IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
February 2022 Vol.:23, Issue:3

Hepatoprotective Potentials of Aqueous Extract of *Alphonsea sclerocarpa* against Thioacetamide-Induced Liver Damage in Albino Wistar Rats

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ISSN 2349-7203
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Submitted: 22 January 2022
Accepted: 27 January 2022
Published: 28 February 2022

Keywords: *Alphonsea sclerocarpa*, Hepatoprotective activity, Thioacetamide, Serum Enzymes, Bilirubin, Total protein, Liver enzymes.

ABSTRACT

Background: To develop an effective treatment for the management of hepatoxicity with the least side effects and to establish the link of the traditional system of medicine of India with the modern system of medicine. **Objective**: The objective of the present investigation was to evaluate the hepatoprotective potentials of aqueous extract of *Alphonsea sclerocarpa* leaves against thioacetamide-induced liver damage in rats. **Methodology**: The acute oral toxicity study was conducted according to guidelines No 425 prescribed by OECD. The extract was proved safe up to the dose of 2000mg/kg. Hepatotoxicity was induced in the animals of all groups except normal control by single-dose administration of Thioacetamide(100mg/kg) on the first day of the study followed by animals were treated daily with standard drug silymarin and aqueous extract of *Alphonsea sclerocarpa* (100mg/kg, 200mg/kg and 400mg/kg) to respective groups for 21 days. Variations in biochemical parameters like alanine transferase (ALT), aspartate transferase (AST), alkaline phosphatase (ALP), total bilirubin, direct bilirubin, albumin, total protein, ions, and other parameters like clotting time and weight of the liver were considered to determine the beneficial effect of the extract. At the end of the study, three animals from each group were sacrificed under ether anesthesia. Two samples from each group were subjected to histopathological evaluation. The third liver sample was homogenized and estimated for various anti-oxidant enzymes. **Results**: In toxic control animals treated with Thioacetamide alone there were variations in the above-mentioned parameters. But in the animals treated with aqueous extract of *Alphonsea sclerocarpa*(AEAS) and standard drug silymarin, all the parameters were normal possibly due to their beneficial property in protecting the liver against thioacetamide-induced hepatotoxicity. **Conclusion**: The results of the present research study suggest that the aqueous extract of *Alphonsea sclerocarpa* possess has significant hepatoprotective activity.

1. INTRODUCTION

The liver diseases such as liver cirrhosis, hepatitis, and hepatotoxicity have become one of the common health problems worldwide due to exposure to human life to various hepatotoxic agents such as drugs, alcohol, toxins, and hepatitis viral infections [1]. The liver is one of the vital organs of the biliary system required to maintain important homeostasis of the body due to its various responsibilities. The liver has got its own importance in the physiological system such as metabolism of ingested substances like carbohydrates, lipids, proteins, blood coagulation, detoxification process, and immune-modulation are the primary functions of the liver[1]. The liver injury is associated with distortion of these metabolic functions 2] and results in disturbance in homeostasis of the body. But till now there is no truly satisfactory liver protective drug in the modern system of medicine that is effective and safe. Hence natural remedies from medicinal plants are considered to be effective and safe alternative drugs for the treatment of hepatotoxicity and a number of medicinal plants in Ayurveda, the Indian system of medicine, are recommended for the treatment of liver disorders [3]. About 600 commercially available herbal preparations with known liver-protecting activity are available globally and about 100 Indian medicinal plants belonging to 40 families are used for herbal formulation [4]. The use of foods and medicinal plants to improve health is nearly as old as humanity. The *Alphonsea* is a genus of the plant which is of Indian origin. The various species of *Alphonsea* are medicinally important and have been proved for their several pharmacological activities [5]. A number of *Alphonsea* species are used as food and for medicinal properties in Ayurvedic and Traditional Chinese Medicine (TCM) for various purposes such as diabetes, hepatitis, kidney disease, cancer, menstrual irregularities especially amongst people where these species grow. These uses, however, originated and are most widely found in the Middle East and South Asian countries [6]. The *Alphonsea sclerocarpa* of family Annonnacea medicinally important plants belonging to the same genus [7]. Their extracts are scientifically proved for many pharmacological activities in animal models. The *Alphonsea sclerocarpa w*as extensively used in Folklore and traditional medicine for the management of liver toxicity and diabetes but lacks the scientific evidence for the same [8]. Hence the present research work aimed to explore the anti-diabetic potentials of leaves of *Alphonsea sclerocarpa*.

