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EVALUATION OF HEPATOPROTECTIVE ACTIVITY WITH LEAF EXTRACT OF *ALANGIUM SALVIFOLIUM WANG* ON CCl_4 INDUCED RATS

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Abstract

Aim

Present study was aimed to Evaluating Hepatoprotective Activity with Leaf Extract of *AlangiumSalvifolium Wang* (Alangiaceae) on CCl_4 induced Rats. Traditionally this plant used in various types of liver disorders and also used as antidiabetic, anticancer, diuretic, cardiotonic, anti-inflammatory, antimicrobial, Laxative, epilepsy, antidote for poisonings, burning sensation, constipation and haemorrhages.

Materials and methods

The leaves of *Alangiumsalvifolium*collected, prepared as ethanolic extracts of *Alangiumsalvifolium*(ASEE),Silymarin, Ethanol, CCl_4, CMC,Olive oil, analytical kits fromstandard companies**.** Albino wistarrats(150-180 g) from TEENA Labs.

Acute toxicity study was conducted in animals to identifying of LD50 value. Two test doses were selected on the basis of LD50 and conducted Hepatoprotective activity by using CCl_4 induced method. The collected blood samples were subjected to various biochemical parameters (SGPT, SGOT, ALP, TB, ALB, TP and CHOL). Dissected the liver for histopathological studies.

Results

Hepatoprotective activity studies, the toxicgroup (CCl_4) shows elevated levels of SGPT, SGOT, ALP, CHOL and TBL where asdecreased TP and ALB levels when compared withthe control group. The pretreatment of ASEE at a dose of 150 and 300 mg/kg exhibited reduction in the serum levels of SGPT($P<0.001$), SGOT($P<0.001$), ALP($P<0.01$),

CHOL($P<0.01$), TB($P<0.001$), and elevated the TP($P<0.01$), ALB($P<0.001$) levels when compare to toxic.ASEE of 300 mg/kg showed results similar to standard results.Histopathologicalstudies reveled that of ASEE exhibited a remarkable recovery as Silymarin does.The results of this study strongly indicate that leaves of *AlangiumSalvifolium*have potent hepatoprotective against CCl_4.

Key Words: *AlangiumSalvifolium,* ethanol, CCl_4, Silymarin.

1. Introduction

Liver is the largest glandular organ of the body, it performs more than 500 metabolic functions, to regulates various physiological processes of internal chemical environment in the body. Liver injury mainly caused by toxic chemicals, excess consumption of alcohol, infections and autoimmune disorders. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages[1]. Now the present generation a number of medicinal preparations are available for the treatment liver disorders, but there are no effective drugs available that stimulate liver function or help to regenerate hepatic cells. In the absence of reliable liver protective drugs in modern medicine there are a number of medicinal preparations in Ayurveda recommended for the treatment of liver disorders and their usage is in vogue since centuries[2]. In India, of the 17,000 species of higher plants, 7500 are known for medicinal uses. The Charkasamhita, an age-old written document on herbal therapy, reports on the production of 340 herbal drugs and their indigenous uses. Currently, approximately 25% of drugs are derived from plants.

In the present study was undertaken to verify the claim and *evaluated for its possible hepatoprotective activities of the plant of* Alangiumsalvifolium *in order to provide scientific evidence for its traditional use in the treatment of Hepatitis* by preparing its various organic extracts.

*Alangiumsalvifolium*Wang (Alangiaceae) is the most widespread species, ranging from Africa to Australia, Fiji, New caledonia and widely cultivated, used in India, China and Phillipines[3,4]. The plant commonly known as Ankola (Sankrit), Uduga (Telugu), Dera (Hindi)[5]. *Alangiumsalvifolium*is a small tree, with more or less spinescent branches. Leaves 7.6-15.2 cm long, narrowly oblong or ovate-lanceolate, glabrous. Flowers –Yellowish white, fragrant, in axillary fascicles. Fruits 1-2 seeded,1cm in length small, nearly globular, purplish-red when ripe, crowened by persistant calyx-limb[6,23].

