Comapartive Study on Hepatoprotective activity of Phyllanthus amarus and Eclipta prostrata against alcohol induced in albino rats

Arun. K, Balasubramanian. U
Department of zoology, A.V.V.M Sri Pushpam College
Poondi, Thanjavur
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ABSTRACT

Our research studies data suggest that, there were significant variations in the observed biochemical parameters. The level of TBARS in ethanol intoxicated rats increased two fold when compared with the control animals. The levels of GSH, SOD and CAT decreased significantly in the ethanol intoxicated rats. The level of GPx was increased in the ethanol intoxicated rats. The value of vitamin E in both plasma and liver samples were less when compared with the control animals. Similarly, the value of vitamin C was also showed decreased level in plasma. Serum iron and copper levels were elevated to a higher level. The therapeutic administrations of Phyllanthus amarus and Eclipta prostrata leaves fine powder greatly change the biochemical parameters in the ethanol intoxicated rats and maintained well to the normal level. These results clearly suggest that, the Phyllanthus amarus and Eclipta prostrata have enormous hepatoprotective value. Among the two plants Phyllanthus amarus has slightly high activity as compare to Eclipta prostrata. These herbal drugs have equivalent therapeutic value with the standards drug Silymarin. Moreover, it is very important to study the specific phytochemical compounds responsible for this hepatoprotective effect.

Keywords: Antioxidants, Phyllanthus amarus, CCI₄, liver damage, Albino rats.

1. Introduction

Liver is an important organ actively involved in many metabolic functions and is the frequent target for a number of toxicants (Meyer et al., 2001). Hepatic damage is associated with distortion of these metabolic functions (Wolf, 1999). Liver disease is still a worldwide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects (Guntupalli et al., 2006). In the absence of a reliable liver protective drug in modern medicine there are a number of medicinal preparations in Ayurveda recommended for the treatment of liver disorders. In view of severe undesirable side effects of synthetic agents.

There is growing focus to follow systematic research methodology and to evaluate scientific basis for the traditional herbal medicines that are claimed to possess hepatoprotective activity. A single drug cannot be effective for all types of severe liver diseases (Shahani, 1999). Therefore an effective formulation has to be developed using indigenous medicinal plants, with proper pharmacological experiments and clinical trials.

The plant Phyllanthus amarus is widely distributed in all tropical regions of the planet. Paleobotanical studies have not found the exact geographic origin of this plant. This plant may be indigenous to the tropical Americas (Cabieses, 1993; Morton, 1981; Tirimana, 1987), the Philippines or India (Cabieses, 1993). Phyllanthus amarus is a common pantropical weed that grows well in moist, shady and sunny places (Cabieses, 1993). Some common names of
Comparative Study on Hepatoprotective activity of *Phyllanthus amarus* and *Eclipta prostrata* against alcohol induced in albino rats

*Phyllanthus amarus* in North, Central and South America are black catnip, carry-meseed, chanca piedra, djari-bitá, egg woman, fini-bitá, flor escondida, gale-of-(the)-wind, hurricane weed, quebra-pedra, quinine creole, quinine weed, seed-under-leaf, stone breaker and yerba de la nina (Morton, 1981). *Phyllanthus amarus* is a member of the Euphorbiaceae family (Spurge family), which groups over 6500 species in 300 genera. Euphorbiaceae is a large family of upright or prostrate herbs or shrubs, often with milky acrid juice (Lewis, 1977) and is mainly a pan-tropical family with some species either more or less temperate. Numerous species of this family are native to North, Central and South America (Unander, 1995).

The plants are monoecious or homogamous; leaves are simple, alternate or opposite, some are leathery; flowers are very small and diclinous, they cluster in cup-shaped structures, greenish, often with glands. The fruit is a three-lobed capsule extending from the cup and commonly the long stalk pendant (Lewis, 1977). The name ‘Phyllanthus’ means “leaf and flower” because the flower, as well as the fruit, seems to become one with the leaf (Cabieses, 1993). *Phyllanthus amarus* is an erect annual herb, 10 to 50 cm high, with smooth cylindrical stem 1.5 to 2 mm thick and deciduous horizontal branchlets 4 to 12 cm long and about 0.5 cm thick, with 15 to 30 leaves (Figure 2.2).

