Research Article

Hepatoprotective Effect of Aqueous Leaf Extract of *Erythrina subumbrans* in Ammonium Acetate Induced Hepatotoxicity in Mice.

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ABSTRACT

The present study was aimed to evaluate the hepatoprotective activity of leaf extract of *Erythrina subumbrans* against ammonium acetate induced hepatotoxicity in albino mice. Hepatoprotective activity was analyzed by determining liver weight of the mice and by estimating serum bilirubin and protein. The ammonium acetate induced groups showed a significant increase in the weight of liver which is due to the blocking of the secretion of hepatic triglycerides into plasma and increased levels of lipids during Hyperammonemia. However aqueous extract of the leaf sample inhibit an increase in liver weight. The levels of total bilirubin were increased significantly in ammonium acetate treated mice. Decrease in the levels of serum bilirubin in the mice treated with leaf extract of plant is an indicative of the regeneration of hepatic damage. Similar findings were noted in liver and serum marker enzymes. The serum protein content of the experimental mice was found to be decreased significantly when compared to normal. Upon treatment, a rise in the protein level was suggesting the stabilization of endoplasmic reticulum leading to normal protein synthesis. This indicates the hepatoprotective effect of plant extract.

Keywords: Hepatotoxicity, *Erythrina subumbrans*, Ammonium acetate, Hyperammonemia, AST and ALT.

INTRODUCTION

Liver diseases have become one of the major causes of morbidity and mortality in man and animals. Over dosage or excess use of the drugs, chemical agents, microcystin generally referred as hepatotoxin which cause hepatotoxicity. Hepatitis, viral infection, toxic industrial chemicals, alcohol, aflatoxin and water pollutants are the major risk factors of liver diseases[1].

High blood levels of ammonia are observed in several pathological conditions such as cirrhosis of the liver and inborn errors of urea cycle. Elevated blood ammonia levels are toxic to the brain and central nervous system and lead to convulsions, coma and death[2].

Numerous medicinal plants and their formulations are used for liver disorders in ethno medical practice as well as traditional system of medicine in India. More than fifteen of these plants are evaluated for their hepatoprotective action in modern medicine[3].

The plant *Erythrina subumbrans* is one of the best shade and live support tree for a wide range of crops. The bark and leaves of the plant are used in the treatment of stomach ache, eye ailment and is given for cough. This also exhibits neuromuscular blocking activities which are caused by alkaloids.

They have been related with antiasthmatic, diuretic, antineoplastic, antibacterial, antiplasmodial, antimyobacterial and cytotoxic activities[4].

An active and safe drug is needed for the treatment of liver diseases. In view of this, the present study was aimed at evaluating the hepatoprotective activity of the leaf of *Erythrina subumbrans* against ammonium acetate induced hepatotoxicity in albino mice.

MATERIALS AND METHODS

Collection of Plant Materials

The leaves of *Erythrina subumbrans* used in this study were collected during the month of December to January in Muthupettai village, Regunathapuram town, Ramanathapuram district of Tamilnadu. A voucher specimen has been identified by the Tamilnadu Agricultural University (TNAU) at Coimbatore for future reference.

Preparation of the Extract

The dried leaves of *Erythrina subumbrans* was powdered and extracted with aqueous using sox let apparatus. The aqueous extract was evaporated under reduced pressure and the yields are calculated. The residue obtained was kept in dry clean bottles till used.

Selection and Grouping of Animals

Healthy albino mice of Wistar strain weighing between 20-25g were selected and obtained from small animal breeding station, Thrisur, Kerala and were acclimatized to the laboratory conditions (12+ 1hr, day and night schedule; temperature maintained between 11-20˚C +2˚C; housed in large hygienic plastic cages). The study protocol was approved by IAEC. Before a week of the experiment, water and natural pellets were provided throughout the experimental period.

The hepatoprotective activity was analyzed by inducing ammonium acetate in mice. Ammonium acetate was

induced in the experimental animals by intraperitoneal injection (100mg/Kg b.wt)[5]. The aqueous extract of *Erythrina subumbrans* was suspended in water and was orally given to animals. After 7 days of administration the hepatoprotective effect of *Erythrina subumbrans* was analyzed on the serum and liver samples of control and experimental animals.

Group 1

Control, normal healthy mice

Group II

Toxic control (ammonium acetate) (100 mg/Kg body weight) intraperitoneally on day1, day 3 and day 5.

Group III

Aqueous extract (100mg/Kg of body weight) given orally on day 2, day 4 and day6.

Group IV

Aqueous extract (100 mg/Kg of body weight) given orally along with ammonium acetate intraperitoneally on day 1 to day 6.

Before treatment, the mice were fasted overnight with free access to water. On the 7th day of the study, the animals were sacrificed and liver and blood samples were collected from each animal to produce the liver and serum biochemical assay.

