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Original Article

Comparison of hepatoprotective activity of *Swertia chirayita* and *Andrographis paniculata* plant of North–East India against CCl_4 induced hepatotoxic rats

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ABSTRACT

Background: The aim of present study was to demonstrate and compare the hepatoprotective activity of ethanol extract of two well-known plants *Swertia chirayita* Buch-Ham and *Andrographis paniculata* (Burm.f.) Nees, in Swiss albino rats.

Method: The hepatotoxicity induced by single dose of CCl_4 dissolved in olive oil (1 ml/kg b.w.; p.o.) while vehicle control given food and water only. Vehicle as well as hepatotoxic rats were divided into groups ($n = 6$). Standard group treated with Silymarin (50 mg/kg b.w.; p.o.) daily for 16 days; and treated group received ethanol extract of plant *A. paniculata* and *S. chirayita* at the dose of 200 mg/kg b.w. p.o. daily for 16 days respectively.

Results: Ethanol extract of plant *S. chirayita* and *A. paniculata*, at a dose of 200 mg/kg body weight exhibited protective lowering effects of the serum enzyme levels SGPT, SGOT, GGTP and SALP to a significant extent. The pronounced activity observed in ethanol extract of *A. Paniculata* with dose of 200 mg/kg (b.w.) however decreases the elevated level of bilirubin, and lipid peroxidase (LPO). The decreased level of TP, GSH, SOD and CAT levels in CCl_4 induced hepatotoxic animal were significantly increase on treatment with ethanol extract of *A. Paniculata* and *S. chirayita* plant. The histopathological studies of liver were also supported hepatoprotective activity of *A. paniculata*.

Conclusion: Since results of biochemical studies conclude that the ethanol extract of *A. Paniculata* showed significant better hepatoprotective as compare to *S. chirayita*.

1. Introduction

Liver is one of the largest organ play vital roles in human body and liver diseases are some of the fatal disease in the world today. A healthy liver is a crucial factor for overall health and well-being because liver involves in metabolism, secretion, storage and excretion. Any injury to liver can result in many disorders ranging from transient elevation in liver enzyme to life threatening liver cirrhosis and hepatic failure. The common causative agents of liver injuries are alcohol, poor drug habits,

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over-the-counter drugs, toxic chemicals (e.g. CCl_4, aflatoxin etc.), therapeutic drugs (e.g. Antibiotics, anti-tubercular drugs etc.) and microbial agents (e.g. hepatic virus, leptospira, malarial parasites) which can eventually lead to various liver ailments like hepatitis, cirrhosis and alcoholic liver disease. So liver has a surprising role to play in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction. The modern medicines have little to offer for alleviation of hepatic diseases but there is not much drug available for the treatment of liver disorders.[1]

The plant *Swertia chirayita* Buch-Ham (Gentianaceae) is one of the oldest herbal medicines used against bronchial asthma and liver disorders from ancient time in western India. It has been widely used in Ayurvedic and Unani medicine system as an anthelmintic, febrifuge and stomach and protective liver tonic.[2,3] The herb containing amarogentin (most bitter compound isolated till date) as main chemical constituent attributed anthelmintic, hypoglycemic and antipyretic properties. Swerchirin a compound with xanthone structure has hypoglycaemic, hepatoprotective activity[4,5] and the xanthone content of *Swertia* is mostly responsible for its hepatoprotective activity.[6]

Andrographis paniculata (Burm. f.) Nees, (family: Acanthaceae) commonly and locally known as "Kalmegh" is an important traditional medicinal plant, occurring wild in different region of India, and is used both in Ayurveda and Unani system of medicine.[7] It is also known as "King of Bitters", and is a member of ancient medicinal herb with an extensive ethnobotanical history in Asia. Modern pharmacological studies indicate that active compound andrographolide are very bitter diterpene lactones protects the liver and gallbladder, and has been found to be slightly more active than Silymarin, a known hepatoprotective drug[8] Neo-andrographolide shows greater activity against malaria[9] while 14-deoxy andrographolide produced a more potent hypotensive effect in anaesthetized rats. The plant having a number of activities studied by different researchers such as, hepatoprotection, vermicidal, antiacne, analgesic, anti-inflammatory, antibacterial, antityphoid, antibiotic activities, hypoglycemic, besides immune enhancement mainly due to andrographolide and related diterpens chemical present in plant.[7,10,11]