The leaves are alternate, on tetioles 0.3 to 0.5 mm long, elliptic, oblong or obovate, 5 to 11 mm long and 3 to 6 mm wide, rounded to slightly point at the tip, scarcely oblique on one side at the base. The flowers are alone or usually one male and one (larger) female are in each leaf axil together. The seed capsules on stalks are 1 to 2 mm long, round, smooth, 2 mm wide, with 6 seeds. When the fruits burst open the seeds are hurled away. Seeds are triangular (like an orange segment), light brown, 1 mm long, with 5 to 6 ribs on the back (Morton, 1981).

*Eclipta prostrata* Linn (Family-Asteraceae) is a common plant and abundantly grows throughout India up to 6000 ft height of hills. It is commonly known as Trailing Eclipta in English, Bhamgra in Hindi and Kayyantakara in Tamil. It is an erect or prostrate annual herb and the leaves are opposite, sessile and lanceolate. The leaves are densely arranged on both sides of the stem and rooting at the nodes and the flower-heads are white (Asolkar et al., 1992). *Eclipta prostrata* Linn has great traditional reputation of being used as a medicinal agent in India. Various parts of the plant is used by the rural people of Tamil Nadu for several human illnesses like kidney and liver weakness, inflammatory conditions, ophthalmic and digestive disorders.

It is also regarded as the best remedy for hair in Ayurvedic medicines and act as haematinic, diuretic and anthelmintic (Anonymous, 1952; Kirthikar and Basu, 1998). The extract of the plant has the ability to act as an antidote for snake venom (Melo et al., 1994; Mors et al., 1989).

Previous studies on this plant proved its usefulness in modification of immune function, cytological responses, serine proteinase inhibition, lipid lowering and liver function (Konarev, 2002; Kumari et al., 2006; Lans, 2001).

Recent reports showed that the triterpenoid saponins isolated from this plant has antimicrobial, immunosuppressant, anti-guardian and anti-venom potentials (Liu et al., 2000; Pithayanukul et al., 2004; Sawangjaroen et al., 2005; Zhang Guo, 2001; Zhao et al., 2001). Phytochemically, *Eclipta prostrata* is rich in wadeoloctone, eclalbasaponin, b- amyrin, stigmasterol and luteolin-7-glucoside ((Asolkar et al., 1992).
Wagner and Fessler (1986) reported the effectiveness of the 5-lipoxygenase inhibition of wedelolactone isolated from Eclipta alba (L.) and Wedelia calendulacea Less in in-vitro porcine-leukocytes test system. With the above scenario, the herbal ethanolic extract made up equal quantities of leaves of *Phyllanthus niruri* and *Eclipta prostrata* were subjected to various assays in order to evaluate their hepatoprotective effect from mixture of these herbs against alcohol toxicity in albino rat.

### 1.2. Materials and Methods

#### 1.3. Preparation of Plant Material

Leaves of *Phyllanthus amarus* and *Eclipta prostrata* were collected from Thanjavur district of Tamil Nadu, India during the months of September - December. Fresh leaves were dried at 45°C for 48 hours, powdered using electric grinder, and stored in a decicator. About 500g of dried powder of each plant was extracted with ethanol by continuous hot percolation using soxhlet apparatus and was concentrated up to 100 ml on Rota vapour under reduced pressure. The concentrated crude extracts were lyophilized in to powder and used for the study.

#### 1.3. Experimental Design

In this experiment twenty four healthy male albino Wistar strains rats, 3 months of age, weighing 300 – 350 g were selected for acclimation for a period of two weeks in laboratory animal house and maintained under standard conditions of temperature 27 ± 2°C, relative humidity of 60 ± 5% and 12: 12 hour light: dark cycle prior to experimentation. The animals were fed with standard pellet diet and water ad libitum.

The experimental animals were divided into four groups (G1, G2, G3 and G4) each contains six animals as per the drug treatment plan. First group served as control and the rest served as experimental groups. The ethics committee of Tamil University, Thanjavur, approved the protocol of the present study. Morphological view of Albino rat were presented in the figure 3.

