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In vivo antioxidant and hepatoprotective activity of *Girardinia heterophylla* against ethanol intoxication in mice

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ABSTRACT

A systemic and scientific investigation of aqueous extract of Girardinia hetrophylla for its antioxidant and hepatoprotective potential against ethanol induced hepatic damage in mice was carried out. Antioxidant property was assessed by using reducing property, superoxide anion scavenging and hydroxyl radical scavenging property. Hepatoprotective property was assessed by measuring the extent of reversal of enhanced biochemical marker for liver such as serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP), bilirubin and also by estimating the tissue Glutathione (GSH), Superoxide dismutase(SOD), Catalase and the tissue lipid peroxidation levels. Aqueous extract of Girardinia hetrophylla at the dose of 1600mg/kg produced significant hepatoprotective effect by decreasing the serum enzymes, bilirubin and tissue lipid peroxidation while it significantly increased the levels of tissue GSH, SOD and catalase.

Key words: Antioxidant, Hepatoprotective, *Girardinia heterophylla*, Free radicals,Transaminase.

INTRODUCTION

Hyper-physiological burden of free radicals causes imbalance in homeostatic phenomena between oxidants and antioxidants in the body. This imbalance leads to oxidative stress that is being suggested as the root cause of aging and various diseases like diabetes, cancer, and liver damage [1]. Liver is the main organ which regulates many important metabolic functions. Liver disorders being a serious health problem and still remain common and unconquered. Modern medicines have little to offer for alleviation of hepatic diseases and only limited numbers of drugs are available for the treatment of liver disorders. The herbal based preparations were effective for the treatment of liver disorders [2,3].Free radicals lead to cell damage through mechanisms of covalent binding and lipid peroxidation with subsequent tissue injury, cellular necrosis, rheumatoid arthritis as well as toxicity of many xenobiotics [4]. Antioxidant agents of natural origin have attracted special interest as they can protect human body from oxidative damage and free radicals [5].

Girardinia heterophylla is a dioecious herb belongs to the family *Urticaceae* is commonly known as bichhoo ghass. This is a much despised plant in the hills of north India. The plant itself has medicinal values. The leaves should not be touched with bare hands, but dried or boiled thoroughly in water, are used as diuretic, anti-allergic and also for lactating mother. The other parts of the plant are also useful for production of oils, biomass and fibre or paper. The present study was done to investigate hepatoprotective and antioxidant activity of *Girardinia hetrophylla* against ethanol induced liver damage in male Wistar Albino Mice.

MATERIALS AND METHODS

Plant Material and Preparation of Extract

Leaves of *Girardinia heterophylla* was washed under running tap water, air dried, and then homogenized to fine powder dissolved in distilled water for two days and centrifuged the solution at 10,000 rpm for 10 minutes. Supernatant was collected and stored at $4^{O}C$.

Experimental Animals

Permission from the institutional ethical committee for laboratory use of animals was duly obtained. The experiments were performed on Wistar albino mice obtained from Indian Veterinary Research Institute, Bareilly. The animals were kept in the experimental lab animal house at our Institute .The mice were housed in clean polypropylene cages and feed *ad libitum* with the commercially available feed and water. The litter was changed after every 5 days.

Labelling and Experimental Design of Animals

The mice were labelled and total of 24 animals were equally divided into 4 groups. Each group contained 6 mice. The details of the groups are given below:

TABLE-1 Allocation of animals according to treatment groups

GROUP	TYPE	TREATMENT
I	Normal	No Treatment
II	Alcohol	5% Alcohol (2 ml /kg body wt.)
III	Positive	5% Alcohol + Liv-52 (2 ml/kg body wt.)
IV	AEGH	5% Alcohol + Aqueous plant extract (1600mg/kg body wt.)

AEGH-Aqueous Extract of Girardinia hetrophylla

Acute Toxicity Studies

Acute oral toxicity (AOT) of alcohol was determined using Wistar albino mice. The animals were administered with single dose of the AEGH along with 5% alcohol and observed for mortality up to 90 days period.

Dissection of the Animal

The animals were sacrificed by chloroform anesthesia. Take anesthetized mice fixed on dissection tray by ventral side. Lay down the mice by pinning it in the dissecting tray ventro -dorsally and pinning it in the limbs. With the help of a fresh sterile syringe withdraw blood from the heart while the heart is pumping. Store the blood in sterile plain vials with label. The liver was cut and removed and washed two or three times with cold n-saline. Liver was weighed and then stored in cold phosphate buffer (50mM pH-7).

