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Research Article

HEPATOPROTECTIVE ACTIVITY OF *AEGLE MARRMELOS* AGAINST ETHANOL INDUCED HEPATOTOXICITY IN RATS

HIRAL MODI[1], VISHNU PATEL[2], KOMAL PATEL[3]

[1] JJT University, Jhunjhunu, Rajsthan, India; [2] APMC college of Pharmaceutical Education & Research, Himmatnagar, Gujarat, India; [3] Saraswati Institue of Pharmaceutical Sciences; **Email:** starhir86@gmail.com

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ABSTRACT

This study investigated heapatoprotective activity of various extracts *of Aegle Marmelos*, belonging to the family Rutaceae, in Wistar Female rats with liver damage induced by ethanol. Herbal drugs play crucial role in treatment of various diseases due to its antioxidant property. It was found that AMCL, AMAL & AMAQ, at a dose of 500 mg/kg body weight exhibited hepatoprotective effect by lowering the Serum Glutamate Pyruvate Transaminase (SGPT), Serum Glutamate Oxaloacetate Transaminase (SGOT), alkaline phosphate and total bilirubin to a significant extent. The groups treated with various extract of *A.Marmelos & Silyamarine* shows significant (P<0.001) restoration of liver weight & liver volume nearer to normal control group. Since results of biochemical studies of blood samples of ethanol treated rats showed significant increase in the levels of serum enzyme activities, reflecting the liver injury caused by ethanol and blood samples from the animals treated with AMCL & AMAQ showed significant decrease in the levels of serum markers, indicating the protection of hepatic cells against ethanol induced hepatocellular injury. The effects of AEAC and AQEAC were comparable with standard drug silymarin.

Keywords: Antioxidant, Heaptotoxicity, *Aegle Marmelos,* Ethanol

INTRODUCTION

Oxidative stress play important role in many diseases including liver diseases. The production of free radicals can be controlled by antioxidant system in living system. The liver is a key organ regulating homeostasis within the body & involved in almost all the biochemical pathway related to metabolism of fats, carbohydrates, proteins, hormones, synthesis and storage of vitamins, formation of bile, manufacture of antibodies, detoxification of drugs and other toxins, excretion of bilirubin & heavy metals. [1] Liver injury caused by toxic chemicals and certain drugs has been recognized as a toxicological problem. Hepatotoxicity means chemical-driven liver damage and chemicals that cause liver injuries are called hepatotoxins. The liver plays a central role in transforming and clearing chemicals and hence it is susceptible to the toxicity from these agents. Such unexpected toxicities appear to be the consequence of the unique vascular, secretary, synthetic, and metabolic features of the liver. Hepatocytes are highly reliant on ATP for ureagenesis, gluconeogenesis, and fatty acid metabolism among many other metabolic processes. So on long term deprivation of oxygen it leads to hepatocellular necrosis. Hepatotoxicity is one of very common aliment resulting into serious debilities ranging from severe metabolic disorders to even mortality. [2]

Jaundice & hepatitis are two major disorders of liver that increase the risk for mortality. Currently treatment options for hepatotoxicity are very limited. Free radicals initiate the damaging process through covalent binding to cell macromolecules leading to lipid peroxidation, oxidation of DNA and protein cause liver damage. Free radicals are found to be responsible for the toxic effects of xenobiotics. Flavonoids are a family of antioxidants that protect the cell from oxidative stress. Flavonoids and phenolic compounds, which have been reported to exert multiple biological effects, including antioxidant, free radical scavenging abilities, anti-inflammatory activity, anticarcinogenic activity etc. Flavonoids used for the prevention and cure of various diseases which is mainly associated with free radicals. [3]

Aegle Marmelos is a fruit-bearing tree indigenous to dry forest on hills and plains of central and southern Asian countries & belongs to the family Rutaceae. It has many Indian names, depending on the geographical region or the language.[4] Different parts of *A. Marmelos* have been investigated by several workers and found to contain coumarins, alkaloids, triterpenes, sterols and essential oils & flavanoids. [5] The objective of the present study was to investigate the hepatoprotective activity of the various leaves extract of the leaves of *Aegle Marmelos* using ethanol induced hepatotoxic rats.

MATERIAL & METHODS

Collection of Plant Material

The fresh leaves of *Aegle Marmelos were* collected from Ayurvedic Botanical Garden, Gandhinagar. Leaves were identified and authenticated. Fresh leaves dried under shade. The coarsely powdered fresh leaves were stored in polythene bags at room temp until required.