#### 1.4. Phytochemical analysis

1.5. Alkaloids

1.6. Meyer’s reagent (Potassium mercuric iodide)

1.36 g of mercuric chloride was dissolved in 60 ml of distilled water and 5 g of potassium iodide in 10 ml of water. The two solutions were mixed and diluted to 100 ml with distilled water. A few drops of the reagent were added to 1 ml of the plant extract. The formation of a pale precipitate showed the presence of alkaloids.

1.7. Flavonoids (Somolenski *et al.*, 1972)

In a test tube containing 0.5 ml of plant extract, 5-10 drops of diluted HCl and a small piece of zinc or magnesium were added, and the solution was boiled for a few minutes. In the presence of flavonoids, are reddish pink or dirty brown colour was produced.

1.8. Saponins (Malick and Singh, 1980)
In a test tube containing about 5 ml of plant extract, a drop of sodium bicarbonate was added. The mixture was shaken vigorously and kept for 3 min. Formation of a honey comb like froth showed the presence of saponins.

1.9. Tannins (Segelman et al., 1969)

1.10. Lead acetate test

In a test tube containing about 500 ml of plant extract, a few drops of 1 per cent solution of lead acetate was added. Formation of yellow or red precipitate indicated the presence of tannins. The result were presented in the Table 1.

1.11. Thin layer chromatographic analysis of Antihepatic activity compounds

The extracts were spotted on the baseline of the silica gel plates at 1.0 cm and then allowed to dry at room temperature. Then the plates were placed in pre-saturated TLC chamber which contains the mobile phase butanol-acetic acid-water (4:1:2). Then the chromatogram was developed and dried for few minutes. It was visualized under ultraviolet (UV) light and spots were marked. The Rf values for each band were measured. The result were presented in the Table 2.

1.12. Anti hepatic Activity of Fractionated Plant Extracts

Ethanol and methanol extracts showed potential activity than other extracts and these extracts were fractionated by column chromatography in silica gel (Anand, 2001). Elution were made with, Ethanol (E), Methanol (M) and ethanol mixed with methanol in various proportion (E:M 18:2, 16:4,14:2, 12:8, 10:10, 8:12, 6:14, 4:16 and 2:18). Eluted fractions were assayed for anti hepatic analysis by injecting animals were considered as hepatic rats and used for the experiment.

1.13. Drug treatment protocol

In order to study the effect of ethanolic and aqueous extract of *Phyllanthus amarus* (200 mg/kg bw) and *Eclipta prostrata* (200 mg/kg bw) in rat were used respectively. Silymarin (2.5 mg/kg bw) was used as a standard drug in this study. Rats were divided into five groups as following protocol.

1.14. Treatment Protocol

**GROUP I**

Normal control (n=6, the animals were given normal saline only)

**GROUP II**

Hepatotoxic control (n=6, the animals were given alcohol for 21 Days)

**GROUP III**

Treatment group (n=6, the animals were given alcohol + *Phyllanthus amarus* for 21 days)

**GROUP IV**

Treatment group (n=6, the animals were given alcohol + *Eclipta prostrata* for 21 days)

**GROUP V**
Standard group (n=6, the animals were given alcohol +Silymarin for 21 days) Rats were treated as per the treatment protocol. Body weights of these rats were monitored sequentially in control and experimental animals for a period of 21 days.

**1.15. Biochemical Assays**

At the end of the drug treatment period, all the animals were anaesthetized by application of light chloroform and blood samples were collected from a group of animals from dorsal aorta by heparinized syringe in vacutainer tubes. Plasma was separated from the collected blood by centrifugation at 3000 rpm for 5 minutes. Separate blood samples were collected from another group of anaesthetized animals in glass test tubes and allowed to coagulate for 30 min. Serum was separated by centrifugation at 3000 rpm for 20 minutes.

Plasma and serum samples were kept at – 20°C for biochemical analysis. The animals were sacrificed by cervical decapitation, the perfused liver of each animal was dissected out and washed with isotonic solution, and their wet weight was recorded. The liver homogenate was prepared using phosphate buffer solution for biochemical analysis. The biochemical parameters analyzed from serum, plasma, and liver homogenate was presented in the tables (1-4).

**1.16. Histopathological studies**

The livers were excised quickly and fixed in 10% formalin and stained with haemotoxylin and eosin and then observed under microscope for degeneration, fatty changes, necrotic changes and evidence of hepatotoxicity if any. Results of the histopathological studies are shown in the figure (4-7).