In recent years, the usages of herbal drugs for the treatment of liver disease have increased all over the world. The herbal drugs are harmless and free from serious adverse reaction and are easily available. The limited therapeutic options and disappointing therapeutic success of modern medicine has increased the usage of alternative medicine including herbal preparations. The present study carried with the objective of evaluation and comparison of hepatoprotective activities of these two well-known medicinal plants.

2. Materials and methods

2.1. Plant materials and preparation of extracts

The whole fresh plants materials of *A. paniculata* (Burm.f.) Nees, (AP) and *S. chirayita* Buch-Ham (SC) were collected from Guwahati in month of Sep.–Oct. The botanical identification of the plant material was confirmed by the Taxonomist Dr. B. K. Sinha (Scientist E-HOD) Botanical Survey of India, Shillong. A voucher specimen (DPSD-04) was deposited in the herbarium of Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh, Assam.

The dried plant materials were pulverized into coarse powder in a grinding machine. The powder plant materials were successive solvent extracted separately in petroleum ether, ethyl acetate and ethanol. The ethanol solvent filtered, squeezed off and evaporated off under reduced pressure in a rotary evaporator to obtain crude extract was used for animal testing.

2.2. Animals

Male albino Wistar rats weighing 150–200 g were used in this evaluation. These rats aged between 2.5 and 3 months were procured from PBRI Bhopal. They were kept in polypropylene cages, under controlled temperature ($24 \pm 2\ ^{\circ}C$), humidity and 12/12 h light/dark cycles. The animals were fed standard diet (golden feed, New Delhi) and water given *ad libitum*. These animal experiments were approved by Institutional Animal Ethics Committee (IAEC) of Pinnacle Biomedical Research Institute (PBRI) Bhopal (Reg No.-1283/c/09/CPCSEA). Protocol Approval Reference No. PBRI/IAEC/11/PN-120.

2.3. Acute toxicity

The oral toxicity was performed according to OECD 423 guideline. All animals were given extract by oral route, and for next 3 h animals were observed for mortality and behavioral changes. Animals were observed for next 48 h for any mortality. Acute oral toxicity of both plants extracts *A. paniculata* and *S. chirayita* in female albino Wistar rat was determined as per reported method.[12]

2.4. Experimental design for hepatoprotective activity

The rats divided randomly into six groups of six rats each. The hepatoprotective activity of the plant extracts tested using CCl_4 model. All animal groups except vehicle control group received carbon tetrachloride (CCl_4) 50% v/v in olive oil at a dose of 0.1 ml/kg body weight intra peritoneal (i.p.) for 16 day. Group I vehicle control received food and water only and plain olive oil orally; Group II CCl_4 toxic control was received CCl_4 dissolved in olive oil at a dose of 0.1 ml/kg b.w. i.p. for 16 days. Group III was standard drug received Silymarin (50 mg/kg b.w.; p.o.) daily for 16 days; Group IV and V was administered orally ethanol extract of plant *A. paniculata* and *S. chirayita* at the dose of 200 mg/kg b.w. orally daily for 16 days respectively. Vehicle, extract and standard drug administered 1 h before CCl_4 administration. After 24 h of last dose, blood collected from overnight fasted rats of each group by cardiac puncture, for estimation of serum biochemical parameters. Then the rats sacrificed after 24 h after induction by cervical dislocation for the study of liver biochemical and histopathological parameters.