2.0MATERIALS AND METHODS

2.1 Chemicals

All the chemicals and reagents used in the present study were of analytical grade. The hepatotoxin Thioacetamide was procured from Sigma-Aldrich chemical Pvt. Ltd., Bangalore.) and standard drug Silymarin was obtained from Himalaya dug company, Bangalore. (Nice chemicals Bangalore) and Estimation Kits for AST, ALT, ALP, serum bilirubin, Sodium, Potassium, glutathione peroxidase were obtained from SPAN diagnostics.

2.2 Plant material: The leaves of *Alphonsea sclerocarpa* had have been collected from Sri Venkateshwara University, Tirupati, India, and shade dried. The leaves were identified, collected, and authenticated by Dr. Madhavachetty Asst. Prof. Dept. of Botany and specimen herbarium were preserved at the institute herbarium library. The authenticated leaves were separated from other plant parts, cleaned, washed, and dried for further use.

2.3 Preparation of aqueous extract of *Alphonsea sclerocarpa*

The shade dried leaves were pulverized into powder and sieved through No. 22 mesh. About 350 g (appx.) of coarse powder was defatted using petroleum ether and the marc leftover was extracted using chloroform water [9].

2.4 Preliminary phytochemical investigation of aqueous extract of *Alphonsea sclerocarpa*

The preliminary phytochemical investigation for the aqueous extract of *Alphonsea sclerocarpa* had been conducted as per the procedure prescribed by Khandelwal [10].

2.5 Drugs and chemicals

The alloxan was procured from Sigma Aldrich and all the chemicals and reagents used in the present investigation were of analytical grade and procured SD Fine chemicals, Bangalore.

2.6 Animals

The healthy albino Wistar male rats were procured from Sri Venkateswara Enterprises, Bangalore housed under standard conditions of temperature (22 ± 10C), relative humidity (55 ± 10%), 12hr light/dark cycles, and fed with standard pellet diet (Amrut, Pranav Agro Industries Ltd., Sangli, India) and water ad libitum. After randomization into various groups

and before initiation of the experiment, the rats were acclimatized for a period of 7 days under the above said environmental conditions. The experimental protocol has been approved by the Institutional Animals Ethics Committee with permission from the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

2.7Acute Oral Toxicity Study

The OECD guidelines 423 (up and down procedure) were used to determine acute oral toxicity for aqueous extract of *Alphonsea sclerocarpa*. A starting dose used was 2000 mg/kg body weight p.o. of extract (AEAS was administered to 3 male rats, observed for 14 days. The experiments were repeated again with the same dose level, 2000 mg/kg body weight p.o. of extracts for 3 days more, and observed for 14 days [11].

2.8 Evaluation hepatoprotective activity

Thioacetamide-induced liver damage in the albino Wistar rats model [12,13] was used for the determination of hepatoprotective activity for the plant extracts. The experimental design was as follows:

Sl.No	Name of Group	Treatment
I	Normal	Treated with normal saline 5ml/kg i.p
II	Toxic control	Treated with Thioacetamide (TAA) (100 mg/kg, b. w., s.c.) as a 2% w/v solution in water for injection on day 1st and then the vehicle for 21 days.
III	Standard Control	Treated with Thioacetamide (TAA) (100 mg/kg b. w., s.c.) as a 2% w/v solution in water for injection on day 1st and then silymarin (25mg/kg per day, p. o.) for 21 days.
IV	AEAS-100mg	Treated with Thioacetamide (TAA) (100 mg/kg b .w.,s.c.) as a 2% w/v solution in water for injection on day 1st and then aqueous extract of low dose (100mg/kg.,p.o) of *Alphonsea sclerocarpa* low dose for 21 days.
V	AEAS-200mg	Treated with Thioacetamide (TAA) (100 mg/kg b .w.,s.c.) as a 2% w/v solution in water for injection on day 1st and then aqueous extract of medium dose (200mg/kg.,p.o) of *Alphonsea sclerocarpa* low dose for 21 days.