**2. Results**

**2.1. Thiobarbituric acid reactive substances (TBARS)**

The thiobarbituric acid assay is the most popular method of estimation of malondialdehyde level, which is an indication of lipid peroxidation and free radical activity. The increase in lipid peroxidation, a degradative process of membraneous polyunsaturated fatty acid has been suggested by the increase in malondialdehyde in ethanol induced toxicity in the liver. The increased lipid peroxidation results in changes in cellular metabolism of the hepatic and extra hepatic tissues, which ultimately leads to the whole cell deformity and cell death.

The levels of TBARS in liver tissues of ethanol intoxicated rats were significantly elevated when compared to the level of TBARS in control animals. The administration of herbal drugs *Phyllanthus amarus* and *Eclipta prostrata* at the therapeutic doses (1g/Kg. b.wt) showed maximum reduction in TBARS level. The standard hepatoprotective drug Silymarin maintained the decreased lipid peroxidation level to the normal limits in the liver. The results indicate that, the herbal drugs *Phyllanthus amarus* and *Eclipta prostrata* has very good hepatoprotective effect in liver damage. The results were presented in the Table 3.

**2.2. Superoxide dismutase (SOD)**

SOD is the major attractive metalloprotein in the antioxidant family. The increased synthesis of superoxide dismutase against superoxide anion radical production is an adaptive response
of the cell to synthesis increased mitochondrial SOD through the stimulation of gene transcription. The enzyme SOD was found to be decreased in ethanol intoxicated rats.

This is due to the low level of Zinc (a metal constituent of the enzyme SOD) in plasma and liver tissues. The low level of zinc was also found in alcoholic liver cirrhosis. In the present study, significant decrease in the activity of liver SOD in ethanol intoxicated rat was observed. The therapeutic treatment with Phyllanthus amarus and Eclipta prostrata herbal drug significantly improved the level of SOD in liver. This result indicates that, the herbal drug promoted the hepatoprotection by elevating free radical scavenging activity. Similar results were also observed in Silymarin treated rats. The results were presented in the Table 4.

2.3. Catalase (CAT)

The protective antioxidant enzyme next to SOD is catalase. CAT traps the harmful hydrogen peroxide and converts into water and oxygen. The activity of catalase was found to be decreased in ethanol intoxicated rats. The inhibition of catalase activity during ethanol induced toxicity may be due to the increased generation of reactive free radicals, which can create an oxidative stress in the cells.

The administration of herbal drugs Phyllanthus amarus and Eclipta prostrata inversed the catalase activity in the liver tissues and protected from the free radical induced oxidative stress. This results supports that, the antioxidant properties of the herbal drug was excellent as compared with the standard drug Silymarin. The results were presented in the Table 4.

2.4. Glutathione Reductase (GSH)

It is an important source of reducing equivalents during oxidative stress generated by reactive oxygen species. The higher level of ethanol intake develops cirrhosis and liver damage by enhancing lipid peroxidation in the liver. Acetaldehyde the toxic metabolite of ethanol depresses the liver and plasma glutathione level by conjugating with the sulphydryl groups of glutathione. In the present research work, we have observed the decreased level of glutathione in ethanol intoxicated rats.

The GSH depletion in hepatic mitochondria is considered the most important sensitizing mechanism in the pathogenesis of alcoholic liver injury. Treatment with Phyllanthus amarus and Eclipta prostrata herbal drugs had significantly improved the level of glutathione both in plasma and liver tissues. Similar results also observed with the standard drug Silymarin. The results were presented in the Table 5.

2.5. Glutathione peroxidase (GPx)

GPx is a selenium dependent enzyme has high potency in scavenging reactive free radicals. In the present experiments, the levels of glutathione peroxidase activity in liver was elevated during alcohol intoxication to compensate the free radical scavenging effect utilized by the GSH as the substrate. When GPx activity in liver increased, the glutathione level is decreased in ethanol fed rats. Treatment with the herbal drug Phyllanthus amarus and Eclipta prostrata significantly decreased the level GPx to normal level. The standard drug Silymarin showed equivalent effect in the GPx level in the ethanol intoxicated rats. The results were presented in the Table 5.
2.6. Vitamin E and C

Vitamin E and C are natural antioxidants found in variety of plant materials. Ascorbic acid is most powerful antioxidant under physiological conditions. It exists mostly in the reduced form. It can directly scavenge superoxide, hydroxyl radicals and single oxygen. The ascorbic acid reduces H$_2$O$_2$ to water via ascorbate peroxidase reaction. Vitamin – E is a chain breaking antioxidant. It can repair oxidizing radicals directly, and preventing the chain propagation step during lipid autoxidation. In our present research work, the decreased level of these vitamins was observed in ethanol intoxicated rats.