2.5. Assessment of hepatoprotective activity

After 24 h of last dose the animals were dissected under ether anesthesia. Blood was collected from overnight fasted rats of

each group by cardiac puncture and collected in previously labeled centrifuging tube stand and allowed to clot for 30 min at room temperature. Serum was separated by centrifugation at 3000 rpm for 15 min. The separated serum was used for the estimation of some biochemical parameters, 10% liver portion was homogenate and used for liver biochemical evaluation.

2.5.1. *Serum biochemical estimation*

Serum was analyzed for various serum biochemical parameters i.e. serum glutamine oxaloacetate transaminase (SGOT or AST), serum glutamine pyruvate transaminase (SGPT or ALT),[13] serum alkaline phosphatase (SALP),[14] serum total bilirubin (TB),[15] γ-glutamate transpeptidase (GGTP)[16] and total protein (TP)[17] content using reported method with the help of commercially available kits (SPAN Diagnostics).

2.5.2. *Liver biochemical estimation*

The homogenate portions of liver used for the estimation of various biochemical parameters like level of lipid peoxidation (LPO)[18] and expressed as nM/mg protein of liver tissue. The reduced glutathione (GSH) content of liver tissue was determined as per reported method[19] and expressed as mM/gm of liver tissue. The catalase (CAT) activities in liver tissue were assayed as per the methods described[20] and expressed in terms of U/mg protein of liver tissue. The superoxide dismutases (SOD)[21] level also estimated according to the prescribed methods.

2.5.3. *Histopathological study*

In histopathological study, liver from each animal removed after dissection and preserved immediately in 10% formalin, dehydrated in ethanol (50–100%). Then representative blocks of liver tissues from each lobe taken and processes for paraffin embedding using the standard microtechnique. Sections (5 μm) of livers stained with hematoxylin and alcoholic eosin dye for photo-microscopic observation for histopathological studies.

3. Statistical analysis

All results were expressed as the mean ± standard error of mean (SEM). The results were analyzed for statistical significance One-way Analysis of Variance (ANOVA) followed by Dunnett's *post hoc* multiple comparison tests using Graph Pad Prism software, $P < 0.01$ was considered as statistically significant.

4. Results

4.1. *Acute toxicity*

The extracts were found non-toxic up to the dose of 2000 mg/kg b.w. Neither mortality nor any significant behavioral changes were observed, thus 2000 mg/kg was considered as NOAEL and 1/10th of these doses is oral LD_{50} in both *A. paniculata* and *S. chirayita* plant was 200 mg/kg b.w.

4.2. *Assessment of hepatoprotective activity*

4.2.1. *Serum biochemical estimation*

Biochemical parameter like SGOT, SGPT, SALP, γ-glutamate transpeptidase (GGTP) and serum bilirubin were significantly ($P < 0.01$) elevated and total protein (TP) level decreased in CCl_4 treated group as compare to vehicle control group indicating liver damage. Treatment with ethanol extract of plant *A. paniculata* and *S. chirayita* at the dose of 200 mg/kg b.w. significantly ($P < 0.01$) reduced the SGOT, SGPT, SALP, γ-glutamate transpeptidase (GGTP). The bilirubin levels towards the normal values and increase in total protein (TP) level however the liver weight of the animals of CCl_4 treated and plant extract treated groups also supports the extract activity. *A. paniculata* showed the more significant effect to reduce the SGOT, SGPT, SALP, γ-glutamate transpeptidase and bilirubin levels (Tables 1 and 2).

