Comparative study on Hepatoprotective activity of Aegle marmelos and Eclipta alba against alcohol induced in albino rats
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doi:10.6088/ijes.00202020002

ABSTRACT

The present study suggest that, Aegle marmelos and Eclipta alba were shows the 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 Aegle marmelos and Eclipta alba 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 Aegle marmelos and Eclipta alba have enormous hepatoprotective value. Among the two plants Aegle marmelos has slightly high activity as compare to Eclipta alba. 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.

Key words: Aegle marmelos, Eclipta alba, hepatoprotective activity

1. Introduction

Aegle marmelos, commonly known as Bael, is a spiny tree belonging to the family Rutaceae. It is an indigenous tree found in India, Myanmar, Pakistan and Bangladesh. The leaves, roots, bark, seeds and fruits are edible and medicinal values. The medicinal properties of this plant have been described in the Ayurveda. In fact, as per Charaka (1500 B.C) no drug has been longer or better known or appreciated by the inhabitants of Indiathan the Bael. The leaves of Bael are astringent, a laxative, and an expectorant and are useful in treatment of ophthalmia, deafness, inflammations, cataract, diabetes, diarrhoea, dysentery, heart palpitation, and asthmatic complications (Kirtikar and Basu, 1993). It has been claimed the leaf of Aegle marmelos posses contraceptive efficacy (Bhattacharya, 1982). Fresh aqueous and alcoholic leaf extracts of Aegle marmelos were reported to have a cardio tonic effects in mammals (Haravey, 1968 and Nadkarni, 2000). Aegle marmelos leaf extract has been reported to regenerate damaged pancreatic beta cells in diabetic rats (Das et al., 1996) and increased the activities of peroxidase in the liver tissues of Isoproterenol treated rats (Rajadurai et al., 2005).

An aqueous decoction of the leaves has been shown to possess a significant hypoglycemic effect (Karunanayeke et al., 1984). Aegle marmelos leaf extract was found to be a potential antioxidant drug, which reduces the blood sugar level in alloxan induced diabetic rats (Sabu and Ramadasan, 2004). It was found to be as effective as insulin in the restoration of blood
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glucose and body weight to normal levels on hyperglycemic state (Seema et al., 1996). The ethanolic extract of Aegle marmelos leaf possesses anti spermatogenic activity (Sur et al., 1999) and aqueous extract of the leaf has anti motility action on spermatozoa in rats (Sur et al., 2002).

Eclipta alba Hassk. (Bhringaraja, Fam : Compositae) is a perennial shrub which grows widely in moist tropical countries. Different uses have been reported for this shrub. It is used as alterative, anthelmintic, expectorant antipyretic, antiasmatic, tonic, deobstruent in hepatic and spleen enlargement, in skin diseases and as a substitute for Taraxacum (a popular liver tonic)1,2. It is good for the diseases of spleen, stomatitis, toothache, hemicrania, fever, pain in liver and cures vertigo (Yunani). Its juice in combination with honey is administered for Cataract and Jaundice1, (Chopra, 1996). In Gujarat district and Punjab, it is used externally for ulcers and as an antiseptic for wounds in cattle. Recently Chandra, 19873, has observed a significant anti-inflammatory activity of the powder in rats. Since no work has carried out on Comparative effects of hepatoprotective activity of these plants. Hence the present study undertaken to evaluate the hepatoprotective of these plants against alcohol Induced in experimental animal models.

2. Materials and Methods

2.1. Preparation of Plant Material

Leaves of Aegle marmelos and Eclipta alba 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 500 g 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.

2.2. Experimental Design

In this experiments 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 ± 20C, 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.

2.3. Nut protocol

In order to study the effect of ethanolic and aqueous extract of Aegle marmelos (200 mg/kg bw) and Eclipta alba (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.

2.4. Treatment Protocol

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

GROUP II

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International Journal of Environmental Sciences Volume 2 No.2, 2011
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Hepatotoxic control (n=6, the animals were given alcohol for 21 Days)

GROUP III
Treatment group (n=6, the animals were given alcohol + Aegle marmelos for 21 days)

GROUP IV
Treatment group (n=6, the animals were given alcohol + Eclipta alba 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.

2.5. 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 and serum samples were separated 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 present in the Graph (1-8).