This may be due to the high level of oxidative stress during the intoxication. The reduced form of glutathione substrate (GSH) is required for the regeneration of vitamin C, which is intern necessary for the regeneration of vitamin E. The ascorbic acid functions as an aqueous phase antioxidant. Therapeutic treatment with the herbal drug *Phyllanthus amarus* and *Eclipta prostrata* in intoxicated rats significantly increased level of vitamin E and C through the influence of GSH regeneration. Thus, the herbal drugs exert a beneficial effect in regenerating the GSH through the recycling mechanism of these vitamins. The standard drug Silymarin has similar effect in GSH regeneration. The results were presented in Table 6.

2.7. Histopathological studies

In histological studies, hepatocytes of the normal control group showed a normal lobular architecture of the liver. Where as, the alcohol treated group the liver showed hepatocytic necrosis and inflammation also observed in the centrilobular region with portal triaditis. The *Phyllanthus amarus* treated group showed minimal inflammation with moderate portal triaditis and their lobular architecture was normal. While, *Eclipta prostrata* treated group showed minimal inflammation with moderate portal triaditis and their lobular architecture was normal. Silymarin treated group showed normal hepatocytes and their lobular architecture was normal. These histopathological results were presented in the Figure 4-7.

<table>
<thead>
<tr>
<th>Table 1: Phytochemical analysis test</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phytochemical Test for:</strong></td>
</tr>
<tr>
<td>Alkaloid</td>
</tr>
<tr>
<td>Flavanoid</td>
</tr>
<tr>
<td>Saponins</td>
</tr>
<tr>
<td>Tannins</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2: Thin Layer Promotography Analysis Solvent front from 16.3 c.m</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rf value</strong></td>
</tr>
<tr>
<td>0.215</td>
</tr>
<tr>
<td>0.319</td>
</tr>
<tr>
<td>0.233</td>
</tr>
<tr>
<td>0.503</td>
</tr>
<tr>
<td>0.184</td>
</tr>
<tr>
<td>0.288</td>
</tr>
<tr>
<td>0.423</td>
</tr>
</tbody>
</table>
Table 3: Effect of Phyllanthus amarus and Eclipta prostrata leaves extract on the Levels of TBARS in plasma and tissues of control and experimental rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Plasma (mg/dL)</th>
<th>Liver (mg/100g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.18 ± 0.012a</td>
<td>0.73 ± 0.05a</td>
</tr>
<tr>
<td>Alcohol control</td>
<td>0.60 ± 0.036b</td>
<td>2.95 ± 0.23b</td>
</tr>
<tr>
<td>Alcohol + Phyllanthus amarus ethanolic flower extract (200 mg/kg bw)</td>
<td>0.35 ± 0.025c</td>
<td>1.25 ± 0.10c</td>
</tr>
<tr>
<td>Alcohol + Eclipta prostrata ethanolic flower extract (200 mg/kg bw)</td>
<td>0.29 ± 0.019d</td>
<td>0.92 ± 0.08d</td>
</tr>
<tr>
<td>Alcohol + Silymarin (2.5 mg/kg)</td>
<td>0.22 ± 0.011a</td>
<td>0.77 ± 0.04a</td>
</tr>
</tbody>
</table>

Values are given as mean ± S.D. (n=6 rats)
Values not sharing a common Superscript letter differ significantly at P <0.05 (DMRT)