4.2.2. *Liver biochemical estimation*

Analysis of LPO levels was significant ($P < 0.01$) increased in CCl_4 treated animal. On treatment with ethanol extract of plant *A. paniculata* and *S. chirayita* 200 mg/kg b.w. dose significantly ($P < 0.01$) reduced the LPO levels as compare to CCl_4 treated as well as normal animal. The level of reduced GSH was significantly depleted in CCl_4 treated animal group. GSH level was found to be significantly elevated towards normal level on administration of *A. paniculata* and *S. chirayita* 200 mg/kg b.w. (Table 3). There were significant reduction in superoxide dismutase (SOD) and catalase (CAT) activities in CCl_4 treated animal group and after treatment with ethanol extract of *A. paniculata* and *S. chirayita* (200 mg/kg b.w.), significantly ($P < 0.01$) elevated SOD and CAT activities

Table 1 – Effects of ethanol extract of *A. paniculata* and *S. chirayita* plant materials on various serum biochemical parameters in rats with carbon tetrachloride induced hepatotoxicity.

Animal groups	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)	Liver wt./100 g body weight (g)
I-Vehical control	34.35 ± 1.31^{b}	47.5 ± 1.66^{b}	11.5 ± 1.64^{b}	4.44 ± 0.17^{b}
II-Standard Drug (Silymarin)	42.58 ± 1.65^{b}	52.16 ± 1.24^{b}	$11.16 \pm 1.04^{a,b}$	4.80 ± 0.11^{b}
III-CCl_4 control	127.5 ± 2.32^{a}	129 ± 2.12^{a}	41.33 ± 1.92^{a}	6.18 ± 0.19^{a}
IV- ETE-AP (200 mg/kg)	$63.16 \pm 3.30^{a,b}$	$70.66 \pm 1.52^{a,b}$	$20.66 \pm 1.94^{a,b}$	$5.30 \pm 0.12^{a,b}$
V- ETE-SC (200 mg/kg)	$59.96 \pm 2.69^{a,b}$	$69.83 \pm 3.38^{a,b}$	$20.00 \pm 2.03^{a,b}$	$5.16 \pm 0.03^{a,b}$

Values are expressed as mean ± SEM ($n = 6$); $^{a}P < 0.01$, vs Vehicle control group; $^{b}P < 0.01$, vs CCl_4 Control group.

Table 2 – Effects of ethanol extract of *A. paniculata* and *S. chirayita* plant materials on various serum biochemical parameters in rats with carbon tetrachloride induced hepatotoxicity.

Animal groups	Total bilirubin (mg/dL)	Total protein (mg/dL)	GGTP (u/L)
I-Vehical control	0.35 ± 0.06^{b}	7.93 ± 0.04^{b}	77.90 ± 0.32^{b}
II-Standard Drug (Silymarin)	$0.48 \pm 0.05^{a,b}$	7.48 ± 0.11^{b}	$95.53 \pm 0.2^{a,b}$
III-CCl_4 control	5.83 ± 0.26^{a}	5.60 ± 0.11^{a}	168.43 ± 0.26^{a}
IV- ETE-AP (200 mg/kg)	0.53 ± 0.01^{b}	$6.55 \pm 0.08^{a,b}$	$95.85 \pm 0.46^{a,b}$
V- ETE-SC (200 mg/kg)	0.511 ± 0.02^{b}	$6.28 \pm 0.11^{a,b}$	$97.83 \pm 0.18^{a,b}$

Values are expressed as mean ± SEM ($n = 6$); $^{a}P < 0.01$, vs Vehicle control group; $^{b}P < 0.01$, vs CCl_4 Control group.

towards normal values were observed as compared to CCl_4 treated animal group as well as vehicle control group.

4.2.3. *Histopathological study*

Results of histopathological studies provided supportive evidence for biochemical analysis. Histology of liver section of normal animal group exhibited normal hepatic cells each with well defined cytoplasm, prominent nucleus, and nucleolus and well brought out central vein (Fig. 1a), whereas that of CCl_4 intoxicated group animal showed presence of normal hepatic cords and total loss of hepatic architecture with centrilobular hepatic necrosis, fatty changes, vacuolization and congestion of sinusoids, Kupffer cell hyperplasia, crowding of central vein and apoptosis (Fig. 1b). Treatment with standard drug Silymarin 50 mg/kg and ethanol extract of *A. paniculata* and *S. chirayita* (200 mg/kg b.w.) showed potential activity in protecting the liver cells from CCl_4-injury (Fig. 1c–e). Among these, two-plant extract, treatment with *A. paniculata* ethanol extract returned the injured liver to quite normal and thus shown very potential hepatoprotective activity.