Traditionally all parts of the plant are used in various types of disorders like in the treatment of liver disorder, Chemically it contains carbohydrates, steroids, glycosides, flavanoids, tannins.The seeds have been reported to exhibit a variety of biological activities, including antidiabetic, anticancer, diuretic, antifertility[7], cardiotonic[8], anti-inflammatory[9], antimicrobia[l0], laxative, and antiepileptic activity[11]. The Root bark is an antidote for several poisons. Fruits are sweet, used to treat burning sensation, constipation and haemorrhage[12].The leaves are used as poultice in rheumatism[13]. The stem barks exerts a biphasic action on the blood pressure in cats at lower doses and marked hypotension in higher doses.

2. Materials and Methods

2.1 Plant materials

*Alangiumsalvifolium*leavescollected from local areas of Peddapalli in the month of March 2013, and it was Authenticated in Department of Botany, Kakatiya University, Warangal. and a specimen voucher was deposited for future reference.

2.2 Preparation of extracts

Alangiumsalvifolium Linn Leaves were made free from foreign material and air dried and coarsely powdered. Prepared a thimble, 200gm of powder, and placed into a Soxhlet apparatus containing the solvent ethanol. The Soxhlet is then equipped with condenser at 37^{o}C temp. The obtained extracts were kept in desiccators to remove moisture and stored properly until used.

2.3 Preliminary Phytochemical Investigation

The extract was subjected to qualitative chemical investigations for identification of different phytoconstituents like sterols,glycosides, saponins, carbohydrates, alkaloids, flavonoids, tannins, proteins and tri-terpenoids[14].

2.4 Animals

Female albino Wistarrats weighing 150-180 gm were purchased from Animal sciences, TEENA Labs, HYD. (Regn.No1533/PO/a/11/CPCSEA.), Hyderabad and maintained in the animal house. Animals were provided with standard rodent pellet diet and the food was withdrawn 18-24 hrs before the experiment, water was allowed *ad libutum*. They were maintained at standarad laboratory conditions(27 ± 2 ^{0}C) 12 hrs light- dark cycle throughout the period of acclimatization and experimentation.

2.5 Acute Toxicity Studies

Healthy Wistar albino mice of 20-30 g either sex were divided into ten groups of six animals each. Acute toxicity study was carried out according to the method described in the literature[15,16].The ethanolic extracts ofAlangiumsalvifolium (ASEE)were suspended in 0.5% of carboxy methyl cellulose in doses of 100, 200, 400, 800, 1000, 1200, 1400, 1800 and 2000 mg/kg were administered orally to albino mice. The animals were observedcontinuously for any change in autonomic or behavioral responses for first few hours and later at 24 hrs intervals for a period of 48 hrs. At the end of this period, the mortality rates in all groups were noted.Mortality was noticed in the dose of 2000 mg/kg. The LD50 of the extracts was found to be 150 mg/kg body weight. One-tenth of this dose was selected as the therapeutic dose for the evaluation

2.6 Hepatoprotective Studies:

In the present study, the animals were pretreated with test extracts before inducing liver damage with CCl_4. Seven days after acclimatization, the rats were divided into five groups (I-V), each group consisting of six animals. All animals were kept on same diet for 7 days.

Group-I served as a normal and received 1ml/kg of 0.5 %w/v Carboxy methyl cellulose in water p.o. for seven days.

Group-II Treated with vehicle (1 ml/kg of 0.5% w/v Carboxy methyl cellulose in water p.o.) daily for seven days followed by CCl_4 on the seventh day.

Group-III (standard-Silymarin) animals were administered with 50 mg/kg of Silymarinp.o. for seven days followed by CCl_4 administration p.o.

Groups IV-V test groups were treated in the similar way using ethanolic extract of Alangiumsalvifolium(ASEE) 150, 300 mg/kg respectively followed by CCl_4 administered p.o on the seventh day.