Extract Preparation

Successive Soxhlet Extraction

The dried and coarsely powdered material of *Aegle Marmelos* (100g) was subjected to successive extraction in a Soxhlet apparatus with different solvents like petroleum ether, chloroform and methanol. After each extraction, the solvent was recovered using distillation assembly. In vacuum after evaporation of ethanol from the ethanolic extract, residues were obtained and were stored in desiccators. Fresh Juice was prepared by just mixing of fine powdered material of *Aegle Marmelos* with water to make juice.

Animals

Wistar rats weighing 150-250g procured from central animal facility of Institute. The animals were maintained in controlled temperature (24 ± 2^{0}C) as well as humidity (60-70%) in 12 –h light –dark cycles with standard diet and water will provide ad *libitium*. The care and the use of these animals were in accordance with the guidelines of the CPCSEA. An experimental protocol was approved by IAEC.

Hepatoprotective Activity

The animals (wistar female rats) weighing between 200 to 250 gm were divided into main four groups, six animals in each group. Animals in Group 1 were treated with Vehicle only twice a day P.O for 25 days served as Normal control Group. Group 2 animals were treated with 40% ethanol 3.76 gm/kg twice a day P.O for 25 days Served as Positive control Group. Others animals were pretreated twice daily with vehicle containing Petroleum Ether extract (AMPE), Chloroform extract (AMCL), Alcoholic Extract (AMAL), Fresh juice (AMAQ) of *Aegle Marmelos* 500mg/kg P.O for 25 days & *Silymarin* 100 mg/kg P.O for 25 days, 1 hour before Ethanol administration. At the termination day, animals were anaesthetized using anesthetic ether and blood collected from retro orbital puncture. The level of SGPT, SGOT, TB and ALP were estimated as per the standard procedures described by manufacturer using serum kit.

Statistical Analysis

The results were expressed as mean ± SEM, where n represents the number of rats. Statistical difference between two means determined by one-way ANOVA followed by Dunnett t-test by using statistical computer software Graph pad Prism 5.0. Only those mean values showing statistical difference P<0.05 was considered as statistically significant.

RESULTS

The results of the present study showed that, the levels of SGPT [137.5±3.48 U/L], SGOT [309.4±4.45], ALP [111.3±3.61], TB [1.89±0.09] were significantly increased in hepatotoxin treated group (P<0.001) (Group 2) when compared with control group (Group 1). Administration various leaf extracts (500 mg/kg) of *A.Marmelos* treated rats showed significant reduction in the level of SGOT, SGPT, ALP, TB when compared with hepatotoxin treated rats. Pretreatment of *A. Marmelos* various leaves extracts (AMCL, AMAL) in ethanol treated rats showed significant (P< 0.001) decrease in level of SGPT, SGOT, ALP as shown in Figure 1. Pretreatment with AMAQ in ethanol intoxicated rats shows significant reduction in SGPT (p<0.001), & SGOT & ALP (p<0.01) shown in Figure 1.

Administration various leaf extracts (500 mg/kg) of *A.Marmelos* treated rats showed significant reduction in the level of TB when compared with hepatotoxin treated rats. Pretreatment of *A. Marmelos* various leaves extracts (AMCL, AMAQ, AMPE, SL) in ethanol treated rats showed significant (P< 0.001) decrease in level of TB as shown in Figure 2. Pretreatment with AMAL in ethanol intoxicated rats shows significant reduction in TB (p<0.01) as shown in figure 2.