5. Discussion

Liver damage induced by CCl_4 is routinely used model for the screening of hepatoprotective drugs. CCl_4 administration causes the acute liver damage mediated changes in liver function that ultimately leads to destruction of hepatocellular membrane. The CCl_4 treatment forms various free radicals, which are activated by the cytochrome P-450 and involved in the pathogenesis of liver damage in chain reaction. This pathogenesis of liver damage arises so many complications like destruction of structures of the endoplasmic reticulum and other membrane, loss of metabolic enzyme activation, reduction of protein synthesis. The loss of glucose-6-phosphatase activation, decreasing level of phospholipids, increasing triglyceride levels, inhibition of calcium pumps of microsomes, covalent binding of macromolecules and disruption of metabolic mechanisms in mitochondria thus leading to necrosis of liver.[22,23]

The acute toxicity study expressed the absence of lethality among the tested animals upon administration of the ethanolic extract both plant as single dose (200 mg/kg). There were no any signs and symptoms of any behavioral changes observed except an increase in urination which decided the safe use of the plant extract.

When rats were treated with CCl_4 it induces hepatotoxicity by metabolic activation, therefore, it selectively causes toxicity in liver cells maintaining semi-normal metabolic function. The liver specific enzymes are the having very sensitive and reliable indices for the necessary hepatotoxic as well as hepatoprotective or curative effects of various compounds. The rise in serum levels of SGOT and SGPT attributed to the damaged structural integrity of the liver, because they are cytoplasmic in location and released into circulation after cellular damages.[24]

The amino transferases contribute a group of enzyme that catalyse the interconversion of amino acids and α-keto acids by the transfer amino groups. Both the enzyme SGOT and SGPT levels increase with the CCl_4 treatment and after treated with *A. paniculata* and *S. chirayita* plant ethanol extract the elevated level were altered which indicates the protective action of plant extract. The enzyme alkaline phosphate (ALP) reaches the liver mainly from the bone. ALP is a membrane bound glycoprotein enzyme with high concentration in sinusoid and endothelium. It is excreted into the bile; on treatment with CCl_4, elevation of serum ALP level due to hepatobiliary disorder. The ALP related to the functioning of hepatocytes and increase in its activity is due to the increased synthesis in presence of biliary pressure. In the present study the treatment with ethanol extract reduce the level of ALP in treated animals. Thus on treatment with extract, probably it stabilizes the hepatic plasma membrane, which is evident of recovery (Table 1).[25]

Table 3 – Effects of ethanol extract of *A. paniculata* and *S. chirayita* plant materials on various liver biochemical parameters in rats with carbon tetrachloride induced hepatotoxicity.

Animal groups	LPO (nM/mg protein)	GSH (mM/gm tissue wt)	Catalase (U/mg protein)	SOD (% inhibition of NBT)
I-Vehical control	2.39 ± 0.20^{b}	18.73 ± 0.4^{b}	34.50 ± 0.41^{b}	71.14 ± 1.49^{b}
II-Standard Drug (Silymarin)	$2.33 \pm 0.16^{a,b}$	18.26 ± 0.34	$33.51 \pm 0.63^{a,b}$	$66.50 \pm 0.83^{a,b}$
III-CCl_4 control	6.82 ± 0.16^{a}	7.01 ± 0.32^{a}	13.83 ± 0.34^{a}	24.08 ± 0.87^{a}
IV- ETE-AP (200 mg/kg)	2.74 ± 0.15^{b}	17.47 ± 0.32^{b}	$28.15 \pm 0.34^{a,b}$	$61.07 \pm 0.83^{a,b}$
V- ETE-SC (200 mg/kg)	2.50 ± 1.15^{b}	17.66 ± 0.62^{b}	$31.98 \pm 0.33^{a,b}$	$63.83 \pm 0.83^{a,b}$

Values are expressed as mean ± SEM ($n = 6$); $^{a}P < 0.01$, vs Vehicle control group; $^{b}P < 0.01$, vs CCl_4 Control group.