Table 4: Effect of Phyllanthus amarus and Eclipta prostrata leaves extract on the activities of superoxide dismutase (SOD), catalase in liver of normal and alcohol -induced hepatotoxicity in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Liver SOD (U/mg/protein)</th>
<th>Liver CAT (U/mg/protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9.37 ± 0.52a</td>
<td>57.0 ± 3.21a</td>
</tr>
<tr>
<td>Alcohol control</td>
<td>4.17 ± 0.18b</td>
<td>38.1 ± 2.15b</td>
</tr>
<tr>
<td>Alcohol + Phyllanthus amarus ethanolic flower extract (200 mg/kg bw)</td>
<td>6.79 ± 0.25c</td>
<td>44.9 ± 3.00c</td>
</tr>
<tr>
<td>Alcohol + Eclipta prostrata ethanolic flower extract (200 mg/kg bw)</td>
<td>9.37 ± 0.43a</td>
<td>57.8 ± 3.05a</td>
</tr>
<tr>
<td>Alcohol + Silymarin (2.5 mg/kg)</td>
<td>5.36 ± 0.30d</td>
<td>50.0 ± 4.06d</td>
</tr>
</tbody>
</table>

Values are given as mean ± S.D. (n=6 rats)
Values not sharing a common Superscript letter differ significantly at P <0.05 (DMRT)
Comparative Study on Hepatoprotective activity of \textit{Phyllanthus amarus} and \textit{Eclipta prostrata} against alcohol induced in albino rats

**Table 5:** Effect of \textit{Phyllanthus amarus} and \textit{Eclipta prostrata} leaves extract on the activities of glutathione peroxidase (GPx) and reduced glutathione (GSH) in liver of normal and alcohol -induced hepatotoxicity in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GPx (U\textsuperscript{c} mg/protein)</td>
</tr>
<tr>
<td>Control</td>
<td>8.37 ± 0.55\textsuperscript{a}</td>
</tr>
<tr>
<td>Alcohol control</td>
<td>3.93 ± 0.20\textsuperscript{b}</td>
</tr>
<tr>
<td>Alcohol + \textit{Phyllanthus amarus} ethanolic flower extract (200 mg/kg bw)</td>
<td>5.35 ± 0.31\textsuperscript{c}</td>
</tr>
<tr>
<td>Alcohol + \textit{Eclipta prostrata} ethanolic flower extract (200 mg/kg bw)</td>
<td>8.40 ± 0.46\textsuperscript{a}</td>
</tr>
<tr>
<td>Alcohol +Silymarin (2.5 mg/kg)</td>
<td>6.77 ± 0.44\textsuperscript{d}</td>
</tr>
</tbody>
</table>

Values are given as mean ± S.D. (n=6 rats)
Values not sharing a common Superscript letter differ significantly at P <0.05 (DMRT)

**Table 6:** Effect of \textit{Phyllanthus amarus} and \textit{Eclipta prostrata} leaves extract on levels of vitamin C and E in plasma of the normal and alcohol-induced hepatotoxicity in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vitamin C (mg/dL)</td>
</tr>
<tr>
<td>Control</td>
<td>1.40 ± 0.09\textsuperscript{a}</td>
</tr>
<tr>
<td>Alcohol control</td>
<td>0.65 ± 0.03\textsuperscript{b}</td>
</tr>
<tr>
<td>Alcohol + \textit{Phyllanthus amarus} ethanolic flower extract (200 mg/kg bw)</td>
<td>0.97 ± 0.06\textsuperscript{c}</td>
</tr>
<tr>
<td>Alcohol + \textit{Eclipta prostrata} ethanolic flower extract (200 mg/kg bw)</td>
<td>1.42 ± 0.08\textsuperscript{a}</td>
</tr>
<tr>
<td>Alcohol +Silymarin (2.5 mg/kg)</td>
<td>1.08 ± 0.07\textsuperscript{d}</td>
</tr>
</tbody>
</table>

Values are given as mean ± S.D. (n=6 rats)
Values not sharing a common Superscript letter differ significantly at P <0.05 (DMRT)
Figure 4: Light microphotographs of HE- stained sections (100X) of the formalin fixed liver of normal control group showing normal hepatic architecture.

Figure 5: Light microphotographs of HE- stained sections (100X) of the formalin fixed liver of alcohol control group showing severe hepatotoxicity
Arun. K, Dr. Balasubramanian. U

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3. Discussion

Alcohol is one of the most important and commonly used hepatotoxic agents in the experimental study of liver related disorders. The hepatotoxic effects of alcohol are largely due to its active metabolite, trichloromethyl radical [24] these activated radicals bind covalently to the macromolecules and induce peroxidative degradation of membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids (PUFA) [25]. This process leads to excessive formation and accumulation of lipids in tissues such as liver. Lipids from peripheral adipose tissue are translocated to liver for accumulation [26].