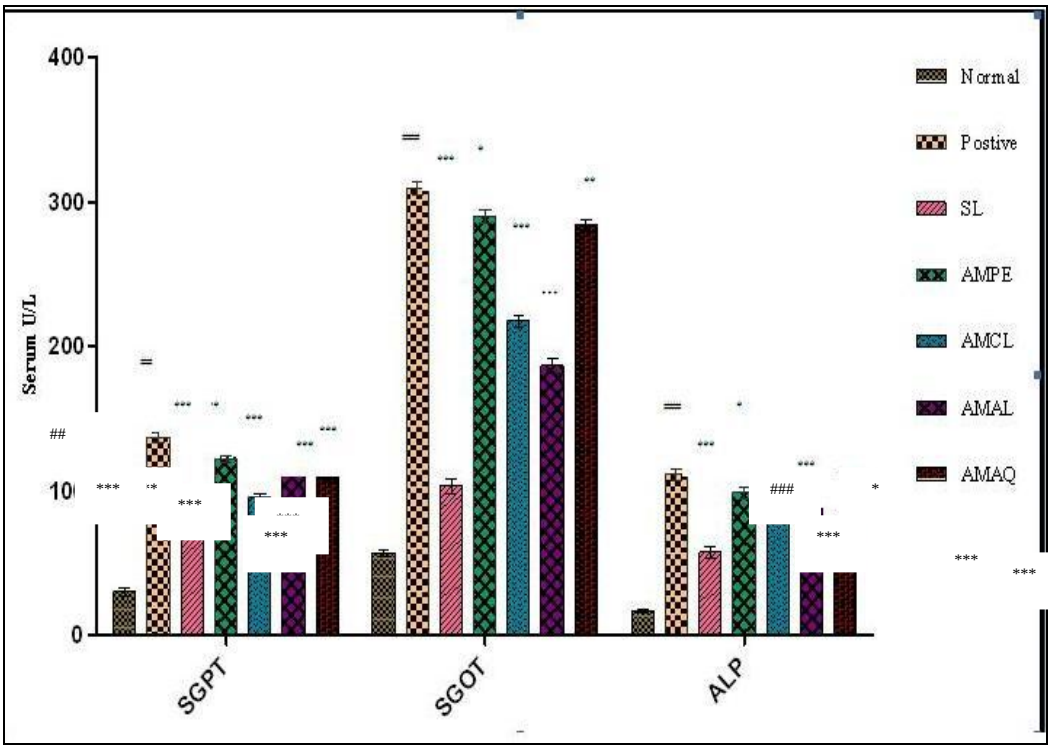

Figure 1: Effect of Various extracts (AMPE, AMCL, AMAL, AMAQ, SL) of *A.Marmelos leaves* on different serum biochemical parameters (SGPT, SGOT, ALP in U/L) in ethanol (50 mg/kg) induced hepatic damage in rats. Group I: Normal control, Group II: Toxin control Ethanol, Group III: SL + Ethanol Group IV: AMPE + Ethanol, Group V: AMCL + Ethanol, Group VI: AMAL+ Ethanol, Group VII: AMAQ+ Ethanol. Values are expressed in Mean ± S.E.M. Where n=5. ### P<0.001 designated as normal control versus disease control group. *p < 0.05, **p < 0.01, ***p < 0.001 as compared with toxin control group.

