur in generalized liver cell injury. CCl_4 interfere with the net uptake of bilirubin by the liver cell and may produce a mild unconjugated hyperbilirubinemia [8]. Bilirubin level rises in diseases of hepatocytes, obstruction to biliary excretion into duodenum, in haemolysis and defects of hepatic uptake and conjugation of bilirubin pigment such as in Gilbert's disease [6]. Significant reversal of elevated bilirubin level in CCl_4 treated animals by EEAS indicated the strong hepatoprotective activity of EEAS.

Cells have a number of mechanisms to protect themselves from the toxic effects of the ROS. SOD removes superoxide (O_2) by converting it to H_2O_2, which can be rapidly converted to water by CAT and Glutathione peroxidase. In addition, a large reserve of reduced glutathione is present in hepatocytes and red blood cells for detoxification of xenobiotics or free radicals. However, oxidative stress results in toxicity when the rate of which the ROS are generated exceeds the cell capacity for their removal. Lipid peroxidation is an autocatalytic process, which is a common consequence of cell death. This process may cause peroxidative tissue damage in inflammation, cancer and toxicity of xenobiotics and aging. MDA is one of the end products in the lipid peroxidation process. In order to elucidate the protection mechanism of EEAS in CCl_4 induced rat liver, lipid peroxide levels and anti-oxidative enzymes activities were analyzed.

GSH is widely distributed in cells. GSH is an intracellular reductant and plays a major role in catalysis, metabolism and transport. It protects cells against free radicals, peroxides and other toxic compounds. GSH is a naturally occurring substance that is abundant in many living creatures. It is well known that a deficiency of GSH within living organisms can lead to tissue disorders and injury. For example, liver injury included by consuming alcohol or by taking drugs like Paracetamol, lung injury by smoking and muscle injury by intense physical activity, all are known to be correlated with low tissue level of GSH.

In our study, elevation in the levels of end products of lipid peroxidation in liver of rat treated with ccl_4 was observed. The increase in MDA level in liver suggests enhanced lipid peroxidation leading to tissue damage and failure of anti-oxidant defense mechanism to prevent formation of excessive free radicals. Treatment with EEAS significantly reversed these changes. Hence it may be possible that the mechanism of hepatoprotection of EEAS is due to its antioxidant effect.

It is well known that there are significant elevations in the levels of serum GPT, GOT and ALP in liver diseases and disorders and in hepatocellular damage caused by a number of agents. An increase in these enzyme levels is also observed with cardiac damage due to myocardial infarction and with liver disorders [9]. Biochemical measurements of these parameters in normal mice treated with ASC showed some extent of increase due to little hepatotoxicity during treatment period but they become normal after completion of treatment schedule. The slight host toxic effects observed in mice during treatment time are mostly reversible. This means that, the treatments of the compound do not cause any acute or permanent damage to the liver. But in case of tumor bearing mice, these parameters were found to be increase more drastically with time due to the acute and permanent toxicities induced by EAC cells on host. After treatment with ASC in the EAC bearing mice these values remain near the normal range in the treated group. From this it follows that the damage generated by EAC was prevented by ASC supplementation.

The development of hypoglycaemia and hyperlipidaemia in experimental animals with carcinoma has been previously reported [10-12]. In this experiment, the reduced glucose level and elevated cholesterol, triglycerides and serum urea were returned to more or less normal levels in ASC-treated mice, thereby indicating a potent antitumour efficacy of ASC.

The histopathology studies of major organs also revealed the relatively less toxic nature of ASC as compared to control group when viewed under microscope. The histopathology of kidney tissues of ASC treated mice did not show any cellular and glomerular infiltration, and there is no sign of tubular necrosis, casts and glomerular congestion. Tissues from brain and lung did not shown any cellular degeneration or regeneration in the treated mice and this is why they have no signs of neurotoxicity and pulmonary toxicity. Treated mice also have not any change in the splenic architecture. The histology of liver showed very little infiltration (inflammation) with no central vein dilation, fatty generation or nodule formation and due to this mild hepatotoxicity some biochemical parameters were deteriorate during treatment period which become normal after closing treatment whereas tissues from EAC bearing mice showed major abnormalities and it is interesting that the hepatic damage induced by EAC cells were nullified by ASC supplementation. All these slight host toxic effects observed in normal mice during

treatment time are mostly reversible and so treatment with ASC do not cause any acute or permanent damage to the host.

The aim of this study was to determine the hepatoprotective effects and sub-acute toxicity of the compound to find out less host toxic potential anticancer agents and did not attempt to identify the specific mechanism involved. This study revealed some interesting features have been presented here. Almost in all cases the effects of EAC cells on biomolecules have been found to be nullified by such treatment. In most cases antagonistic effects have been found instead of additive effects. Further elevation of glucose levels of EAC bearing mice by the treatment of the compound probably indicates their partial recovery from tumour growth.

As the major organs of the treated mice do not show any histopathological abnormalities, these findings in conjunction with those obtained from the measurement of serum biomolecules definitely give positive support to conclude that ASC is an effective antineoplastic agent with comparatively less toxic effects in our experimental model. However, further chronic toxicological studies and its anti-tumor activity should be carried out against other tumor cell lines which may bring promising results in cancer chemotherapy.

Rats treated with carbon tetrachloride showed a significant hepatic damage as observed from elevated serum level of hepatospecific enzymes as well as severe alteration in different liver parameters. It can be concluded that EEAS posses marked hepatoprotective activity with minimal toxicity and thus has a promising role in the treatment of acute hepatic injury induced by hepatotoxins. Further the present investigation provides a scientific base for the use of the *Allophylus Serratus* plant in treatment of jaundice in Indian folklore medicine.

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