Hepatoprotective activity of any drug is the ability of its constituents to inhibit the aromatase activity of cytochrome P450 thereby favoring liver regeneration [27]. Free radicals induced lipid peroxidation is believed to be one of the major causes of cell membrane damage leading to a number of pathological situations. Liver is particularly susceptible to chemically induced injury due to its extensive metabolic capacity and cellular heterogeneity. Oxidative stress occurs, when there is an imbalance between reactive oxygen species (ROS) formation and scavenging by antioxidants. Excess generation of ROS can cause oxidative damage to biomolecules resulting in lipid peroxidation, mutagenesis and carcinogenesis [28].

The degree of lipid peroxidation in liver tissue was determined by measuring the levels of thiobarbituric acid reactive substances (TBARS). In the present study, significantly elevated levels of lipid peroxidation products such as TBARS observed in alcohol administered rats, which may be due to excessive formation of free radicals and activation peroxidation system resulting in hepatic and other cellular damage. On treatment with Phyllanthus amarus and Eclipta prostrata for a period of 21 days significantly decreased the levels of these lipoperoxidative products in plasma and tissues of alcohol induced rats. Researcher have reported that, Rubia cordifolia, Foeniculum vulgare, Swertia chirata, Eclipta alba, Myrica nagi, Terminalia chebula, Curcuma longa and Cuminum cuminum have possesses hepatoprotective activity in vivo and in vitro [29, 30, 31, 32, 33, 34, 35]. It’s well known that,
Ayurvedic medicines could reduce the generation of free radicals and increase free radicals scavenging mechanism.

Antioxidants or free radical scavengers are very important in protecting the cells against any damage induced by free radicals, which are produced continuously in cells either during phagocytosis or accidentally as by-product metabolites. Each biological system has certain antioxidant defense mechanisms against the aggregations of such free radicals. The balance of prooxidant-antioxidant system must exist in the cell, while the disturbance of antioxidant-proxidant balance causes oxidative stress [36].

The mechanisms to prevent or neutralize the free radical induced damage. This is accomplished by a set of endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and reduced glutathione (GSH). If the balance between ROS and antioxidant defenses is lost, the oxidative stress occurs which is through a series of events deregulates the cellular functions leading to various pathological conditions [37]. SOD is a sensitive index in hepatocellular damage and is the most sensitive enzymatic index in liver injury. It scavenges the superoxide anion to form hydrogen peroxide and thus diminishing the toxic effect caused by radical [38]. CAT widely distributed in all animal tissues, and which decomposes hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals [39]. GPx is a seleno-enzyme two third of which (in liver) is present in the cytosol and one third in the mitochondria. It catalyses the reaction of hydroperoxides with reduced glutathione to form glutathione disulphide (GSSG) and the reduction product of the hydroperoxides [13]. In the present study, decline in the activities of antioxidant enzymes like SOD, CAT, GPx, and GSH were observed in liver of alcohol treated rats, is a clear manifestation of excessive formation of free radicals and activation of lipid peroxidation system resulting in tissue damage.

GSH constitutes the first line of defense against free radicals and is a critical determinant of the tissue susceptibility to oxidative damage. Previous studies on the mechanism of alcohol induced hepatotoxicity reported that GSH plays a key role in detoxifying the reactive toxic metabolites of alcohol and that liver necrosis begins when the GSH stores are depleted [13]. Vitamin E (a-tocopherol) functions as antioxidant, mainly in and around the membrane/lipid bilayers acting as the chain breaking antioxidant [40]. It is considered the most important lipophilic antioxidant in biological tissues [41]. Ascorbic acid is an important dietary antioxidant. It significantly decreases the adverse effect of reactive species such that can cause oxidative damage to macromolecules such as lipids, DNA and protein [42].

