

HEPATOPROTECTIVE EFFECT OF *MUSSAENDA ERYTHROPHYLLA* AND *AEGLE MARMELOS*
IN ETHANOL INDUCED RAT HEPATOTOXICITY MODELRojin T.S¹, Sukanya Shetty^{2*} and Rajendra Holla¹¹Department of Pharmacology, KSHEMA, Nitte University, Mangalore, Karnataka, India-575018²Department of Biochemistry, KSHEMA, Nitte University, Mangalore, Karnataka, India-575018*Corresponding Author: E-mail: tsrojin@yahoo.co.in**ABSTRACT**

Aim: To assess the protective effect of ethanolic leaf extract of *Mussaenda erythrophylla* and *Aegle marmelos* in ethanol induced hepatotoxicity.

Methods: The ethanolic extract *M. erythrophylla* (ME) and *A. marmelos* (AM) studied for its hepatoprotective effect on alcohol induced acute liver damage on Wistar albino rats. The degree of protection was measured by using biochemical parameters such as serum glutamate oxalate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), total bilirubin (TBL), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione (GSH), and total antioxidant (TAC) levels.

Results: Alcohol treated group had enhanced levels of SGPT, SGOT, total bilirubin ($p < 0.05$) and decreased levels of GSH, SOD and GPx ($p < 0.05$) when compared with control group. Treatment with silymarin and 200mg/kg of *M. erythrophylla* and *A. marmelos* leaf extract had significantly ($p < 0.01$) brought down the elevated levels of SGPT, SGOT, and total bilirubin and an increase in the levels of GSH, SOD, GPx ($p < 0.001$) and total antioxidant. ($p < 0.0001$)

Conclusion: The results showed that ethanolic extract of *M. erythrophylla* and *A. marmelos* leaves extracts possesses significant hepatoprotective activity.

Key words: Alcohol, *Aegle marmelos*, *Mussaenda erythrophylla*, Hepatotoxicity.

INTRODUCTION

Liver is the major drug-metabolizing and drug-detoxifying organ of the body. It is continuously been expose to environmental toxins, carcinogens, drugs, insecticides, industrial chemicals hepatotoxins, which lead to impairment of its functions (Preussmann R, et al, 1978). These agents cause liver damage by lipid peroxidation, oxidative damage, and free radical generation. Even though free radicals are essential components to many biochemical processes and an important factor of aerobic metabolism but can be deleterious to health (Khalid Rahman, 2007). Generation of free radicals results in DNA, protein and lipid damage, which causes chromosomal defects. Oncogene activation, atherosclerosis, rheumatoid arthritis, leukemia, malignant neoplasia, degeneration process of aging and cardiovascular disease have been found in relation with free radical generation (Lea AJ, et al, 1966). Antioxidants have been reported to prevent oxidative damage caused by free radicals by interfering with the oxidation process through radical scavenging (Buyukokuroglu ME et al, 2001). Alcohol is one of the main causes of end stage liver disease worldwide. Alcoholic liver disease is the second most common reason for liver transplantation in US. (Mandayam S, et al 2004). Chronic alcoholism is one of the common cause for free radical insult in the liver. Alcohol metabolism is directly involved in the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), which can lead to generation of free radicals and increased peroxisomal oxidation of fatty acid, which would affect functions of the antioxidant system in our body (Das SK, et al 2007, Wang XD et al 1999) Therefore, antioxidant status of the living system is very much important in regulating and restoring the normal functions of the body.

Herbal drugs are playing an important role in health care programs worldwide. There is a resurgence of interest in herbal medicines for treatment of various diseases has increased and the active plant extracts are frequently screened for new drug discoveries (Sabu MC et al 2004). The present study has undertaken to evaluate the hepatoprotective effect of *M. erythrophylla* and *A. marmelos* in ethanol induced hepatic damage, in experimental animal models.