VI	AEAS-400mg	Treated with Thioacetamide (TAA) (100 mg/kg b .w.,s.c.) as a 2% w/v solution in water for injection on day 1st and then aqueous extract of high dose (400mg/kg.,p.o) of *Alphonsea sclerocarpa* low dose for 21 days.

After 21 days of experimental period blood samples had been collected individually for all the animals by retro-orbital puncture method and estimated for Aspartate aminotransferase, Alanine aminotransferase, Alkaline Phosphatase, Total bilirubin, Total protein, Glutathione peroxidase, Sodium, and Potassium. The clotting time was determined for blood samples by the capillary tube method[14,15]. Later all the animals were sacrificed by cervical dislocation, liver samples were collected and the individual weights of the livers were estimated.

2.9 Determination of liver enzymes

The liver samples were dissected out and washed using ice-cold saline solution. The pieces of liver samples were subjected to homogenization using a tissue homogenizer within 0.1M Tris- HCl buffer (at pH 7.4). The homogenate was centrifuged and a collected supernatant solution was used for the determination of liver antioxidant enzymes such as Glutathione Peroxidase (GPX), Catalase Peroxidase (CAP), Glutathione S transferase (GST), Superoxide dismutase (SOD), and Glutathione reductase (GRD). The homogenate was also determined for the activity of Lipid Peroxidation (LOP) in the liver [15,16].

2.10 Histopathological evaluation

At the end of the research work, 2 animals from each group were sacrificed by euthanasia. After exsanguinations of the liver were removed immediately and washed with ice-cool saline. The liver samples were fixed with 10% formaldehyde, dehydrated in a graded series of alcohol, and embedded in paraffin wax before sectioning. The tissue was cut into sections approximately 5µm thick, dewaxed, and rehydrated. The sections were then stained with hematoxylin-eosin dye and studied for histopathological changes using a light microscope. Each sample was observed at a magnification of 100X.

2.11 Statistical analysis

The data obtained from the study were subjected to statistical analysis by one-way ANOVA followed by Turkey multiple comparisons test, and results were expressed in terms of Mean±SEM values. Statistical analysis was performed using GraphPad prism.

3.0 RESULTS

3.1 Phytochemical investigation

The aqueous extract of *Tephrosia calophylla* Linn. was subjected to different preliminary chemical tests to determine the chemical constituents present in the extracts. The results of the study suggested that aqueous extract consists of alkaloids, flavonoids, tannins, and phenolic compounds.

3.2 Acute oral toxicity

The results of the acute oral toxicity study suggested that the extract of *Alphonsea sclerocarpa* (AEAS)was safe up to 2000mg/kg. As per the above study, dose fixation was done and hence low dose was decided as 200mg/kg and the high dose was decided 400mg/kg for the above extract.

3.3 Evaluation of the hepatoprotective activity of aqueous extract of *Alphonsea sclerocarpa*.

3.3.1 Effect of AEAS on liver weight

In the present study, a significant increase in weights of rat liver was observed which may be due to damage induced by administration of thioacetamide in toxic control animals as compared to normal animals. In animals treated with reference standard Silymarin and AEAS (200mg/kg and 400mg/kg), there was a significant ($P<0.001$) reduction in liver weight compared to toxic animals (see Table No: 1).