2.6. 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.

3. Results

3.1. Thiobarbituric acid reactive substances (TBARS)

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 Aegle marmelos and Eclipta alba 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 Aegle marmelos and Eclipta alba has very good hepatoprotective effect in liver damage Figure 1).

3.2. Glutathione Reductase (GSH)

In the Glutathione Reductase test, showed the decreased level of glutathione in ethanol intoxicated rats. Treatment with Aegle marmelos and Eclipta alba herbal drugs had significantly improved the level of glutathione both in plasma and liver tissues. Similar results also observed with the standard drug Silymarin. and this results were presented in the Figure 5.
3.3. Superoxide dismutase (SOD)

The significant decrease in the activity of liver SOD in ethanol intoxicated rat was observed. The therapeutic treatment with *Aegle marmelos* and *Eclipta alba* 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. and these results were presented in the Figure 6.

3.4. Catalase (CAT)

The administration of herbal drugs *Aegle marmelos* and *Eclipta alba* 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( Figure 7).

3.5. 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 *Aegle marmelos* and *Eclipta alba* 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 Figure 8.

3.6. Vitamin E and C

The herbal drug *Aegle marmelos* and *Eclipta alba* 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 (Figure 6 &7)

3.7. Iron and Copper

The therapeutic treatment with herbal drugs *Aegle marmelos* and *Eclipta alba* decreased the level of serum iron and copper level to the normal level found in control animals. The action of standard drug Silymarin in serum iron and copper level in ethanol intoxicated rat was equivalent to the *Aegle marmelos* and *Eclipta alba* herbal drug (Figure 8).

3.8. 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 andinflammation also observed in the centrilobular region with portal triaditis. The *Aegle marmelos* treated group showed minimal inflammation with moderate portal triaditis and their lobular architecture was normal. While, *Eclipta alba* 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 Figures (9-12).
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**Figure 1:** Effect of *Aegle marmelos* and *Eclipta alba* leaves extract on the leaves of TBARS in plasma and tissues of control and experimental rats

**Figure 2:** Effect of *Aegle marmelos* and *Eclipta alba* leaves extract on the activities of Glutathione Reductase (GSH) catalase in liver of normal and alcohol-induced hepatotoxicity in rats
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**Figure 3:** Effect of Aegle marmelos and Eclipta alba leaves extract on the activities of Superoxide Dismutase (SOD) catalase in liver of normal and alcohol-induced hepatotoxicity in rats

**Figure 4:** Effect of Aegle marmelos and Eclipta alba leaves extract on the activities of catalase in liver of normal and alcohol-induced hepatotoxicity in rats
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**Figure 5:** Effect of *Aegle marmelos* and *Eclipta alba* leaves extract on the activities of Glutathione Peroxide (GPx) and reduced Glutathione (GSH) in liver of normal and alcohol-induced hepatotoxicity in rats.

**Figure 6:** Effect of *Aegle marmelos* and *Eclipta alba* leaves extract on the activities of Vitamin E in plasma of the normal and alcohol-induced hepatotoxicity in rats.
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Figure 7: Effect of Aegle marmelos and Eclipta alba leaves extract on the activities of Vitamin C in plasma of the normal and alcohol-induced hepatotoxicity in rats

Figure 8: Effect of Aegle marmelos and Eclipta alba leaves extract on the activities of Fe & Cu in experimental and control rats
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Figure 9: Light microphotographs of HE- stained sections (100X) of the formalin fixed liver of normal control group showing normal hepatic architecture.

Figure 10: Light microphotographs of HE- stained sections (100X) of the formalin fixed liver of alcohol control group showing severe hepatotoxicity

Figure 11: Light microphotographs of HE- stained sections (100X) of the formalin fixed liver of Aegle marmelos leaf extract pre-treated groups (200mg/kg, p.o.)
Figure 12: Light microphotographs of HE- stained sections (100X) of the formalin fixed liver of Eclipta alba leaf extract pre-treated groups (200mg/kg, p.o.)