MATERIALS AND METHODS

Instruments

The instruments which have been used for the study are

Soxhlet apparatus

Homogenizer

High speed cold refrigerated centrifuge

Spectrophotometer

Animals

Wistar albino rats of either sex weighing between 150-250g were chosen for the study. They were housed in polypropylene cages with paddy husk bedding under controlled temperature and humidity. The animals were fed with standard pellet diet and water ad libitum. Institutional Animal Ethics Committee of KSHEMA reference number AEC/15/2010 approved the experimental protocols and procedures employed in this study.

Preparation of extract:

Aegle marmelos leaf extract

The fresh leaves of *A. marmelos* were dried under shade and then powered with a mechanical grinder to obtain a coarse powder, which was then subjected to extraction in a soxhlet apparatus using 90 % of ethanol. The extract was concentrated by using rotary evaporator. The percentage yield of the extract was 10%. The extract was stored in the refrigerator at 4°C. From this stock, the extract was diluted freshly according to the need to perform the experiment.

Mussaenda erythrophylla leaf extract

A weighed quantity of 200 g of leaf powder was taken and extracted by using 90% of alcohol by using soxhlet apparatus. The extract was concentrated by using rotary evaporator. The yield of the extract was 15%. The extract was stored in the refrigerator at 4°C and from this stock the extract was diluted freshly according to the need to perform the experiment.

Acute toxicity study

Wistar albino rats weighed 100-150g were used for testing acute oral toxicity. It was performed on the basis of OECD guideline no:423 (OECD, 2001). Overnight fasted animals were administered with *A. marmelos* leaf extract and *M. erythrophylla* leaf extract orally as single dose at five different dose levels of 200, 400, 800, 1600 and 3200 mg/kg body weight. The rats were observed continuously for 2 h for any behavioural changes and toxicity, and occasionally observed for 4 h, finally checked for overnight mortality. There after the animals were kept for 14 days and checked for mortality.

Treatment protocol

In this study 30 healthy wistar rats weighed 200g- 250g were used. The animals were randomly allotted into 5 groups of 6 rats each and treated orally as below for 21 days.

Group I: Served as normal control and received distilled water

Group II: Administered 50% of alcohol in 4g/kg body wt.p.o

Group III: 200 mg/kg *Aegle marmelos* leaf extract + 4g/kg alcohol

Group IV: 200mg/kg *Mussaenda erythrophylla* leaf extract + 4g/kg alcohol

Group V: 50 mg/kg Silymarin + 4g/kg alcohol

Experimental methodology

Ethanol induced hepatotoxicity:

Group I received distilled water and considered as control. Group II received 50% of alcohol in 4g/kg body wt p.o for 21 days. Group III received 200mg/kg of *A. marmelos* leaf extract and alcohol. Group IV received 200mg/kg of *M. erythrophylla* leaf extract and alcohol. Group V received silymarin 50 mg/kg and alcohol, which was used as a standard drug. The drug treatment duration for all the groups was 21 days.

Biochemical estimations

At the end of drug treatment period all the animals were sacrificed by using ether. Blood was collected by cardiac puncture, allowed it to clot for 30 minutes and serum was separated by centrifugation at 3000 rpm for 15 minutes. Liver was dissected out, rinsed with water, weighed and homogenized by using 0.1M Tris-HCl buffer of pH 7.5. The resultant homogenate was centrifuged and the supernatant was collected. The serum as well as liver homogenate was used for determining the biochemical analysis of liver serum marker enzymes as well as oxidative stress parameters like serum glutamate pyruvate transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), total bilirubin(TBL), glutathione peroxidase (GPx), glutathione reductase (GSH), superoxide dismutase (SOD) and total antioxidants as per standard procedure.

Statistical analysis

Statistical analysis done by One Way Analysis Of Variance (ANOVA) followed by Tukey Kramer multiple group comparison test performed by using Graph pad Prism software. Analysis between 2 groups to compare study group and control done by Paired t test. All values are expressed as mean \pm standard deviation (SD). $p < 0.05$ was considered as significant.