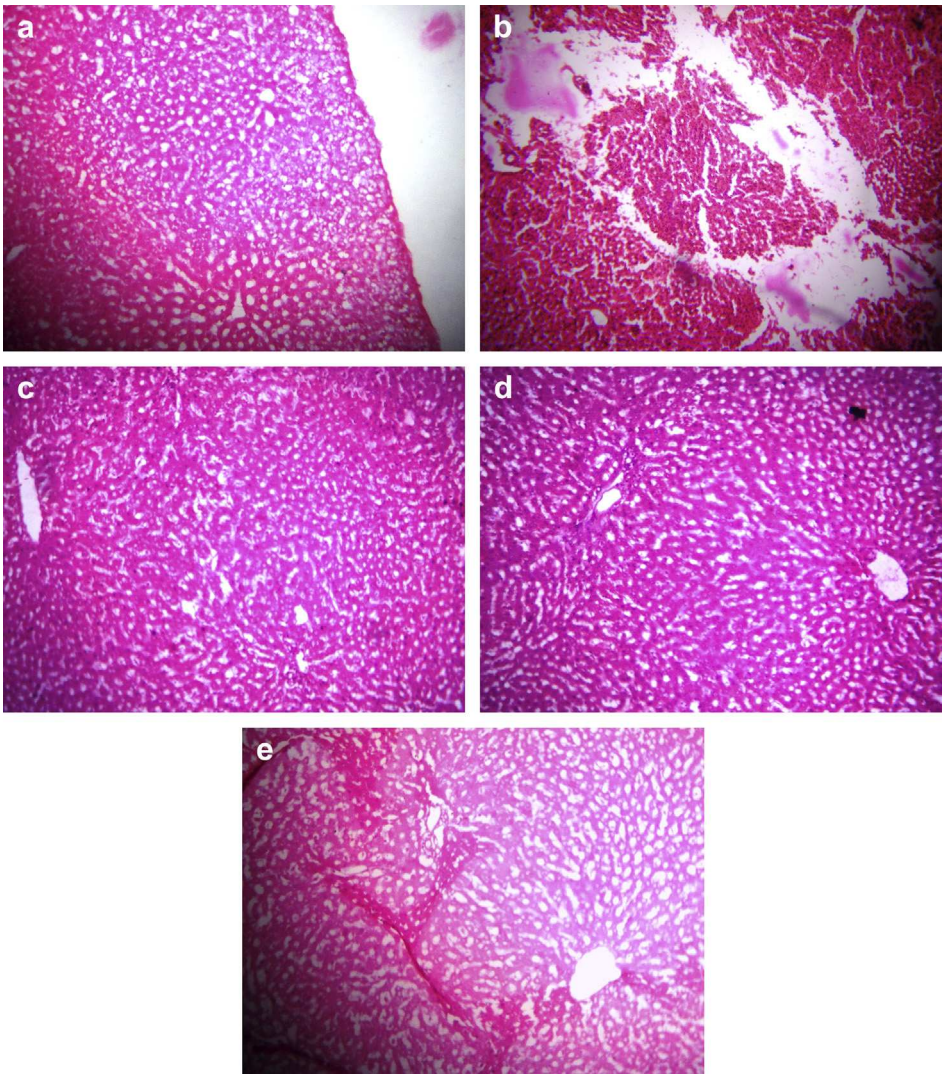

Fig. 1 – a. Microscopic view of liver tissue of vehicle control rat b. Microscopic view of liver tissue of CCl_4 induced control rat c. Microscopic view of liver tissue of standard drug Silymarin treated rat d. Microscopic view of liver tissue of ethanol extract of *A. paniculata* treated rat e. Microscopic view of liver tissue of methanol extract of *S. chirayita* treated rat.