3.3.2 Effect of AEAS on Clotting time

In the current study, the prothrombin time was prolonged due to deficiency of clotting factors in toxic animals compared to the normal group as a result of thioacetamide-induced liver injury. The dose-dependent significant ($P<0.001$) reduction in clotting time was observed in animals treated with standard silymarin while AEAS (200mg/kg & 400mg/kg) (Table No: 1).

Animals treated with silymarin and aqueous extract have shown a significant decrease in clotting time compared to positive toxic animals indicating that aqueous extract can reverse complications of hepatotoxicity.

3.3.3 Effect of AEAS on Serum Enzymes

In our study, the serum enzymes ALT, AST and ALP were significantly (P<0.001) elevated toxic control group due to administration of Thioacetamide compared to animals of the normal group as a result of liver damage while AEAS (200mg/kg and 400mg/kg) and standard drug silymarin significantly (P<0.001) reduced concentration serum enzymes in therapeutic animals. The effect of aqueous extract was comparable to standard drug and it was dose (see Table No: 1). Treatment with silymarin and aqueous extract significantly reduced serum concentrations of enzymes ALT, AST, and ALP and also reduced bilirubin levels in blood compared to toxic animals.

3.3.4 Effect of AEAS on direct bilirubin, total bilirubin & Total bilirubin

In the current investigation, the administration of Thioacetamide induced hepatic injury serum direct bilirubin and total bilirubin were significantly increased in toxic control animals as compared to the normal group of animals while there was significant (P<0.001) reduction of direct bilirubin and total bilirubin was observed in animals treated with standard drug silymarin and AEAS (200mg/kg and 400mg/kg) compared to toxic alone animals. The results were equivalent to normal the effect of aqueous extract was dose-dependent (see Table No: 1). AEAS reduced bilirubin levels in blood shows the increased detoxification in therapeutic animals compared to the toxic group which could be due to possible protection given by aqueous extract.

3.3.5 Effect of AEAS on Total protein and Albumin

In drug-induced liver toxicity leads to a reduction in total protein is observed due to decreased albumin synthesis due to cirrhosis. In the present study, in toxic control group animals administered with thioacetamide, significant reduction of serum total protein and albumin was observed due to liver damage compared to normal animals but administration of silymarin and AEAS (200mg/kg and 400mg/kg) caused dose-dependent significant (P<0.001) rise in total protein and albumin therapeutic group compared to toxic animals and the results (see Table No: 2).

3.3.6 Effect of AEAS on serum ions

In the current research, Thioacetamide induced liver damage may cause ascites, and hence there was a significant reduction in serum ionic concentration was observed in toxic control animals when compared to animals of the normal group but serum ionic concentrations were significantly ($P<0.001$) increased in animals of therapeutic groups treated with silymarin and AEAS (200mg/kg and 400mg/kg) when compared to toxic animals. The effect of the extract was dose-dependent and comparable to standard (see Table No: 2).

3.3.7 Effect on liver antioxidant enzymes

There was found to be a significant ($P<0.001$) reduction concentration of liver antioxidant enzymes GPX, CAP, GST, SOD, and GRD in toxic control animals treated with Thioacetamide alone compare to normal animals. While animals of therapeutic groups treated with silymarin and AEAS (200mg/kg and 400 mg/kg) have exhibited a significant ($P<0.001$) rise in liver antioxidant enzyme compared to toxic animals (see Table No: 4).

3.3.8 Effect on lipid peroxidation in liver

The activity of enzyme lipid Peroxidase significantly increased in thioacetamide alone treated toxic animals compare to normal animals while the concentration of lipid peroxidase was significantly reduced in therapeutic groups treated with standard drug silymarin and TCME (200mg and 400mg/kg) indicating the ability of the extract to reduce free radical-mediated damages (See Table No.:3).

3.3.9 Histopathological evaluation

The histopathological examination of liver samples revealed the protection to the liver tissue by the aqueous extract against thioacetamide-induced damage.