RESULTS

Acute toxicity study:

There was no mortality among the graded dose groups of animals and not showed any toxicity or behavioral changes at a dose level of 3200 mg/kg of leaf extracts. This finding suggests that *A. marmelos* and *M. erythrophylla* were safe and non-toxic to rats up to 3200 mg/kg.

Effects of *A. Marmelos* and *M. erythrophylla* leaf extract in ethanol induced hepatotoxicity

In the present study ethanol administration (group II) resulted in hepatic damage as evidenced by significant increase in the levels of TBL, SGPT, SGOT ($p < 0.05$) as compared to the normal control group. The above biochemical parameters were significantly reversed in rats which received *A. marmelos* + alcohol, *M. erythrophylla* +alcohol & Silymarin + alcohol ($p < 0.01$). There is a very significant ($p < 0.0001$) reduction in SGPT levels group IV and SGOT levels in group III has been observed which is summarized in Table-1

Table 1. Effect of the *A. marmelos* and *M. erythrophylla* leaf extract on biochemical parameters in alcohol induced hepatotoxicity.

Groups	TBL (mg/dl)	SGPT (U/L)	SGOT(U/L)
Group I	0.524 \pm 0.191	49.84 \pm 1.24	115.97 \pm 2.788
Group II	1.324 \pm 0.264 ^a	71.25 \pm 8.98 ^a	189.56 \pm 7.25 ^a
Group III	0.168 \pm 0.08 ^b	50.11 \pm 1.19 ^b	117.16 \pm 5.8 ^c
Group IV	1.79 \pm 0.103	44.91 \pm 2.6 ^c	128.05 \pm 25.8 ^b
Group V	0.91 \pm 0.05 ^c	47.12 \pm 0.12 ^b	94.8 \pm 1.6 ^b
a: $p < 0.05$ when group II compared with control group, b: $p < 0.001$, c: $p < 0.0001$ when group III, group IV & group V compared with group II(alcohol treated group).			

Effect of the *A. marmelos* and *M. Erythrophylla* leaves extracts on hepatic oxidative stress parameters

After intoxication with ethanol there is a significant reduction in the levels of GSH, SOD, GPx and total antioxidant ($p < 0.05$) have been noticed when compared with control group. Co-administration of *A. marmelos* in 200 mg/kg and *M. erythrophylla* 200mg/kg with alcohol caused significant elevation in the levels of SOD, GPx and total antioxidant ($p < 0.001$) in group III & IV when compared with alcohol treated group. These results were consistent with standard drug silymarin (group V). There is a very significant ($p < 0.0001$) elevation in the levels of GSH in group in group III and group IV, SOD in group III and total antioxidant in group IV. This indicates the protective effect of plant extract on alcohol induced toxicity which is summarized in Table 2.

Table 2. Effect of the *A. marmelos* and *M. erythrophylla* leaf extract on oxidative stress parameters in alcohol induced hepatotoxicity.

Groups	GSH (umoles/ml)	SOD (U/ml)	GPx (umoles/g)	TAC (mg/dl)
Group 1	1.16±0.257	2530.15±72.009	21.154±2.090	6.327±1.622
Group II	0.0524±0.0021 ^a	748±0.061 ^a	10.6±3.334 ^a	0.262±0.019 ^a
Group III	1.586±3.84 ^c	4843.628±326.45 ^c	28.088±1.560 ^b	3.236±0.126 ^b
Group IV	1.873±3.12 ^c	2976.73±290 ^b	25.99±4.92 ^b	3.56±0.090 ^c
Group V	2.29±1.6 ^b	3059±147.80 ^b	28.51±3.3 ^b	3.21±0.04 ^b

a:p <0.05 when group II compared with control group, b: p <0.001, c: p <0.0001 when group III, group IV & group V compared with group II(alcohol treated group).