After 24hrs following CCl_4 administration, the blood was collected from the retro-orbital plexus under ether anaesthesia. After blood collection, the blood samples were allowed to coagulate at room temperature for at least one hour. Serum was separated by centrifugation at 3000 rpm for 30 minutes and then analyzed of serum was separated and used for determination of SGPT, SGOT,ALP, TB, ALB,TP and CHOL levels were estimated by their specific methods. Then animals were then dissected and the livers were carefully removed and washed with 0.9% saline solution and preserved in formalin solution (10% formaldehyde) for histopathological studies.

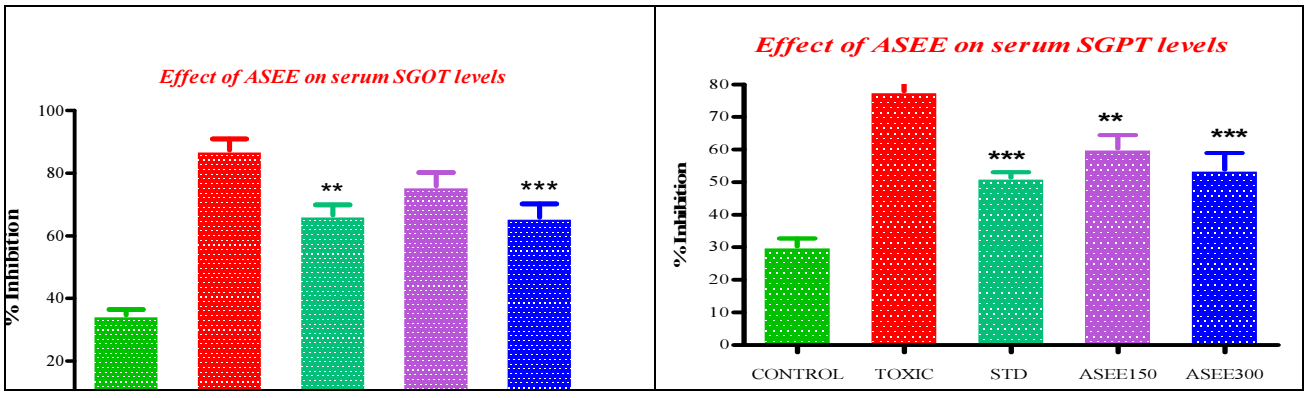
2.7 Histopathological examination: For histopathological studies the liver sections were prepared 3-5 mm thick, stained with alum hematoxylin and eosin (Okuno et al., 1986) and examined microscopically for histopathological changes[15].