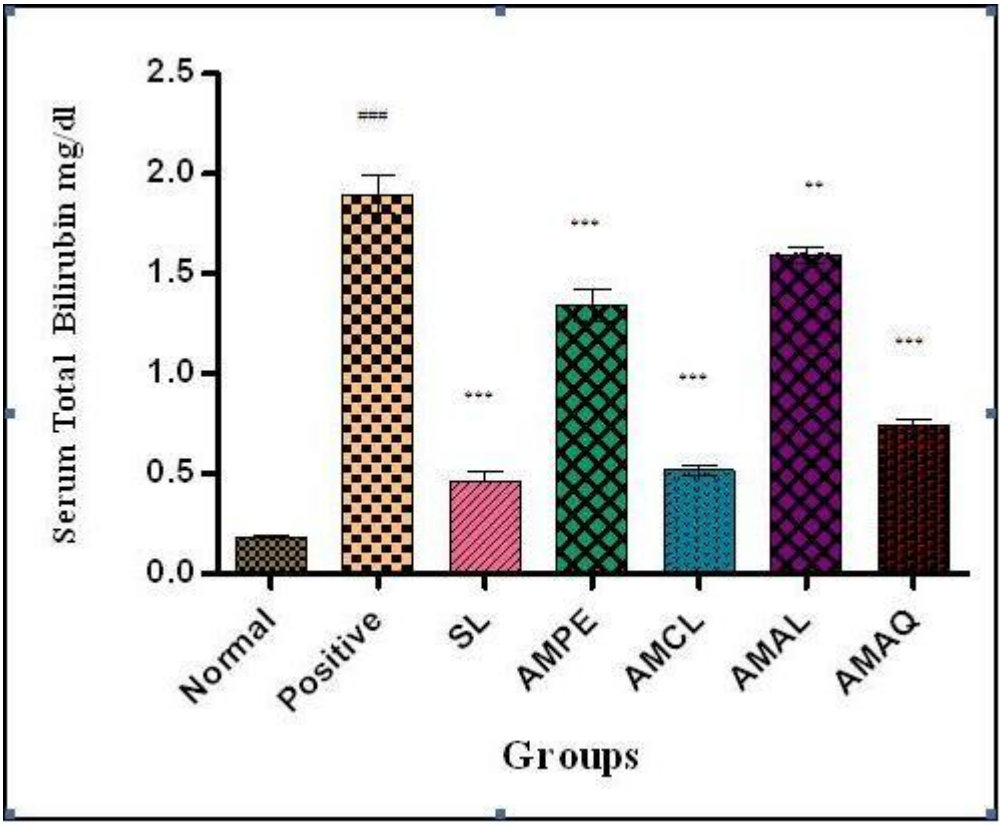

Figure 2: Effect of Various extracts (AMPE, AMCL, AMAL, AMAQ, SL) of *A.Marmelos leaves* on different serum biochemical parameters Total Bilirubin mg/dl in ethanol (50 mg/kg) induced hepatic damage in rats. Group I: Normal control, Group II: Toxin control Ethanol, Group III: SL + Ethanol Group IV: AMPE + Ethanol, Group V: AMCL + Ethanol, Group VI: AMAL+ Ethanol, Group VII: AMAQ+ Ethanol. Values are expressed in Mean ± S.E.M. Where n=5. ### P<0.001 designated as normal control versus disease control group. *p < 0.05, **p < 0.01, ***p < 0.001 as compared with toxin control group.

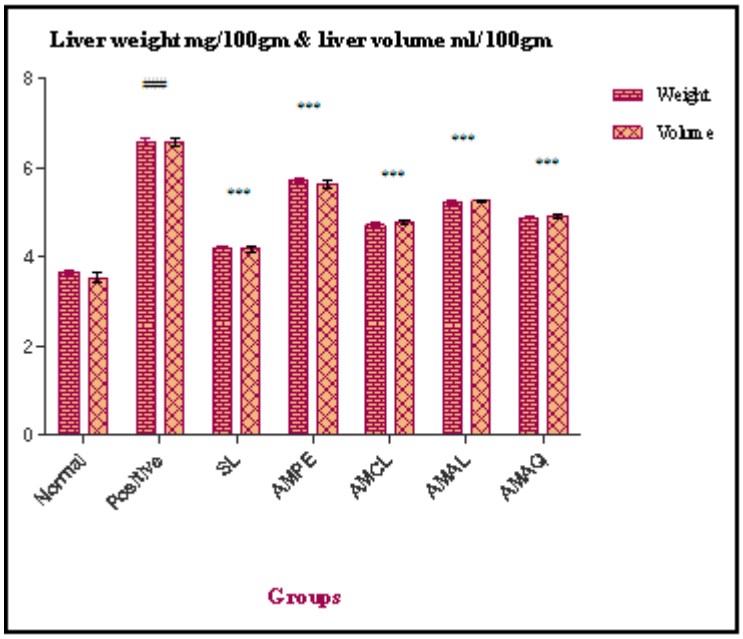

Figure 3: Effect of Various extracts (AMPE, AMCL, AMAL, AMAQ, SL) of *A.Marmelos leaves* on different Physical parameters Liver weight & Volume in ethanol (50 mg/kg) induced hepatic damage in rats. Group I: Normal control, Group II: Toxin control Ethanol, Group III: SL + Ethanol Group IV: AMPE + Ethanol, Group V: AMCL + Ethanol, Group VI: AMAL+ Ethanol, Group VII: AMAQ+ Ethanol. Values are expressed in Mean ± S.E.M. Where n=5. ### P<0.001 designated as normal control versus disease control group. ***p < 0.001 as compared with toxin control group.

The groups treated with various extract of *A.Marmelos & Silyamarine* shows significant (P<0.001) restoration of liver weight & liver volume nearer to normal control group. The results are shown in Figure 3.