Preparation of the Sample for Biochemical Analysis

The blood samples after coagulation were centrifuged at 3000rpm for 10-15 minutes and the sera isolated were used for estimation of the biochemical markers of liver damage[6]. The liver tissues from the animals were surgically removed, blotted and weighed. 0.5 gm was crushed in a mortar and then homogenized in phosphate buffered saline.

This formed the whole liver homogenate and an aliquot of this was used for the total protein estimation. The reminder of the liver homogenate was centrifuged at 2000rpm for 20 minutes in a refrigerated centrifuge. The supernatant was separated and immediately used for assay of liver marker enzymes.

Determination of Biochemical Parameters

Serum bilirubin was determined by the method of (Dangerfield and Finlayson)[7]. Total protein was estimated by the method of (Lowry)[8]. Aspartate transaminase, alanine transaminase and alkaline phosphatase in both serum and liver was determined by the method of (Reitman and Frankel)[9].

Statistical Analysis

Statistical significance of the data was assessed by analysis of variance (one way ANOVA). The toxic control was compared with the normal control group and all other treatment groups were compared with the toxic control group.

RESULTS AND DISCUSSION

Determination of Liver Weight of Mice

The dry weight of the liver from different groups of experimental animals was recorded as given in the Table 1.

Table 1: Weight of the liver sample in different groups of mice

Treatment Groups	Liver Weight(g)
I-control	0.44g
II-ammonium acetate control	0.67g
III-ammonium acetate + plant extract	0.40g
IV-plant extract	0.42g

Values are expressed as mean of 4 mice

The ammonium acetate induced group showed a significant increase in the weight of the liver which is due to the blocking of the secretion of hepatic triglycerides into plasma. Increases in liver weight have been attributed to the injured structural integrity of the liver as they are released into the circulation after cellular damages. The weight gain was also due to the increased levels of lipids during hyperammonemia. It has been reported that carnitine transport defect is associated with hyperammonemia[10]. As a consequence of carnitine transport defect the fatty acids are accumulated in the cytosol, which may be the reason for the weight gain absorbed in ammonium acetate treated mice. However aqueous extract of the leaf sample of *Erythrina subumbrans* seemed to inhibit an increase in liver weight in Group III when compared with group II. This shows that phytoconstituents in the plant extract maintains proper catabolism of lipids and it prevents their accumulation in liver[11]. This indicates the hepatoprotective effect of the plant extract.

Estimation of Serum Bilirubin and Total Protein

Table 2 and shows the bilirubin content in serum and protein content in liver sample of different groups of mice.

Table 2: Serum bilirubin and total protein content in experimental mice on treatment with aqueous leaf extract of *erythrina subumbrans*

Treatment Groups	Serum Bilirubin (mg/dl)	Total Protein (g/dl)
I-control	0.875 ± 0.004	0.567 ± 0.011
II-ammonium acetate control	1.694 ± 0.005	0.187 ± 0.011
III-ammonium acetate + plant extract	0.985 ± 0.004	0.220 ± 0.017
IV-plant extract	0.565 ± 0.004	0.233 ± 0.005
CD%	0.0087	0.0231

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001). Values are expressed as mean ± S.D of 4 mice. Mean values followed by different alphabets differ significantly.

Table 3: Effect of aqueous leaf extract of *erythrina subumbrans* of serum enzymes in different groups of mice

Treatment Groups	SGO (u/l)	SGPT (u/l)	ALP (u/l)
I-control	14.70 ± 0.086	36.33 ± 0.577	0.713 ± 0.005
II-ammonium acetate control	65.70 ± 0.260	54.33 ± 0.577	5.300 ± 0.260
III-ammonium acetate + plant extract	23.06 ± 0.462	48.33 ± 0.577	3.767 ± 0.028
IV-plant extract	18.68 ± 0.277	33.33 ± 0.577	0.340 ± 0.052
CD%	0.569	1.087	0.251

IU- Concentration of enzyme that catalyze the formation of 1µmole of product per minute.

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference. (P = <0.001).

Values are expressed as mean ± S.D of 4 mice.

Mean values followed by different alphabets differ significantly.

Table 4: Effect of aqueous leaf extract of *erythrina subumbrans* on liver enzymes in different groups of mice

Treatment Groups	SGOT (U/L)	SGPT (U/L)	ALP (U/L)
I-control	24.57 ± 0.543	26.33 ± 0.577	3.180 ± 0.277
II-ammonium acetate control	46.64 ± 0.572	75.33 ± 0.577	10.247 ± 0.219
III-ammonium acetate + plant extract	29.54 ± 0.577	57.33 ± 0.577	5.933 ± 0.057
III-plant extract	26.67 ± 0.543	24.33 ± 0.577	3.247 ± 0.219
CD%	1.052	1.087	0.395

Values are expressed as mean ± S.D of 4 mice. The differences in the mean values among the treatment groups are greater than would be Expected by chance; there is a statistically significant difference (P = <0.001).