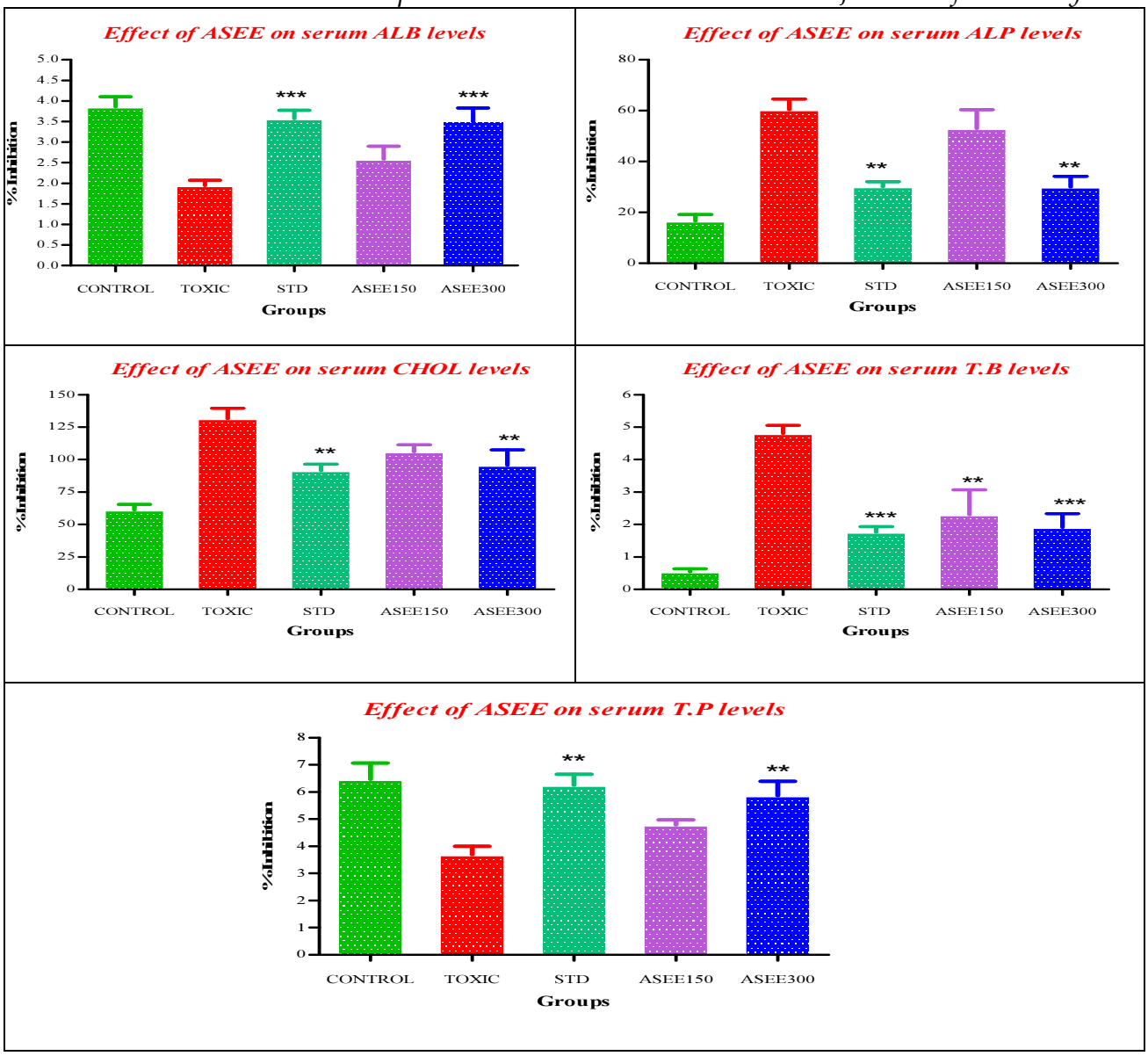
2.8 Statistical analysis: Values are expressed in Mean±S.E.M. for six animals in each group and statistically assessed by one-way analysis of variance (ANOVA) and subjected to Dunnett's test. The $P<0.05$ was considered significant.

3. Results

The preliminary phytochemical analysis of ethanolic extract of Alangiumsalvifolium shows the presence of Flavonoids, Glycosides, Steroids, Tannins and Terpenoids. The data mentioned in Table-1.The Biochemical Parameters of the hepatoprotective studies are given in Table-2 and Fig-1. From the above resultsCCl_4i.e., The administration of CCl4 induced acute liver damage which was well indicated by increased SGPT, SGOT, ALP , CHOL and TBL when compared with the control group. As well as those the animals receive CCl4 cause to decrease in the total protein and albumin levels. The pretreatment of ASEE at a dose of 150 and 300 mg/kg exhibited reduction in the serum levels of SGPT($P<0.001$), SGOT ($P<0.001$), ALP ($P<0.01$), CHOL ($P<0.01$) and TB ($P<0.001$). The TP ($P<0.01$) and ALB ($P<0.001$) levels were also increased and statistically significant when compared to the toxic groups.The increase in dose levels of Alangiumsalvifolium had exhibited an increase in efficacy which was reflected in the value of biochemical parameters

Figure: 1 Column diagrammatic representation of Effect of ASEE Hepatoprotective on different biochemical parameters in CCl_4 induced liver damage in rats.