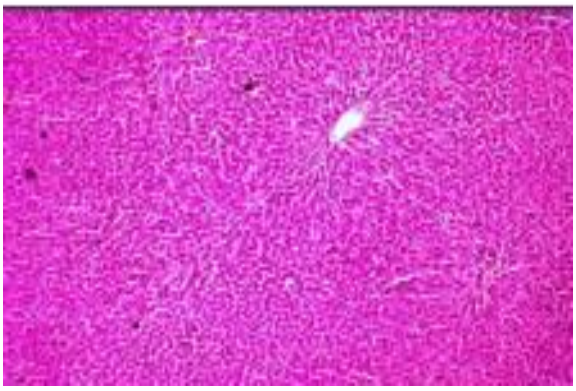
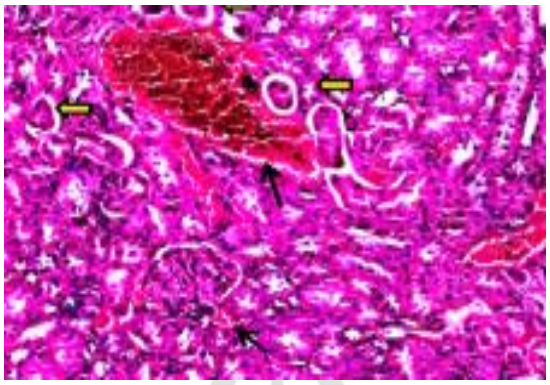


Figure 1: Histopathology of liver Figure



2: Histopathology of liver

Sample from Normal group sample from Toxic group

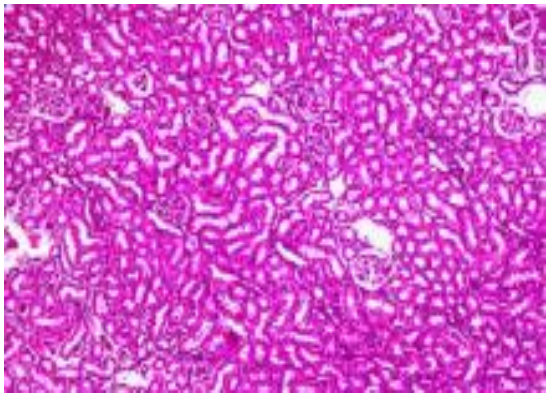


Figure 3: Histopathology of liver

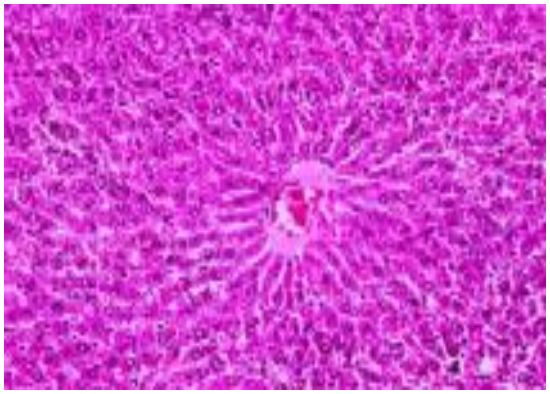


Figure 4: Histopathology of liver sample

Sample from the standard group from AEAS (200mg/kg)

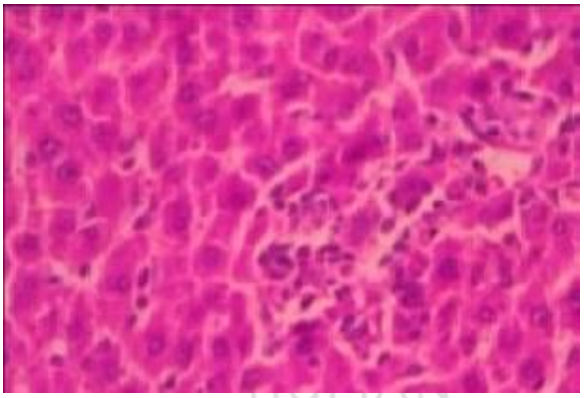


Figure 5: Histopathology of liver sample from AEAS (400mg/kg)

