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# ALPHONSEA SCLEROCARPA AND ITS BIOLOGICAL ACTIVITY

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## ABSTRACT

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) at first day of the study followed by animals were treated daily with standard drug sylimarin 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 bilurubin, direct bilirubin, albumin, total protein, ions and others parameters like clotting time and weight of the liver were considered to determine 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 were homogenized and estimated for various anti-oxidant enzymes. 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. The results of the present research study suggesting that, the aqueous extract of *Alphonsea sclerocarpa* possess significant hepatoprotective activity.

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

## 1. INTRODUCTION

Liver is one of the vital organ of biliary system required to maintain important homeostasis of the body due its various responsibilities [1]. 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 into disturbance in homeostasis of the body. But till now there is no truly satisfactory liver protective drug in the modern system of medicine which 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 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 belongs to the same genus [7]. Their extracts scientifically proved for many pharmacological activities in animal models. The *Alphonsea sclerocarpa w*as extensively used in Folklore and traditional medicine for the management liver toxicity and diabetes but lacks the scientific evidence for the same [8]. Hence the present research work aimed to explore anti-diabetic potentials of leaves of *Alphonsea sclerocarpa*.

## 2.0 MATERIALS AND METHODS

### 2.1 Chemicals

All the chemicals and reagents used in present study were of analytical grade. The hepatotoxin Thioacetamide was procured from Sigma-aldrich chemcal Pvt. Ltd., Bangalore.) and standard drug Silymarin was obtained from Himalya dug company, Bangalore. (Nice chemicals Bangalore) and Estimation Kits for AST, ALT, ALP, serum bilirubins, Sodium, Potassium,

glutathione peroxidase were obtained from SPAN diagnostics

**2.2 Plant material**: The leaves of *Alphonsea sclerocarpa* had been collected from Sri Venkateshwara University, Tirupati, India and shade dried. The leaves were identified, collected and authenticated by Dr. Madhava chetty Asst.Prof. Dept. of Botany and specimen herbarium were preserved at 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 procedure prescribed by Khandelwal [10].

**2.5 Drugs and chemicals**

The alloxan was procured from Sigma Aldrich and all the chemical 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%), 12 hr 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 experiment, the rats were acclimatized for a period of 7 days under above said environmental conditions. The experimental protocol has been approved by the Institutional Animals Ethics Committee with the permission from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

### 2.7 Acute 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 albino wistar rats model [12,13] was used for 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 1$^{st}$ and then 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 1$^{st}$ 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 sample had been collected individually for all the animals by retro-orbital puncture method and estimated for Aspartate amino transferase, Alanine amino transferase, Alkaline Phosphatase, Total billirubin, Total protein, Glutathione peroxidase, Sodium and Potassium. The clotting time was determined for blood samples by 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 tissue homogenizer with in 0.1M Tris-Hcl buffer (at pH 7.4). The homogenate was centrifuged and 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 activity of Lipid Peroxidation (LOP) in the liver [15, 16].

## 3. 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 study suggested that aqueous extract consist of alkaloids, flavonoids, tannins and phenolic compounds.

### 3.2 Acute oral toxicity

The results of 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 high dose was decided 400mg/kg for the above extract.

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

#### 3.3.1 Effect of AEAS on liver weight

In the present study, 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 significant (P<0.001) reduction 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 toxic animals compared to normal group as a result of thioacetamide induced liver injury. The dose dependent significant (P<0.001) reduction in clotting time was observed animals treated with standard silymarin while AEAS (200mg/kg & 400mg/kg) (Table No: 1). Animals treated with silymarin and aqueous extract have shown significant decrease in clotting time compared to positive toxic animals indicating that aqueous extract can reverse complications of hepatotoxicity.

#### 3.3.1 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 compare to animals of normal group as a result 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 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 compare to toxic animals.

#### 3.3.2 Effect of AEAS on direct bilirubin, total bilirubin & Total bilirubin

In 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 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 toxic group which could be due to possible protection given by aqueous extract.

**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.

### 3.3.3 Effect of AEAS on Total protein and Albumin

In drug induced liver toxicity leads to reduction in total protein is observed due decreased albumin synthesis due to cirrhosis. In 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)

### 3.3.4 Effect of AEAS on serum ions

In current research, Thioacetamide induced liver damage may cause ascites and hence there was significant reduction serum ionic concentration was observed in toxic control animals

when compared to animals of 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 extract was dose dependent and comparable to standard (see Table No: 2).

**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 vs toxic control.

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

### 3.3.5 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 concentration of lipid peroxidase was significantly reduced in therapeutic groups treated with standard drug sylimarin and TCME (200mg and 400mg/kg) indicates the ability of the extract to reduce free radical mediated damages (See Table No.:3).

### 3.3.6 Effect on liver antioxidant enzymes

There was found to be 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 sylimarin and AEAS (200mg/kg and 400 mg/kg), have exhibited significant (P<0.001) rise in liver antioxidant enzyme compare to toxic animals (see Table No: 3).

**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 vs toxic control.

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

## 4. DISCUSSION

The fungicidal drug Thioacetamide get converted into potent hepatotoxins sulfine and sulfene metabolites after biotransformation in liver by cytochrome P450 systems which and 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. The liver damage is always followed by disturbances in the several function of live such as metabolism of nutrients, storage functions, synthetic function, detoxification process and etc [17]. Administration of aqueous extract of *Alphonsea sclerocarpa* (AEAS) could normalize various biochemical parameters in experimental animal's induced liver damage by thioacetamide as follows:

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

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

Storage of various serum enzymes like ALT, AST and ALP is one of the important functions of liver. ALT and AST transaminases that are involved in transamination reactions of various amino acids while alkaline Phosphatase (ALP) is isoenzyme synthesized mainly by liver and has important role in dephosphorylation of biomolecules. These enzymes are leaked into blood in hepatotoxicity due to liver parenchyma damage and hence their concentrations in serum found to be elevated [19,20]. Another very important role of liver is detoxification of bilirubin which is 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 enzyme glucuronyl transferase. Later conjugated bilirubin gets excreted through bile. In liver toxicity total bilirubin and direct bilirubin concentration are increased in serum due to 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 toxic group which could be due to possible protection given by aqueous extract.

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

The two main complications of hepatotoxicity are ascites and edema which are due to accumulation of fluids in extra-vascular sites of the body. In these complications serum ions sodium, potassium and chlorides moves of blood into extra-vascular tissues and hence finally lead to reduction in these ionic concentrations in blood [24]. In our study, animals treated with aqueous extract and sylimarin exhibited significant increase of ions sodium, potassium and potassium which shows 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 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 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 been reported for its antioxidant activity. In the present study maqueous extract of *Alphonsea sclerocarpa* could reduce the 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 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 due to its potent antioxidant activity. The Histopathological studies supported the results of biochemical tests, showing less damage in the cyto-architecture of the liver.

## 5. CONCLUSION

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

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