Homogenization of Tissue and Serum Separation

The small piece of liver was transformed to homogenizing tube to prepare homogenate in 50mM phosphate buffer saline in cold conditions. The homogenate was centrifuged at 3000rpm for 10 minute at $4^{0}C$ then collected the supernatant for further tests. The blood samples were allowed to clot at room temperature for 45 min. The serum was separated by centrifugation at 2500 rpm at room temperature for 15 min and was stored at $4^{0}C$.

Biochemical Test in Tissue Homogenate

Assay of lipid peroxidation was done by Esterbauer and Cheeseman method [6], Superoxide Dismutase **(**SOD**)** by Mishra and Fridovich method [7], Catalase activity was measured by the method of Beers and Sizers [8] and Glutathione (GSH) was done by Ellman method [9].

Blood Serum Enzyme Assay

Liver function tests as Alanine transaminase (ALT)/SGPT, Aspartate transaminase (AST)/SGOT, Alkaline Phosphatase (ALP) and Bilirubin Total were performed by standard Autopeck kit methods.

Histopathological Examination

The method described by Baker and Silverton[10] was adopted in the preparation of slices or fixed tissues (liver) for histological examinations. The liver was removed immediately after blood collection and a part of liver was sliced and fixed in 10% buffered formal saline. Following decalcification, dehydration, impregnation, embedding and

section cutting. The tissues were stained using the Mayer's acid alum haematoxylin and eosin staining method[11] and mounted in neutral balsum. The slides were then examined microscopically for histological changes and micrograph of each section taken.

RESULTS AND DISCUSSION

Although oxygen is essential for life, its transformation to reactive oxygen species (ROS) may provoke uncontrolled reactions. Such challenges may arise due to exposure to radiation, chemicals or by other means. Antioxidants may offer resistance against the oxidative stress by scavenging free radicals, inhibiting lipid peroxidation and some other mechanism[12].

MDA is one of the end products in the lipid peroxidation process [13]. In our *in vivo* study elevation in levels of end products of lipid peroxidation in liver of mice treated with ethanol were observed. The increase in MDA level in liver suggests enhanced lipid peroxidation leading to tissue damage. Pretreatment with AEGH significantly reversed these changes. Hence it may be possible that the mechanism of hepatoprotection of extract is due to its antioxidant effect.

The level of MDA in liver was elevated (51.16 nmol/L) in ethanol intoxicated mice. In mice treated with AEGH, the level was reduced to near normal (20.39 nmol/L) (Table-2).

It is well documented that hepatocellular enzymes (SOD, CAT) serve as biomarkers of hepatocellular injury due to alcohol and drug toxicity [14]. So the studies on antioxidant enzymes (SOD, CAT) have been found to be of great importance in assessment of liver damage. The effect of AEGH on SOD and CAT activity in liver is shown in (Table-2). SOD and CAT activity of the liver homogenate in alcohol treated group was examined to be lower than in normal group. SOD and CAT activity in AEGH was observed to be higher than in alcohol intoxicated group. SOD activity of AEGH was improved by 47.3 % and CAT activity in AEGH is increased by 63.2%, as compared to alcohol treated group.

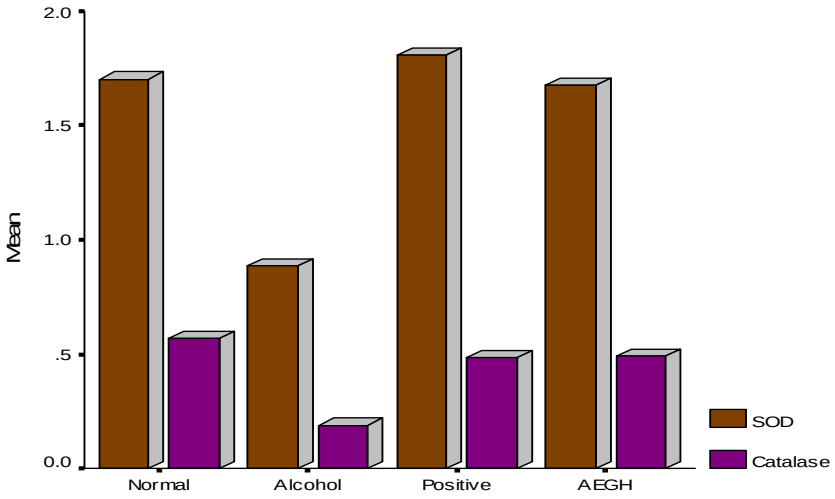
GSH is a naturally occurring substance that is abundant in many living creatures. It is widely known that a deficiency of GSH within living organisms can lead to tissue disorder and injury. For example, liver injury included by consuming alcohol or by taking drugs like acetaminophen, lung injury by smoking and muscle injury by intense physical activity [15], all are known to be correlated with low tissue levels of GSH. From this point of view, AEGH supplementation provide a mean of recover reduced GSH levels and to prevent tissue disorders and injuries. In the present study, we have demonstrated the effectiveness of the extract by using ethanol intoxication in mice. Therefore, the levels of glutathione are of critical importance in liver injury caused by ethanol. Our results found that the supplementation of AEGH was increased by 29.5% as compared to the alcohol treated group in mice (table-2).