The administration of thioacetamide was caused the complete loss of the normal architecture of livers in positive control animals with the appearance of vacuolated hepatocytes and degenerated nuclei. The pathological changes like vacuolization, fatty degenerations, and coagulative necrosis of liver cells were found to be severe in the centrilobular region. The hepatotoxic metabolite thioacetamide produced excessive formation and deposition of fibrous tissue and resulted in the development of scars. The nodular transformation of rat liver treated with AEAS 100mg/kg has shown, large septa of fibrous tissue flowing together which penetrated the parenchyma cells were found. But sections of liver samples belonging to therapeutic groups treated with high doses of the aqueous extract showed an almost normal lobular pattern with tiny and a mild degree of fatty degenerations, necrosis, and infiltration of lymphocyte which was more or less comparable to the standard drug silymarin treated groups.

4. 0 DISCUSSION

The fungicidal drug Thioacetamide gets converted into potent hepatotoxins sulfine and sulfene metabolites after biotransformation in the liver by cytochrome P450 systems which produce centrilobular necrosis of hepatic cells. Administration of a single large dose of thioacetamide 100mg/kg is followed by degenerative changes in liver cells of rats leads to centrizonal necrosis. The pre-necrotic changes include loss of glycogen and acidophilic degeneration of cells in the central zone. Liver damage is always followed by disturbances in the several functions of life such as metabolism of nutrients, storage functions, synthetic function, detoxification process, etc [17]. Administration of aqueous extract of *Alphonsea sclerocarpa* (AEAS) could normalize various biochemical parameters in experimental animals induced liver damage by thioacetamide as follows:

The disturbance in the metabolism of carbohydrates, fats, and proteins is the main consequence of liver toxicity which leads to fatty change or fatty characterized by the deposition of fat in the liver. Hence the total weight of the liver increases due to the deposition of fat and triglycerides in drug-induced hepatic damage [18]. But the administration of aqueous extract and silymarin could be able to normalize the weight of livers in therapeutic groups indicating their liver-protective properties.

The liver produces all the clotting factors associated with the blood clotting mechanism and it has a main role in regulating normal prothrombin time or clotting time. In liver disorders, the synthesis of clotting factors will be affected and hence clotting time is prolonged [18]. Administration with silymarin and aqueous extract has normalized clotting time.

Storage of various serum enzymes like ALT, AST, and ALP is one of the important functions of the liver. ALT and AST transaminases are involved in transamination reactions of various amino acids while alkaline Phosphatase (ALP) is an isoenzyme synthesized mainly by the liver and has an important role in the dephosphorylation of biomolecules. These enzymes are leaked into the blood in hepatotoxicity due to liver parenchyma damage and hence their concentrations in serum were found to be elevated [19,20]. Another very important role of the liver is detoxification of bilirubin which is a breakdown product of haem an iron component of hemoglobin. The bilirubin uptake by liver parenchyma cells from the blood and conjugates with glucuronic acid in presence of the enzyme glucuronyltransferase. Later conjugated bilirubin gets excreted through bile. In liver toxicity total bilirubin and direct

bilirubin concentration are increased in serum due to the reduced ability of liver parenchyma cells [21].

Treatment with silymarin and aqueous extract significantly reduced serum concentrations of enzymes ALT, AST, and ALP indicating the enhanced storage function and also reduced bilirubin levels in blood shows the increased detoxification in therapeutic animals compared to the toxic group which could be due to possible protection given by aqueous extract.

Serum total protein also called total protein or plasma total protein is synthesized by the liver and is an important biochemical test for assessing liver function. The albumin and globulin that are produced in the liver are the main components of total protein in the plasma [22]. The total protein and serum albumin levels were increased by aqueous extract-treated animals indicating its ability to reverse the hepatic damage caused by thioacetamide.