DISCUSSION

The liver is an extremely resilient organ to resist toxic insults because of its unique cellular attributes. Despite this resiliency, the liver is vulnerable to injury because it is frequently exposed to agents in their most reactive toxic forms. The major functions of the liver are carbohydrate, protein and fat metabolism, detoxification, secretion of bile and storage of vitamin. Maintenance of a healthy liver is a crucial factor for overall health and well being (Koda –Kimble young et al. 2015)

The hepatoprotective index of a protective agent is considered its capacity to maintain the normal hepatic physiological mechanisms, which has been disturbed by hepatotoxins. Chronic and excessive ethanol consumption is associated with cellular proliferation, fibrosis, cirrhosis, cancer of the liver, tissue lipid peroxidation and depletion in the tissue GSH levels (Vipul Gujrati, et al 2007) In addition, serum levels of many biochemical markers like SGPT, SGOT, cholesterol, bilirubin, and alkaline phosphatase are elevated. During hepatic damage, these cellular enzymes leak into the serum, resulting in elevation in their concentrations. The biochemical mechanism of hepatotoxicity by ethanol has been reviewed earlier. Alcohol dehydrogenase is the enzyme that is responsible for conversion of alcohol to acetaldehyde. This acetaldehyde is metabolized to acetate by acetaldehyde dehydrogenase enzyme. These two enzymes cause the reduction of nicotinamide dinucleotide (NAD) to NADH. That results an alteration in the ratio of NAD/NADH, which leads to steatosis or fatty liver (Grant BF, et al 1988, Stewart S, 2001) .This causes impairment of carbohydrate metabolism, lipid metabolism, gluconeogenesis and finally results in the diversion of metabolism to ketogenesis and fatty acid synthesis. (Lieber CS, 2000) Long term exposure to alcohol causes the activation of kupffer cells that induce the generation of reactive oxygen species and finally precipitate to oxidative stress, this in turn promotes hepatocyte necrosis, apoptosis, lipid peroxidation, inflammation and fibrosis.(A. gramenzi, et al 2006) Increased alcohol intake also found to be related with the enzymatic induction of CYP2E1 enzymes with cytochrome reductase that contribute to the increased production of superoxide radicals and reactive oxygen species generation which may indirectly contribute to development of alcoholic liver disease.(Lieber CS,1999).

The serum marker enzymes (SGPT & SGOT) are considered the commonest enzymes employed as indicators of hepatocellular damage. These agents are cytoplasmic in nature, but on liver injury, these enzymes enter into circulatory system due to direct permeability of membrane. Increases in serum enzyme activities are roughly proportional to the extent of tissue damage (Hoek JB et al 2004). Bilirubin is the end product of heme catabolism. Bilirubin exists in two forms such as unconjugated bilirubin and conjugated bilirubin. The serum conjugated bilirubin level does not become elevated until the liver has lost at least one half of its excretory capacity. The elevated levels of serum bilirubin could be due to hemolysis and hepatitis. (Johnson DE, et al 1999)

In present study administration of ethanol (50% in 4g/kg bw) for 21 days increased serum enzymes like SGPT, SGOT and total bilirubin which indicated the ability of alcohol to produce hepatic damage. Co-administration of *A. marmelos* leaf extract and *M. erythrophylla* leaf extract with alcohol (group III & group IV) reduced the elevated levels of SGPT, SGOT, and total bilirubin levels indicated their hepatoprotective effect against alcohol induced liver cell damage.

The findings on the effect on liver function tests were consistent with the previous study conducted by Nadeem Ahmad et al hepatoprotective activity of the methanolic extract of *A. marmelos* leaves (MEAML) was examined on carbon tetrachloride (CCl₄) intoxicated rats. The findings of their investigation revealed that the MEAML possess significant hepatoprotective activity by suppressing CCl₄ induced cellular oxidative stress by restoring the levels of SGPT, SGOT, bilirubin, albumin, GSH (Nadeem Ahmad Siddique, et al 2011).