Serum bilirubin levels and γ-glutamate transpeptidase (GGTP) levels also have specific marker of functional status of hepatic cell. The CCl_4 induced hepatotoxicity increases the serum enzyme γ-glutamate transpeptidase (GGTPT) and bilirubin levels.[26] Treatment with both *A. paniculata* and *S. chirayita* ethanol extract reduces the level, which indicates preservation of structural and functional integrity of the hepatocellular membrane in rats. The reduction in the total protein (TP) is attributed to the initial damage produced and localizes in the endoplasmic reticulum which results in the loss of cytochrome-450 enzyme indicating the functional failure of protein synthesis and accumulation of triglycerides leading to fatty liver.[27] Treatment with both *A. paniculata* and *S. chirayita* plant extract enhances the total protein level accelerate the regeneration and protection of liver cells that is clearly demonstrated in Table 2, and the increase level of total protein in serum indicates the hepatoprotective activity of plants.

Glutathione (GSH) is the endogenous non-enzymatic antioxidant in our body system and Lipid peroxidase (LPO) responsible for the oxidative stress and it is protective against chemically induced hepatotoxic condition and oxidative stress.[28] Lipid peroxidation is a process involved in peroxidative loss at unsaturated lipids, causing cellular lipid degradation and disordering membrane. Elevated lipid peroxidation causes tissue injury and damage macromolecules of cell by generation of reactive oxygen species (ROS), which increase the risk of tissue damage. CCl_4 treatment induced lipid peroxidation in rats indicates that the dose of CCl_4 produced highly hepatotoxic. The level of GSH decrease and the LPO increase on treatment with CCl_4 treatment. Animals treated with plant extract significantly restore the hepatic GSH and LPO content toward normal level and present work support Janero et al, work.[29]

Superoxide dismutase (SOD) and catalase (CAT) is endogenous enzyme present in all oxygen metabolizing cells and

antioxidants properties involved in the clearing of superoxide and hydrogen peroxide. The suppression of SOD and CAT activities as an indication of liver damage on CCl_4 treated animal groups and present study support Duairaj et al, work.[30] On the administration of ethanol extracts of plants significantly overcome the Superoxide dismutase (SOD) and catalase (CAT) activities towards normal when compared to CCl_4 and normal animal groups (Table 3).

The histopathological examinations of all groups along with the level of different biochemical marker and serum parameter in circulation were assessing by the hepatic leakage and restoration of hepatic cells. The animal treated with CCl_4 induce hepatic toxicity which evidenced by cellular necrosis, ballooning degeneration, nodal formation, profound steatosis and fibrosis as compared to normal hepatic architecture of normal animal group, which are clearly shown in Fig. 1a & b. On treating with *A. paniculata* and *S. chirayita* extract the animal showed recovery of damaged parenchyma, which was comparable to that of the standard drug Silymarin treated animal group (Fig. 1c–e)

The hepatoprotective drug efficacy can be due to either restoring the normal hepatic physiology or reducing the harmful effect, which has been disturbed by hepatotoxic agent. The *A. paniculata* and *S. chirayita* extract treated group and standard drug Silymarin treated animal group, restore the CCl_4 induced serum enzyme levels that indicates the protection of structural integrity of hepatic cell membrane or damaged liver cell regeneration.

6. Conclusion

The above study suggested that the oral administration of *A. paniculata* and *S. chirayita* plant ethanol extracts having good hepatoprotective properties however, it also prevent lipid peroxidation and arrest free radicals. On study of several parameters, it can conclude that *A. paniculata* plant having the better hepatoprotective activity than the *S. chirayita* plant.

Conflicts of interest

All authors have none to declare.

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