Alcohol induced rats showed significant decrease in the levels of these enzymatic and nonenzymatic antioxidants in plasma and tissues. Rats treated with *Phyllanthus amarus* and *Eclipta prostrata* significantly increased the levels of these non-enzymatic antioxidants in tissues and plasma in alcohol induced rats. This could be due to free radicals scavenging property of *Phyllanthus amarus* and *Eclipta prostrata*, which indirectly helps to increase the antioxidants levels in, by decreasing the levels of lipid peroxidative products. It’s well known that, plant extract that contain falvonoids, alkaloids, tannins and coumarins, which posses free radical scavenging and antioxidant properties.

Further in the present investigation, phytochemical analysis of leaf extract revealed the presence of flavonoids, phytosterol, saponins, tannins and phenolic compounds and carbohydrates. flavanoids (Yoshikawa , Morikawa, Matsuda, Kashima 2003) and saponins (Baek, Kim, Kyung, Park 1996) are well known for their antioxidant and hepatoprotective activities. The literature has already documented the flavanoids are isolated from the leaves.
of Himalayan rhododendrons were found to have potent antioxidant property. Since Quercetin 3-O- beta-D-glucopyranosyl [1->6]-Oalpha-L-rhamnopyranoside, pectolinarinigen 7-O-rutinoside, 7,2’-dimethoxy-4’,5-methylenedioxyflavanone and related flavonoids are present in both plants (Kamil, Shafiullah, 1995).

The mechanism by which quercetin, a natural antioxidant, inhibit lipid peroxidation by blocking the enzyme xanthine oxidase (Cheng and Breen, 2000), Chelating iron (Da Silva, Diskala, Yamamoto, Moon, Tero, 1998) and directly scavenging hydroxyl, peroxy and superoxide radicals (De Whalley, Rankin, 1990) reveals its antioxidant properties. Quercetin also protects antioxidative defense mechanism by increasing the absorption of Vitamin C (Vinson and Bose, 1998). Quercetin has been shown to inhibit structural damage to proteins (Salvi, Carrapt, Tillement, Testa, 2001), the release and the protection of oxidative products generated by the respiratory burst in phagocytes (Zielińska, Kostrzewa, Lognatowicz, 2000). Quercetin has recently shown to be an INOS inhibitor, resulting in reduced nitric oxide (NO) and peronitrate generation (Autore, Rastrelli, Lauro, Marzocco, Sorrentino, 2001).

*Phyllanthus amarus* and *Eclipta prostrata* to rats didn’t show any alterations in the histology of liver, which shows these plants extract didn’t posses any toxic effect. Alcohol treated rats liver shows micro to macrovesicular fatty changes in the cytoplasm of hepatocytes shows the severe tubular degeneration and presence of desquamated lining epithelium in the lumen. Administration of *Phyllanthus amarus* and *Eclipta prostrata* to alcohol induced rats showed, moderate congestion and mild fatty changes of hepatocytes and mild congestion and haemosiderin pigments in brownish color in liver. Rats treated with both plat extract significantly minimized the pathological alterations in the histology of the liver, which shows the protective action of these plant extracts. Protective effects of *Phyllanthus amarus* and *Eclipta prostrata* observed on liver could be due to free radical scavenging, antioxidant and membrane stabilizing properties of the drug.

In conclusion, our research studies data suggest that, there were significant variations in the observed biochemical parameters. The level of TBARS in ethanol intoxicated rats increased two fold when compared with the control animals. The levels of GSH, SOD and CAT decreased significantly in the ethanol intoxicated rats.

The level of GPx was increased in the ethanol intoxicated rats. The value of vitamin E in both plasma and liver samples were less when compared with the control animals. Similarly, the value of vitamin C was also showed decreased level in plasma. Serum iron and copper levels were elevated to a higher level. The therapeutic administrations of *Phyllanthus amarus* and *Eclipta prostrata* leaves fine powder greatly change the biochemical parameters in the ethanol intoxicated rats and maintained well to the normal level. These results clearly suggest that, the *Phyllanthus amarus* and *Eclipta prostrata* have enormous hepatoprotective value.

Among the two plants *Phyllanthus amarus* has slightly high activity as compare to *Eclipta prostrata*. These herbal drugs have equivalent therapeutic value with the standards drug Silymarin. Moreover, it is very important to study the specific phytochemical compounds responsible for this hepatoprotective effect.

4. References

Comapartive Study on Hepatoprotective activity of Phyllanthus amarus and Eclipta prostrata against alcohol induced in albino rats


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