DISCUSSION

Liver is one of the important organs of the body hence damage to the liver leads to sever pathological problems or to the end of life. Mitochondria are prominent targets for the hepatotoxicity of many drugs. Dysfunction of these vital cell organelles results in impairment of energy metabolism and an intracellular oxidant stress with excessive formation of reactive oxygen species and peroxynitrite. Formation of reactive oxygen species (ROS) oxidative stress and hepatocellular injury have been implicated to alcoholic liver disease. Ethanol produced to constellation of dose related deleterious effect in liver. In chronic, an alcoholic, heaptomegaly occurs due to accumulation of lipids & proteins in hepatocytes with an impaired secretion by hepatocytes. Water is retiand in the cytoplasm of heaptocyted leading to enlargement of liver cells, resulting in increase in total liver mass, volume.[6] Oxidative stress is one major factor in etiology of liver injury mainly by kuffer cells thorough the action of Substance endotoxin, which is released by certain gm-ve bacteria which I present in intestine, activate the kuffer cells to generate ROS & proinflammatory cytokines (TNF-alpha ,IL-1) both of them leads to liver injury.[7] Two major enzyme systems are involved in the metabolism of alcohol in the liver: ADH and the microsomal ethanol-oxidizing system (MEOS). ADH converts alcohol to acetaldehyde by removing hydrogen. Then a second enzyme, aldehyde dehydrogenase in hepatic mitochondria, oxidizes acetaldehyde to acetate by removing additional hydrogen and adding oxygen. Acetaldehyde promotes cell death by depleting glutathione levels which impairs a major defense mechanism against oxidative damage. Lipid peroxidation results in the formation of more free radicals which can further damage cell and organelle membrane causing more liver cell injury[8] inducing oxidative damage.

Furthermore, in present study in Ethanol intoxicated (Positive Control) group increase in the levels of serum Total bilirubin reflected the level hepatic damage and increase of transaminases level were the clear indications of cellular leakage and loss of functional integrity of cell membrane. Formation of ROS, oxidative stress and hepatocellular injury has been implicated to alcoholic liver disease. [9]

In present study significant (P< 0.001) increase in level of bilirubin, ALP SGPT & SGOT was found in ethanol intoxicated rats compare to normal rats which clearly indicates cellular damage and loss of functional integrity of hepatic cell membrane. Serum level of SGPT can increase due to damage of the tissue producting acute hepatic necrosis, such as viral hepatitis & cholestatis. Pretreatment of *Aegle Marmelos* various leaves extracts (AMCL, AMAL, AMAQ) in ethanol treated rats showed significant (P< 0.001) decrease in level of SGPT. Pretreatment with AMPE in ethanol intoxicated rats shows significant (P< 0.01) decrease in SGPT compare to ethanol treated rats which is indication of hepatoprotective activity.

SGOT is mithochondrial enzyme released from heart, liver, skeletal muscle; kidney .Liver toxicity elevated the level of serum SGOT due to damage of the tissue producting acute hepatic necrosis, such as viral hepatitis & cholestatis. Pretreatment of *Aegle Marmelos* various leaves extracts (AMCL, AMAL) in ethanol treated rats showed significant (P< 0.001) decrease in level of SGOT. Pretreatment with AMAQ in ethanol intoxicated rats shows significant (P< 0.01) decrease in SGOT compare to ethanol treated rats while pretreatment with AMPE in ethanol intoxicated Rats show significant (P<0.1) decrease in SGOT which is indication of hepatoprotective activity.

In case of toxic liver, ALP (Alkaline Phosphate) level are very high, which may be due to defective hepatic excretion or by increased production of ALP by hepatic parenchymal or duct cells.[10] Pretreatment of *Aegle Marmelos* various leaves extracts (AMCL, AMAL, AMAQ) in ethanol treated rats showed significant (P< 0.001) decrease in level of ALP. Pretreatment with AMPE in ethanol intoxicated rats shows significant (P< 0.1) decrease in ALP compare to ethanol treated rats.

In case of toxic liver, bilirubin level raised due to impaired hepatic uptake of unconjugated bilirubin.Such situation can occur in generalized liver injury, obstruction to biliary excretion into duodenum, in haemolysis & defects in hepatic uptake & conjugation of bilirubin pigment such Gibert's Disease. Pretreatment of *Aegle Marmelos* various leaves extracts (AMCL, AMPE, and AMAQ) in ethanol treated rats showed significant (P< 0.001) decrease in level of ALP. Pretreatment with AMAL in ethanol intoxicated rats shows significant (P< 0.01) decrease in ALP compare to ethanol treated rats.

Phytochemical screening revealed that AMAL, AMCL & AMAQ contains active pharmacological constituents such as flavonoids, alkaloids, phytosterols and phenolic compounds. However, it has been already reported that such phyto constituents like phenolic compounds, flavonoids, tannins[11] are known to possess hepatoprotective activity in various experimental models. Therefore it has been suggest that the better hepatoprotective activity shown by the AMCL and AMAQ can be because of these active phyto constituents present in the plant which is being also confirmed by the biochemical and physical parameters.

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