TABLE-2 Effect of AEGH on ethanol intoxication and antioxidant enzyme in liver homogenate

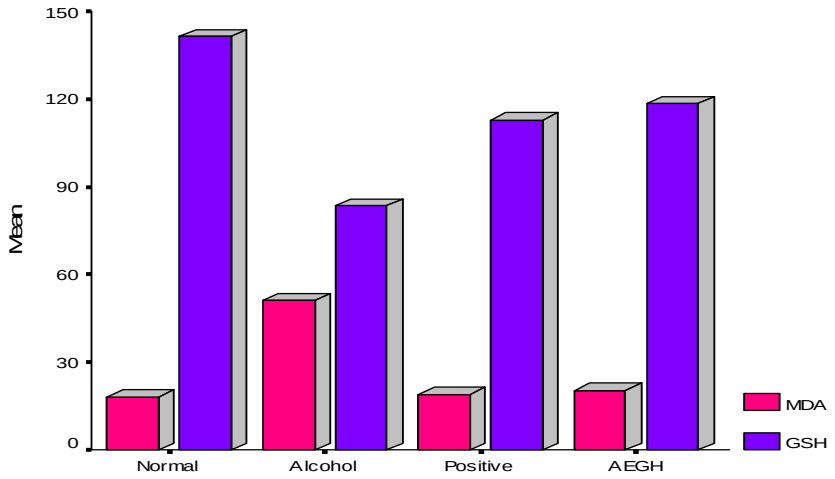
Group	Treatment	MDA[a]	SOD[b]	CAT[c]	GSH[d]
I	**Normal**	18.18 ± 1.78	1.70 ± 0.14	0.57 ± 0.04	141.27 ± 10.42
II	**Alcohol**	51.16 ± 2.80*	0.88 ± 0.12*	0.18 ± 0.08*	83.46 ± 03.64*
III	**Positive**	18.96 ± 2.11**	1.80 ± 0.05**	0.48 ± 0.03**	112.81 ± 03.63**
IV	**AEGH**	20.39 ± 3.84**	1.67 ± 0.06**	0.49 ± 0.03**	118.42 ± 02.76**

Values are mean ±SE, N=6 mice in each group.
**p < 0.05 as compared with Group I*
***p < 0.05 as compared with Group II.*
a= n mole of MDA/mg of protein.
b= Units/mg of protein
c= μ mole of H_2O_2 consumed/min/mg of protein.
d= μg/mg of protein.

Graph-A



Graph-B



Graph-1:-Effect of AEGH on (A) SOD and Catalase (B) MDA and GSH content of ethanol treated mice liver. Results are presented as the mean (n=6).

Biochemical Test in Blood Serum

Serum activities of transaminase (AST/SGOT, ALT/SGPT) and ALP, serum total bilirubin are given in (Table-3).Mice subjected to ethanol only, developed significant (p<0.05) hepatocellular damage as evident from significant increase in serum activities of AST, ALT, ALP and bilirubin concentration as compared to normal control group, which has been used as reliable marker of hepatotoxicity. Oral administration of AEGH exhibited significant reduction in ethanol-induced increase in levels of AST, ALT, ALP and bilirubin concentration. Treatment with Liv-52 also reversed the hepatotoxicity significantly (p<0.05).

Necrosis or membrane damage releases the enzyme into circulation and therefore, it can be measured in serum. High level of AST indicate liver damage, such as that due to viral hepatitis as well as cardiac infarction and muscle injury. ALT catalyses the conversion of alanine to pyruvate and glutamate, and is released in a similar manner. Therefore, ALT is more specific to the liver, and is thus a better parameter for detecting liver injury [16]. Our result demonstrate that the aqueous extract of *G. hetrophylla* caused significant reduction in the level of AST and ALT. Serum ALP and bilirubin levels on the other hand, are related to the function of hepatic cell. Our results also demonstrate that the aqueous extract of *G. hetrophylla* caused significant decrease serum ALP and bilirubin levels.