According to Favier oxidative stress is an abnormal phenomenon occurring inside cells when production of oxygen radicals exceeds their antioxidant capacity. Excess of free radicals damage essential macromolecules of the cell, leading to abnormal gene expression, disturbance in receptor activity, proliferation or cell death, immunity perturbation, mutagenesis, protein or lipofuscin deposition. There is an innate antioxidant defense mechanism in living tissues. The major antioxidant enzymes, superoxide dismutase (SOD), glutathione peroxidase (GPx) which catalyzes decomposition of reactive oxygen species. (Favier A, 2006) SOD protects the cells from the toxic effects of the endogenously generated superoxide radicals. Reduction in the activities of SOD could be deal with accumulation of highly reactive free radicals that can cause toxic effects such as loss of integrity and function of cell membrane (Chrobot M et al 2000). From this point of view in the current study administration of ethanol caused reduction in SOD, which indicated the hepatotoxic potential of alcohol. However, after co-administration of *A. marmelos* leaf extract and *M. erythrophylla* leaf extract with alcohol the hepatotoxicity is prevented by increasing the levels of SOD and total antioxidants.

GPx is an enzyme that protects the cells from oxidative injury by free radicals hydrogen peroxides and lipid peroxides. GPx catalyses the reduction of peroxides using glutathione as a reducing substrate. Glutathione is an antioxidant enzyme & is a naturally occurring tripeptide (Halliwell B 1994) Its main role is to protect the cells from free radical damage, environmental toxins and chemicals, drugs and alcohol as well as various toxins. A decrease level of serum GSH has been associated with an enhanced level of lipid peroxidation (Broid E, 2000). In this study, there is a significant ($p < 0.0001$) reduction of GSH was observed after intoxication with ethanol that showed the alcohol induced deleterious effect on liver. Co-administration of *A. marmelos* plant extract and *M. erythrophylla* leaf extract with alcohol showed increase in the levels of GSH and GPx, which indicated its ability to protect against oxidative stress.

The phytochemical analysis of *Aeglemarmelos* and *Mussaendaerythrophylla* has been done earlier. The quantitative phytochemical estimation specifies that aqueous and methanolic leaf extract of *Aeglemarmelos* found to have a significant amount of alkaloid, flavonoids, phenolic, saponins, and phytosterols (Shailesh Kumar et al 2013) Various parts of the plant have been reported to have a number of bioactive compounds and secondary metabolites mainly marmenol (Ali MS et al 2004), marmin, (Chatterjee A et al 1959) marmelosin (MandalPK, et al 1981) marmelin, (Sharma BR et al 1981,) fagarine (Govindachari TR, et al 1983) anhydromarmelin, (Manandhar MD et al 1978) b-carotene, limonene, marmesin, (GoswamiS, et al 2005) imperatorin, (Atta-Ur-Rehman et al 2004).

Previous studies on preliminary phytochemical analysis of ethanolic and chloroform extract of *Mussaenda erythrophylla* leaves indicated the presence of flavanoids, steroids, glycosides, tritenpens (Venkatesh et al 2013). In one another study the stem of ethyl acetate extract of stem of *Mussaenda erythrophylla* found to have the presence of β -sitosterol, 5 hydroxy-7, 4'-dimethoxy flavones, 3- iso cumaryloxy – cyclopropane-1-oleic acid, 4 -hydroxy-3-methoxy cinnamic acid. (Chinna Eswaraiah M et al, 2011). The above reviewed phytoconstituents in both the plant extract could be responsible for their hepatoprotective activity.

CONCLUSION

The observations illustrate that ethanolic extract of *M. erythrophylla* and *A. marmelos* leaf extract possesses significant hepatoprotective activity against alcohol induced hepatotoxicity.

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