Significant*(P <0.05), ** (P<0.01), *** (P<0.001) compared with standard and toxic group

The administration of CCl4 to the animals resulted in a marked increase in total bilirubin, serum amino transaminases (SGPT and SGOT) cholesterol and serum alkaline phosphatase activities. However, the serum total protein level and albumin was decreased. The toxic effect of CCl4 was controlled in the animals treated with the ethanolic extract by the way of restoration of the levels of the liver function biochemistry similar to that of the standard drug silymarin (Table 1). Among the extract treated groups, significant hepatoprotective activity, was observed in those treated with ethanol extract. Histological profile mentioned in fig-2, Group I control animals showed normal hepatocytes. Group II animals liver section showed centrilobular necrosis, Haemmahroages and Inflammatory cells and macrovesicular fatty change . Group III animals treated with standard drug silymarin 50 mg/kg, its liver section showed significantly normal architecture reduced necrotic areas, *which was similar* to that of control. Group IV animals treated with

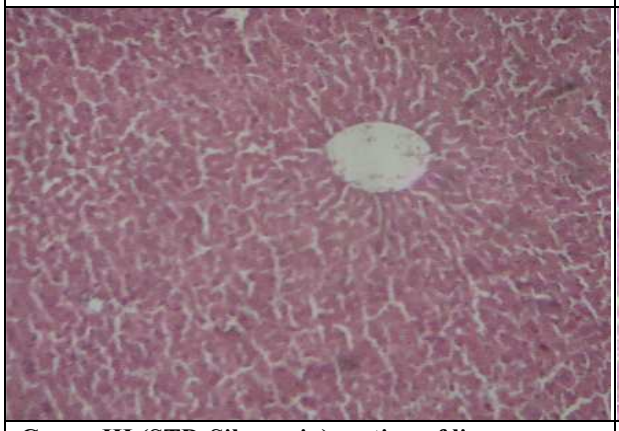
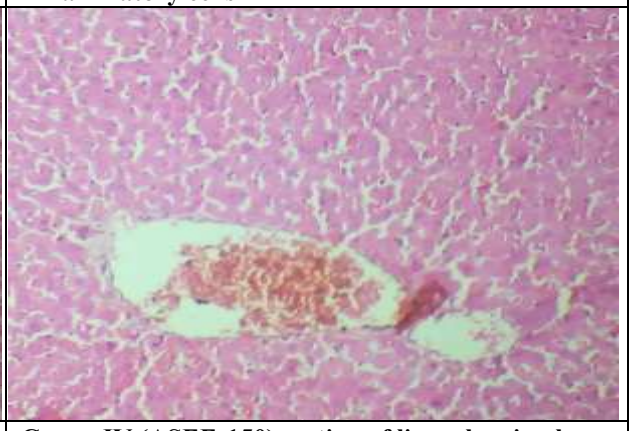
ethanolic extract of *Alangium salvifolium*(ASEE) 150 mg/kg exhibited significant liver protection against the toxicant by showed less reduced Haemmahroages, absence of necrotic areas and normal hepatic cords. As well as Group V animals treated with ethanolic extract of Alangium salvifolium(ASEE) 300 mg/kg exhibited normal architecture and reduced necrotic and moderate accumulation of fatty lobules .

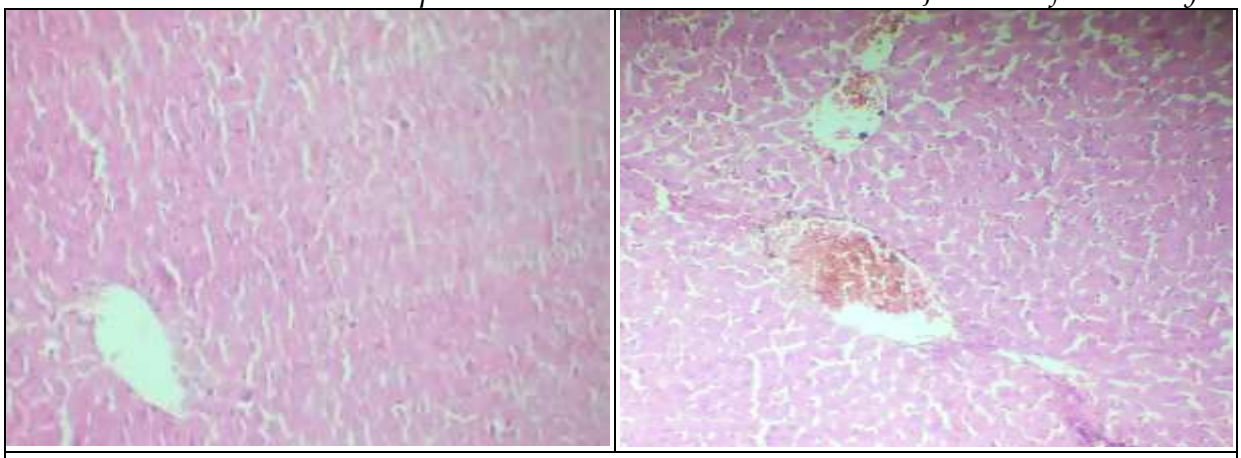
Table-1: Phytoconstituents of *Alangium salvifolium wang.*