The two main complications of hepatotoxicity are ascites and edema which are due to the accumulation of fluids in extra-vascular sites of the body. In these complications serum ions sodium, potassium and chlorides move blood into extra-vascular tissues and hence finally lead to a reduction in these ionic concentrations in blood [24]. In our study, animals treated with aqueous extract and silymarin exhibited a significant increase of ions sodium, potassium, and potassium which shows the property of the aqueous extract to reduce ascites and edema may be by regenerating the liver cells.

The ability of the living system to counteract free radical-mediated damages is a natural antioxidant mechanism in which glutathione Peroxidase, Catalase Peroxidase, Glutathione S transferase, glutathione reductase, and Lipid peroxidase are produced in the affected organ/tissue [25]. The liver antioxidant enzymes such as Glutathione Peroxidase (GPX), Catalase Peroxidase (CAP), Glutathione S transferase (GST), Superoxide dismutase (SOD), and Glutathione reductase (GRD). In the present study, there was a significant increase in the synthesis of liver antioxidant enzymes found in animals treated with AEAS indicating its potential to protect the liver cells against thioacetamide-induced free radical damage.

The drug-induced hepatotoxicity is mainly due to oxidative stress and free radicals mediated damage. Hence free radical scavenging and antioxidant mechanisms are more important to reverse or prevent drug-induced liver toxicity [26,27]. The extract of *Alphonsea sclerocarpa* had has been reported for its antioxidant activity. In the present study aqueous extract of *Alphonsea sclerocarpa* could reduce most of the complications of thioacetamide-induced

hepatotoxicity and also significantly increased liver antioxidant enzymes such as glutathione Peroxidase, Catalase Peroxidase, Glutathione S transferase, glutathione reductase, and lipid peroxidase which may be the possible mechanism of action of the extract. Further studies are required to correlate the hepatoprotective potentials of the extract with increased glutathione concentrations and also to isolate and evaluate hepatoprotective principles from the aqueous extract.

Hence, in conclusion, the possible mechanism of beneficial liver-protecting property of our extract is due to its potent antioxidant activity. The Histopathological studies supported the results of biochemical tests, showing less damage in the cytoarchitecture of the liver.

5.0 CONCLUSION

The results obtained from the estimation of biochemical parameters suggest that the aqueous extract of *Alphonsea sclerocarpa* leaves possess significant hepatoprotective property in thioacetamide-induced liver toxicity in the rats model.

CONFLICT OF INTEREST: We hereby declare that there is no conflict of interest.

ACKNOWLEDGEMENTS: The authors of the manuscript are thankful to The principal and management of OJPS University, Churu, Rajasthan for providing facilities to conduct this research work.

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Table No 1: Effect of aqueous and aqueous extracts of *Alphonsea sclerocarpa*on liver weight and blood parameter against thioacetamide-induced liver toxicity in Wistar rats

Treatment	Serum parameters						
	Liver Weight (g)	Clotting Time (Secs)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Direct Bilirubin (mg/dl)	Total Bilirubin (mg/dl)
Normal Control	7.797±0.84	194.5± 7.47	64.18±3.59	133.1± 3.83	81.87±4.62	0.02667± 0.01	0.3787±0.03
Toxic Control	13.23^{+++}± 1.05	443.2^{+++}± 13.9	153.7^{+++}± 2.22	240.6^{+++}± 8.84	210.4^{+++}± 7.66	0.3195^{+++}± 0.02	0.8745^{+++}±0.03
Standard (Silymarin)	8.850***± 1.34	194.7***± 6.99	64.67***± 5.1	132.4***± 6.03	88.67***± 5.35	0.07637***± 0.01	0.3738***± 0.0131
AEAS 100 mg/kg	10.17±1.02	436.8± 15.38	155.6± 4.49	216.9± 3.09	196.3±4.64	0.2897± 0.016	0.9225±0.10
AEAS 200 mg/kg	8.677** ± 0.58	316.2** ± 7.92	132.5*± 5.95	197.8** ± 6.59	133.3** ± 3.44	0.1455** ± 0.016	0.8235** ± 0.15
AEAS 400 mg/kg	7.248***± 0.57	235.7***± 9.591	88.45***± 5.104	131.9***± 5.497	106.4***± 21.27	0.0881***± 0.02	0.4850***± 0.12

Values are mean ± S.E.M, n=6 symbols represent statistical significance.