4. Discussion

The ability of a hepatoprotective drug to reduce the injurious effects or to preserve the normal hepatic physiological mechanisms, which have been disturbed by a hepatotoxin, is the index of its protective effects. Although serum enzyme levels and ascorbic acid in urine are not a direct measure of hepatic injury, they show the status of the liver. The lowering of enzymes level are definite indication of hepatoprotective action of the drug. The present investigation also revealed that the given dose of alcohol produced significant elevation in TBARS, GSH, SOD, CAT, GPx, Vitamin E and C and reduced Iron and Copper levels indicating all impaired liver function and these parameters have been reported to sensitive indicator of liver injury (Molander et al., 1955; Zimmerman and Seeff, 1970). The massive production of reactive species may lead to depletion of protective physiological moieties (glutathione and tocopherols etc) and ensuing widespread propagation of the alkylation’s as well as peroxidation, causing damage to the macromolecules in vital biomembranes.

The present study reveals that the effect of pretreatment of ethanolic extract of leaves of Aegle marmelos and Eclipta alba had been effective in offering protection, which is comparable to Silymarin. Alcohol treatment caused classical fatty liver as indicated by significant in tissue cholesterol. The tissue cholesterol levels reduced after natural recovery and Silymarin treatment. Aegle marmelos and Eclipta alba treatment significantly improved TBARS, GSH, SOD levels in alcohol treated animals. The disturbance in the transport function of the hepatocytes as a result of hepatic injury causes the leakage of enzymes from cells due to altered permeability of membrane (Recnagel, 1983). Hepatotoxic action of alcohol begins with changes in endoplasmic reticulum which result in loss of metabolic enzymes located in the intracellular structure (Conney and Burns, 1959).

The ethanolic extract of leaves of Aegle marmelos and Eclipta alba when administered orally to rats showed a significant dose dependent hepatoprotective activity. A very important observation with these Aegle marmelos and Eclipta alba extract at higher dose in improvement of highly effective in CAT, GPx, Vitamin E and C and decreased Iron and Copper levels. The histopathological studies are the evidence of efficacy of drug as protectant. Simultaneous treatment of ethanolic extract with alcohol exhibits less damage to...
the hepatic cells as compared to the rats treated with pure alcohol alone. Intralobular veins though are damaged but to a lesser extent. Endothelium is disrupted at places. Hepatic cells adjoining to intralobular vein show atrophy. The sections of the liver treated with ethanolic extract of leaves of both plants and alcohol reveals better hepatoprotective activity. Almost negligible damage to a few hepatocytes present in the close vicinity of intralobular vein is observed. Endothelium lining is almost smooth except one or two places. Hepatocytes show normal appearance only some cells show higher numbers of vacuoles in the cytoplasm. The results of histopathological study also support the results of biochemical parameters.

Ascorbic acid is formed as a metabolite of glucose and galactose in rat liver microsomes via the glucoronic acid pathway and is excreted in urine. The enzyme UDP glucose dehydrogenase and UDP glucuronide transferase are responsible for its formation in the liver microsomes. Its formation and excretion is altered by several drugs and substances that affect the drug metabolizing enzyme systems (Conney and Burns, 1959; Satyanarayana et al., 1988). Reduction in ascorbic acid excretion in alcohol treated rats may reflect the inhibition of such enzymes. Alteration in urinary ascorbic acid excretion appears to be reflecting ascorbic acid level in liver. Hence, the reduction in urinary ascorbic acid excretion can be used as an index for alcohol produced hepatotoxicity (Hesse and Klinger, 1969). The ethanolic extract of Aegle marmelos and Eclipta alba it might antagonize alcohol produced inhibition of enzymes responsible for ascorbic acid formation similar to Silymarin. The results in the present study indicate that reduction in urinary ascorbic acid excretion by Aegle marmelos and Eclipta alba extract as an index for hepatoprotective activity against alcohol induced hepatotoxicity.

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 et al., 2003) and saponins (Baek et al., 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, pectolinarigenin 7-O-rutinoside, 7,2'-dimethoxy-4',5-methylenedioxyflavanone and related flavonoids are present in both plants (Kamil et al., 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 et al., 1998) and directly scavenging hydroxyl, peroxo and superoxide radicals (De Whalley et al., 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 et al., 2001), the release and the protection of oxidative products generated by the respiratory burst in phagocytes (Zielinska et al., 2000). Quercetin has recently shown to be an INOS inhibitor, resulting in reduced nitric oxide (NO) and peronitrate generation (Autore et al., 2001).

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