It is obvious from the Table 2 that the levels of bilirubin were increased significantly due to the ammonium acetate induced hepatotoxicity. Decrease in the levels of serum bilirubin in the mice treated with the aqueous extracts of the leaf of plant is an indicative of the regeneration of hepatic damage.

Hyperbilirubinemia was observed due to excessive heme destruction and blockage of biliary tract. As a result of blockage of the biliary tract there is a mass inhibition of the conjugation reaction and release of unconjugated bilirubin from damaged and dead hepatocytes[12].

The presence of phytoconstituents and antioxidants in the plant sample helps in protecting the liver from hepatic damages. This indicates the antihepatotoxic effect of the plant extract[11].

Ammonium acetate has an ability to inhibit protein synthesis in liver with early hepatic damage. The serum protein content of the experimental mice was found to be decreased significantly when compared to that of normal. Hypoproteinemia is a feature of liver damage due to significant fall in protein synthesis. Upon treatment with the leaf extract of different concentrations the level was increased which was compared with the standard value. The rise in the protein level was suggesting the stabilization of endoplasmic reticulum leading to normal protein synthesis[13]. The presence of proteins, alkaloids and steroids in plant extract enhances the production of protein content in mice. Thus a healthy functioning liver increase synthesis of serum proteins[12].

From the above findings the protective effect exhibited by aqueous leaf extract of *Erythrina subumbrans* is established.

Assessment of the Serum Marker Enzymes

Elevation of serum levels of these enzymes is considered as an index of liver damage. The significant acute hepato cellular damage and biliary obstruction was indicated by the elevated level of SGPT, SGOT, and ALP. Ammonium acetate and its metabolites causes cell membrane damage and mitochondrial damage in liver cells respectively and leads to release of more than 80% of total hepatic AST enzymes from the mitochondria[15]. The activity of serum marker enzymes namely Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), alkaline phosphatase (ALP) were assessed in serum samples of different groups of mice and is depicted in Table 3.

There was a significant elevation of all the serum enzymes studied in group II which is intoxicated with Ammonium acetate. From the Table III, It is evident that group III which received the aqueous leaf extract of *Erythrina subumbrans* demonstrated a dose dependent inhibition of elevation of serum transaminase activity. The leaf extract shows an inhibition of elevation of AST comparable to control group. A similar trend was noticed in the activities of the other enzymes studied such as ALT and ALP in the serum samples of the various groups of mice.

The group III shows reduction in the levels of SGOT, SGPT and ALP, which clearly shows the antihepatotoxic potential of the plant sample. The presence of Alkaloids, steroids, carbohydrates and protein constituents in our plant shows hepatoprotective activity against Ammonium acetate. The activity of antioxidant enzymes (SOD, CAT, and GSH) suggests that the reduction of oxidative stress in this scenario likely plays a role in the mechanism of its hepatoprotective effect[1].

Reduction in the levels of SGPT and SGOT towards the normal value is an indication of regeneration process[16].

Assessment of Liver Marker Enzymes

The rise in Liver enzymes levels of SGOT, SGPT, and ALP has been attributed to the damaged structural integrity of the liver because these are normally located in the cytoplasm and are released into the circulatory system

after cellular damage. The activities of the liver marker enzymes (AST, ALT and ALP) in the liver samples of the various groups of mice are indicated in Table IV.

The liver marker enzymes namely ALT, AST and ALP were also analyzed in the different groups of mice to evaluate the extent of hepatoprotective effect of the aqueous leaf extract of plant. It is obvious from the result that the activities of all the enzymes were found to be significantly higher in group II when compared with the control. Thus the Ammonium acetate induced group shows increased activities of all enzymes. In group III the plant extract inhibits the activity of enzymes and decreased activities are seen here when compared with group II. Treatment with aqueous extract of plant sample recovered the injured liver to normal when given at a dose of (100mg/kg b.wt) which indicates that *Erythrina subumbrans* leaf have antihepatoprotective effect.

The group III shows the depletion of Liver marker enzymes SGPT, SGOT and ALP. This is due to the effect of phytoconstituents such as steroids, alkaloids and flavanoids. The increased activity of the antioxidants such as glutathione, catalase, peroxidase also acts against cellular damages[14]. The plant sample seems to preserve the structural and hepatocellular membrane damages and detoxifies ammonia.

Alkaline phosphatase concentration is related to the functioning of hepatocytes, high level of alkaline phosphatase in the blood serum is related to the increased synthesis of it by cells lining bile canaliculi usually in response to cholestasis and increased biliary pressure[17]. Thus *Erythrina subumbrans* acts as a hepatoprotective effect against ammonium acetate.

CONCLUSION

To conclude, it can be suggested that the aqueous leaf extract of *Erythrina subumbrans* chosen for the study has a great potential to act as hepatoprotective agent against ammonium acetate induced liver damage in Swiss albino mice. Hence the aqueous extract selected can be considered as a source of natural antioxidants and effective hepatoprotective agent.

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