Chemical tests	**Ethanolic Extract of *Alangium salvifolium***
Alkaloids	+ +
Carbohydrates	+ + +
Steroids and sterols	+ +
Glycosides	+ +
Flavanoids	+ + +
Tannins	+ +
Proteins and Amino Acids	+
Saponins	-
Fixed oils	-

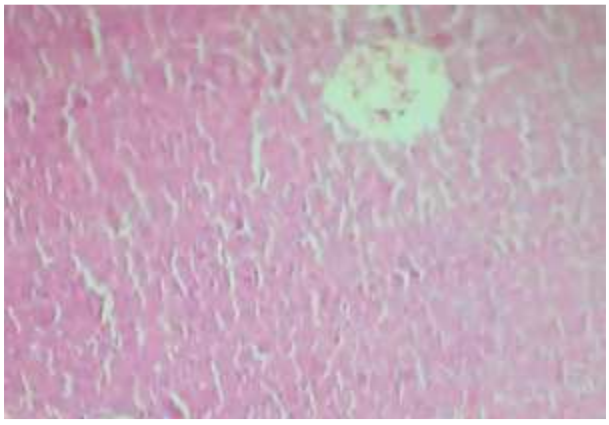
'+' represent presence and '-'represent absence of phytoconstituents.

Figure-2: Effect of ASEE Hepatoprotective activity on histopathalogical changes in CCl_4 induced liver damage in rats.

Group I (Control) section of liver with normal cell structure	**Group II (Toxic) section of liver showing centrilobular necrosis, Haemmahroages and Inflammatory cells**
	
Group III (STD-Silymarin) section of liver showing significantly normal architecture reduced necrotic area	**Group IV (ASEE-150) section of liver showing less reduced Haemahroages, necrotic area**



Group V (ASEE-300) section of liver showing significantly normal architecture and reduced necrotic area



4. Discussion

The preliminary studies on ethanolic extract of leaves of *Alangium salvifolium*conducted in our laboratory by earlier works revealed that ASEE has hepatoprotective activity. ASEE exhibited a significant protective effect when tested with CCl_4 induced Hepatotoxicity in rats.

Alangium salvifolium extract was subjected to identify various biochemical parameters by test tube reactions and TLC methods. ASEE was also assayed for anti oxidant activity like DPPH and Metal Chelating activity methods. The plant leaves of *Alangium salvifolium* demonstrated good radical quenching activity against DPPH. DPPH is relatively stable and free radical which encounters proton donors such as antioxidants, it gets quenched and the absorbance decrease. Results indicated definite scavenging activity of the extract towards DPPH radical in comparison with Ascrobic acid[17, 18]. Metal chelation method indirectly evaluates the antioxidant activity. Iron chelation method evaluates the reducing power. O-Phenanthroline is a selective chelating agent for ferrous ion. It is used for determination of extent of reduction of ferric ions to ferrous ions by antioxidants. *Alangium salvifolium* extract showed dose dependent increase in absorbance, which indicates conversion of ferric ions to ferrous ions.

ASEE was found to be safe up to a dose level of 2000 mg/kg b.w.p.o by using acute toxicity method, as no mortality or symptoms of toxicity were observed within 72 hours.