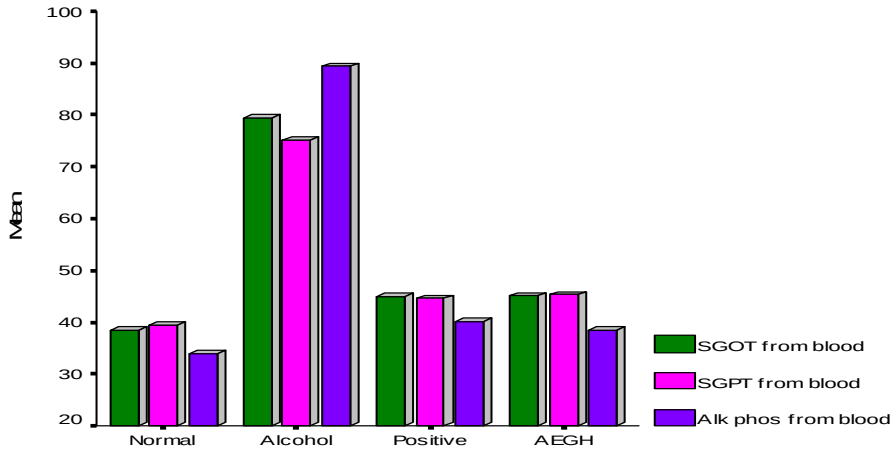
Effective control of alkaline phosphatase activity and bilirubin level points towards an early improvement in secretory mechanism of hepatic cells

TABLE-3: Effect of AEGH on serum AST, ALT, ALP and bilirubin levels on ethanol treated mice

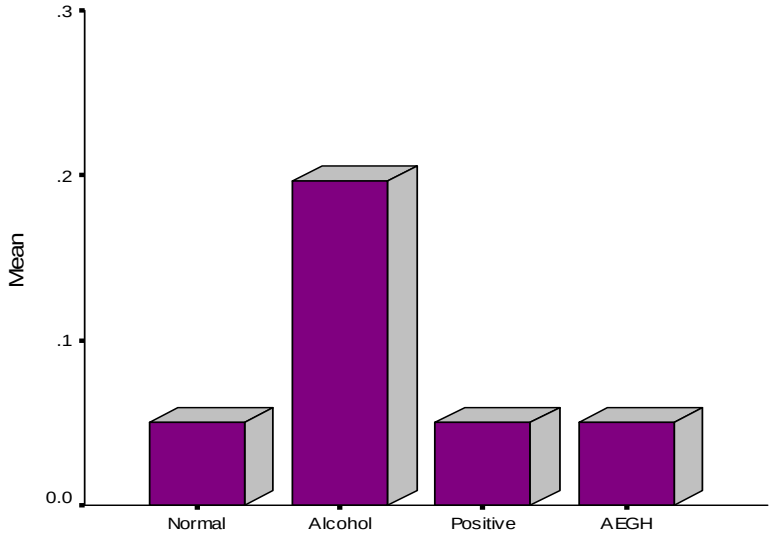
Group	Treatment	AST (U/L)	ALT (U/L)	ALP (U/L)	T. Bilirubin (mg/dl)
I	**Normal**	38.47 ± 0.79	39.43 ± 0.45	33.87 ± 0.62	0.05 ± 0.006
II	**Alcohol**	79.47 ± 1.99*	75.17 ± 3.60*	89.38 ± 0.63*	0.19 ± 0.008*
III	**Positive**	44.88 ± 0.51**	44.58 ± 0.61**	40.23 ± 0.53**	0.05 ± 0.006**
IV	**AEGH**	45.05 ± 1.05**	45.32 ± 0.74**	38.55 ± 0.55**	0.05 ± 0.007**

Values are mean ±SE, N=6 mice in each group.
**p < 0.05 as compared with Group I*
***p < 0.05 as compared with Group II.*

Graph-A



Graph-B



Graph-2: Effect of AEGH on (A) AST, ALT and ALP (B) Bilirubin levels of ethanol intoxicated mice
Results are presented as the mean (n=6)

HISTOPATHOLOGICAL EXAMINATIONS OF LIVER TISSUE

The histopathology of the liver sections of control animal showed normal hepatic cells with well preserved cytoplasm, prominent nucleus, nucleolus and visible central veins. Whereas ethanol treated groups show severe necrosis, degeneration and broad infiltration of the lymphocytes and the loss of cellular boundaries. The positive control groups treated with Liv-52 which is a good hepatoprotective agent and shows almost less harmful effects on liver while aqueous extract of *Girardinia heterophylla* shows protective effects and almost comparable to the control and Liv-52 treated groups.