$^{ns}p>0.05$, * $p<0.05$, ** $p<0.01$, ***$p<0.001$ vs diabetic control.

$^{ns}p>0.05$, $^{+}p<0.05$, $^{++}p<0.01$, $^{+++}p<0.001$ normal control vs positive control.

Table No 2: Effect of aqueous and aqueous extracts of *Alphonsea sclerocarpa* on blood parameter against thioacetamide-induced liver toxicity in Wistar rats

Treatment	Serum parameters				
	Albumin (mg/dl)	Total Protein (mg/dl)	Sodium mE/L	Potassium mE/L	Chlorides mE/L
Normal Control	4.690±0.07358	5.367±0.1175	135.5±1.299	5.732±0.7372	80.76±3.025
Toxic Control	2.390^{+++}±0.1241	2.757^{+++}±0.09793	236.1^{+++}±4.866	3.070^{+++}±0.8783	140.4^{+++}±3.911
Standard (Silymarin)	4.538***±0.1914	5.230***±0.04712	137.8***±3.383	5.840***±0.8990	83.68***±3.229
AEAS 100 mg/kg	2.407±0.1103	3.293±0.08184	215.7±1.948	3.257±0.8202	135.3±4.292
AEAS 200 mg/kg	3.61**5±0.05252	3.857**±0.1712	184.6**±3.034	4.347**±0.8560	114.4**±3.779
AEAS 400 mg/kg	4.877***±0.07233	4.853***±0.03547	140.1***±2.854	5.755***±0.8131	84.44***±3.892

Values are mean ± S.E.M, n=6 symbols represent statistical significance.

^{ns}p>0.05, * p<0.05, ** p<0.01, ***p<0.001 vstoxic control.

^{ns}p>0.05, $^{+}$ p<0.05, $^{++}$ p<0.01, $^{+++}$p<0.001 normal control vs positive control

Table No 3: Effect of aqueous extract of *Alphonsea sclerocarpa*on blood parameter against thioacetamide-induced liver toxicity in Wistar rats

Treatment	Liver tissue antioxidant enzymes				
	Glutathione Peroxidase	Catalase Peroxidase	Glutathione-S-Transferase	Glutathione Reductase	Lipid Peroxidase
Normal Control	12.40 ±1.202	61.51 ±3.466	7.925 ±0.8321	4.878 ±0.7889	8.323 ±0.9576
Toxic Control	7.573^{+++}±0.8262	34.03^{+++}±4.447	4.272^{+++}±0.8050	2.567^{+++}±0.2548	17.89^{+++}±0.9845
Standard (Silymarin)	11.88 ***±1.081	53.50 ***±4.283	7.402 ***±0.8858	4.685 ***±0.8065	10.94 ***±0.7684
AEAS 100 mg/kg	7.547 ±1.015	35.78 ±2.613	4.450 ±1.018	3.837 ±0.9699	16.27±0.7094
AEAS 200 mg/kg	9.077 **±0.7279	48.04 **±3.668	6.048 **±0.9477	4.523 **±1.106	13.84 **±0.9559
AEAS 400 mg/kg	11.65 ***±1.010	53.49 ***±3.817	7.833 ***±1.202	5.463 ***±0.9619	11.01 ***±1.296

Values are mean ± S.E.M, n=6 symbols represent statistical significance.

^{ns}p>0.05, * p<0.05, ** p<0.01, ***p<0.001 vstoxic control.

^{ns}p>0.05, $^{+}$ p<0.05, $^{++}$ p<0.01, $^{+++}$p<0.001 normal control vs positive control.