CCl_4 is one of the most commonly used hepatotoxin in the experimental studies of Liver diseases. CCl_4 is complex, multi-factorial and not completely understood the mechanism of action. CCl_4 is Biotransformed by the cytochrome P450 system to produce the trichloromethyl free radicals in hepatic parenchymal cells. These free radicals reacts with molecular oxygen by lipid peroxidation process to produce peroxy radicals which destructs polyunsaturated fatty acids, i.e., peroxy radicals destructs Ca2+ homeostasis, and finally results in cell death. This result in changes the structures of endoplasmic reticulum and other membranes, which leads to elevation of liver hepato-specific enzymes (SGPT,SGOT and ALP) in serum[19]. Elevation of TB level occurs in CCl_4 which induces hepatotoxicity due to defective excretion of bile by the liver[20]. Elevation of Cholesterol levels is also observed due to chemical hepatotoxin, results in defective biosynthesis of bile acids from cholesterol by the liver.Treating with ASEE at 150 and 300 mg/kg b.w significantly reduced the levels of the parameters. The reduction levels of the enzymes may be a consequence of stabilization of plasma membrane as well as repairs the damaged hepatic tissues[21].In CCl_4 hepatotoxicity, the decreasing levels of total proteins are observed due to the change of protein biosynthesis. This is due to the disruption and disassociation of polyribosomes from endoplasmic reticulum[22]. As well as decreasing the levels of Albumin, which is produced only in the liver is the major plasma proteins that circulates in the blood stream.Treating with ASEE at 150 and 300 mg/kg b.w significantly increasing the levels of total proteins and Albumin.Above all the parameters results in indicating that the extracts have shown hepatoprotective activity, which may be restoring the normal architecture of the liver to the extent possible.

Table-2: Hepatoprotective activity of Ethanolic extract leaves of *Alangium salvifolium* on different biochemical parameters in CCl_4 induced liver damage in rats

GROUPS	SGOT (IU/L)	SGPT (IU/L)	ALB (gm %)	ALP (KA/dL)	CHOL (mg/dL)	TB (mg/dL)	TP (gm %)
NORMAL CONTROL	34.77±1.76	30.26± 2.50	3.86±0.25	16.58±2.51	61.09±4.29	0.54±0.09	6.46±0.61
TOXIC (CCl_4)	87.39±3.51	77.98±2.71	1.95±0.13	60.28±4.23	131.6±8.01	4.81±0.24	3.69±0.32
STANDARD (Silymarin-50)	66.60±3.28**	51.43±1.67***	3.57±0.21***	30.08±1.93**	91.48±4.99**	1.75±0.18***	6.24±0.42**

ASEE-150	75.92±4.27	60.40±4.03**	2.59±0.31	52.96±7.41	106.0±5.23	2.30±0.77**	4.79±0.18
ASEE-300	65.89±4.31***	53.93±5.01***	3.50±0.33***	29.88±4.24**	95.51±11.97**	1.91±0.43***	5.87±0.53**

n=6, values expressed as Mean ± S.E.M. Significant*(P <0.05), ** (P<0.01), *** (P<0.001) compared with standard and toxic group.

The histopathological profiles in the livers of CCl_4 administered rat's revealed that drastic alterations of centrilobular necrosis, fatty changes, dilation of sinusoidal spaces, ballooning degeneration and bleeding area in hepatic lobes. In hepatoprotective studies, of ASEE at 150 and 300mg/kg test dose shown a definite sign of protection and recovery against CCl_4 injury. In this ASEE 300mg/kg exhibited a remarkable recovery, towards normalization of histological architecture of livers of the rats, which was almost similar to that of Silymarin (50mg/kg).

5. Conclusion

The Ethanolic extracts of the leaves of *Alangium salvifolium* is widely used in folk medicine for treatment of liver disorders. From the above studies of ASEE possessed strong hepatoprotective activity in CCL4 induced rat models and also antioxidant activity models. The hepatoprotective activity of ASEE is may be due to its free radical-scavenging and antioxidant activity, resulting from the presence of flavonoids and triterpenoide compounds in the extracts. Additional studies are in progress to better understand the mechanism of action of ASEE that is responsible for the hepatoprotective and antioxidant effects.

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