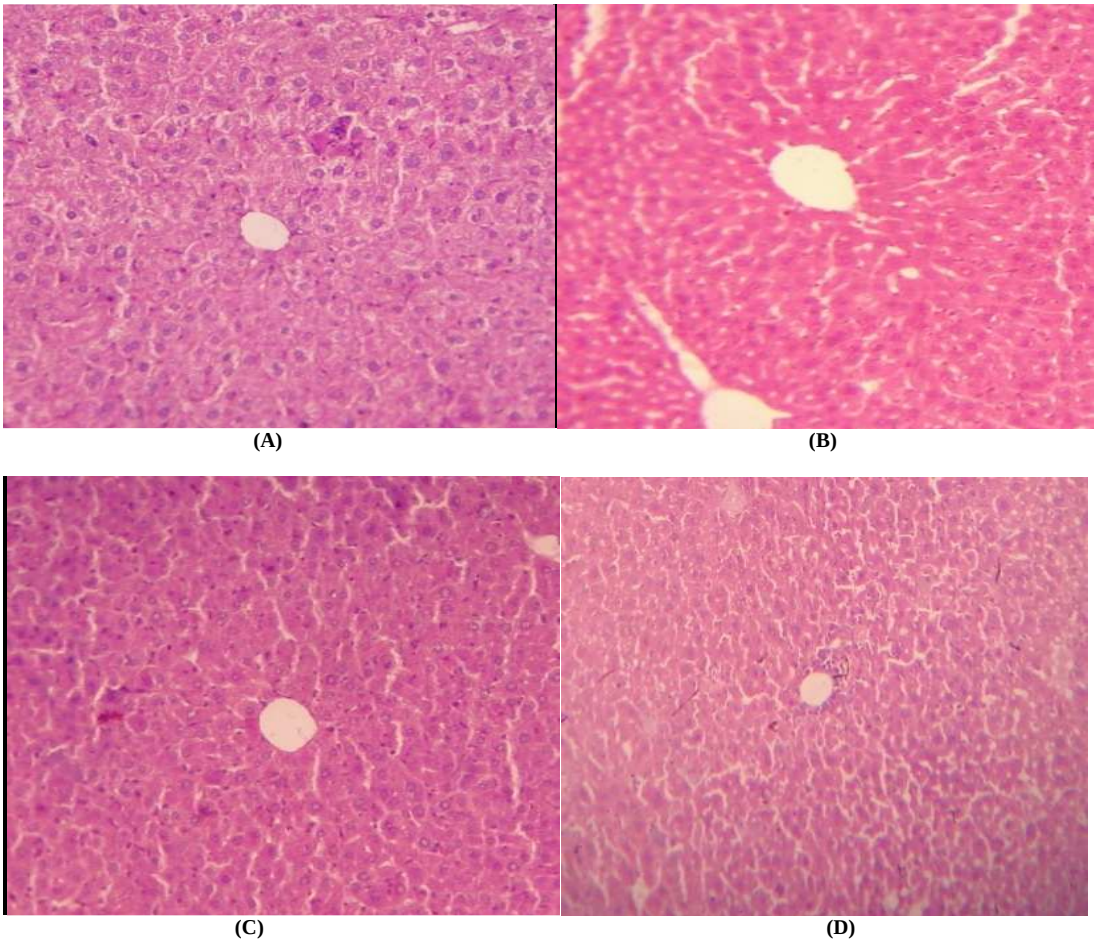


Plate-1: Histological Section of Liver: (A) Normal (B) Treated with ethanol (C) Treated with Liv-52 (D) Treated with AEGH

CONCLUSION

In conclusion, the hepatoprotective and antioxidant effects of aqueous extract of *Girardinia hetrophylla* were evaluated. Our result demonstrate that aqueous extract of *Girardinia hetrophylla* possess significant protection against ethanol-induced hepatotoxicity, which was due to its antioxidant properties through scavenging free radicals to ameliorate oxidative stress and inhibit lipid peroxidation.The observations made *in vivo* were also confirmed by histopathological studies.

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