Kuncha Jayachandra et al /J. Pharm. Sci. & Res. Vol.3(7), 2011,1360-1363

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Hepatoprotective Effect of *Aegle Marmelos (L.) Corr.* Leaf Powder (Crude) Against Carbon Tetrachloride-Induced Hepatic Damage in Albino Rats

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Abstract
The present study appraised hepatoprotective activity of *Aegle marmelos (L.) corr.* leaf powder (crude) against carbon tetrachloride-induced hepatic damage in albino rats. The effect of the leaf powder on functioning against carbon tetrachloride (CCl_4)-induced hepatic damage was studied by assessing the biochemical parameters such as alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total bilirubin (TB) and uric acid in serum of the rats. The concentrations of the hepatic markers were remarkably increased in the CCl_4-induced hepatic rats when compared to rats of normal control. After the *Aegle marmelos* leaf powder administration for 14 days to the rats with hepatic damage induced by CCl_4, it was found that the concentrations of the hepatic enzymes in the rats were comparable to that of healthy control rats. Strikingly, it has also been noticed that concentrations of the hepatic markers of rats treated with standard drug (Liv.52) and that of rats treated with the crude leaf powder are similar, within statistical errors, to each other indicating that *Aegle marmelos* leaf powder may contain lead phytochemicals that may presumably pave the way of designing highly potent hepataprotective compounds/drugs.

Key words: *Aegle marmelos*, Carbon tetrachloride, Hepatic markers, Hepatoprotective activity and Hepatotoxicity.

1. INTRODUCTION

Liver diseases constitute a major health problem in worldwide population as liver is the vital organ of metabolism and excretion in human beings. A statistical report alerts of about 20,000 deaths every year due to liver disorders [1]. Liver injury is induced by various pathogenic factors such as viral hepatitis, ethanol and hepatotoxicants etc [2]. The lipid peroxidation is a destructive process, which alters the structure and functions of cellular membrane [3]. The disrupted tissues are known to undergo lipid peroxidation at faster rate than normal lipid peroxidation (oxidative damage). This oxidative damage causes change in the structure, fluidity and permeability of membrane and also inactivate a few number of membrane bounded enzymes [4,5,6].

In traditional medicine, many plants (either as whole or part) and metabolic products of plants are shown to protect cells and organs of human system by their capability of exerting their antioxidant effect against deleterious effect of free radicals mediated hepatotoxicity. These observations imply that phytochemicals play vital roles in protecting various organs including liver against various toxic compounds [7]. In the present study, we have studied the effect of hepatoprotective property of crude leaf powder of *Aegle Marmelos (L.) Corr.* against CCl_4-induced hepatic damage in albino rats. We have used CCl_4 as a hepatotoxin in the animal models since the mechanism by which CCl_4 induces hepatotoxicity has been well documented in the literature [8,9,10]. The data obtained from the animal models suggest that the leaf powder of *Aegle Marmelos (L.) Corr*. may presumably contain lead anti-oxidant compounds. Moreover, it is interesting to mention that various parts of *Aegle marmelos* have been traditionally used to treat various human ailments. The bitter pungent leaf juice of the plant mixed with honey is given orally for fever and the leaf juice mixed with black pepper and honey is prescribed to relieve from jaundice and constipation [11]. The leaf juice also shows anti-inflammatory and antipyretic activity [12]. The fruits and roots of the plant are being used for treating wound healing, ulcer [13, 14] and diabetic disorder [15] and also for improving the sperm motility [16]. In these backgrounds, the importance of the findings of the present study has been discussed in view of *de novo* drug designing in detail.

2. MATERIALS AND METHODS

Plant materials and chemical reagents: The plant material of *Aegle marmelos* was collected from the surroundings of Thanjavur districts, Tamil Nadu, India and was authenticated by Mr. Balasubramanium, senior taxonomist, Department of Botany, Ponnaiah Ramajayam College, Thanjavur. A voucher specimen (PRCB/BC/153) of the plant has been stored in the departmental herbarium. The plant material was shade dried, powdered and sieved and then, the nice powder was packed in airtight container, which was used for further studies. CCl_4 (Merck), liquid paraffin (Sigma), and all other chemicals used in the biochemical assay of this study were purchased from Ponmani chemicals,

Trichy. All chemicals used in the study were analytical grade. Double distilled water was used for preparation of all chemical reagents.

Animal studies: Albino rats (5-6 weeks of either sex) weighing 180-210 g were obtained from Indian Institute of Science, Bangalore. The animals were housed in polypropylene cages and maintained under controlled conditions (25 ^{0}C – 27 ^{0}C) at 12 hrs dark/light cycle and the rats were fed with standard rat pellet diet (Hindustan lever, India) and water *ad libitum.* The total number of 24 albino rats used in the study was divided into four groups as shown herein. Six rats were considered for each group.

Group I: Healthy rats (Normal control; fed with pellet food and water *ad libitum).*

Group II: Hepatic rats (intoxicated by CCl_4 and Liquid paraffin - 1:2 v/v; 1 ml/kg).

Group III: Hepatic rats treated with Standard drug (Liv.52 drug; 500 mg/kg).

Group IV: Hepatic rats treated with *Aegle marmelos* leaf powder (1000 mg/kg).

Liver damage was induced to rats of Group II, III & IV by intraperitonial administration of mixture containing carbon tetrachloride (CCl_4) and Liquid paraffin (1:2 v/v; 1ml/kg) on the first and seventh day of the experiments. The standard drug/leaf powder (200 mg/ml) suspended in the vehicle acacia mucilage were administered to the rats of Group III/IV for 14 continuous days after hepatatoxic induction, respectively [17]. The dose of 1000 mg/kg was given by oral route using an intragastric tube in both cases. After completion of experimental regimen, rats were fasted over night and blood samples were collected by puncturing of orbital sinus, under light chloroform anesthesia. On the last day, the rats were sacrificed by decapitation [18]. Serum was separated by centrifugation at 2500 rpm for 15 minutes and biochemical parameters such as SGOT, SGPT, alkaline phosphatase, bilirubin and uric acid were carried out as reported elsewhere. Estimation of SGOT (AST) and SGPT (ALT) was performed as per Reitman and Frankel method [19], and concentration of alkaline phosphatase was determined by Kind method [20]. The total bilirubin and uric acid in serum of the rats were estimated according to Malloy and Evelyn method [21], and Caraway method [22], respectively. The data of biochemical estimations were reported as mean ± SD (at the 67% confidence level). Significant inter group difference were determined statistically by subjecting the data to ANOVA followed by Dunnett`s test. Multiple comparison test and $P<0.01$ were considered significant in the analysis [23].

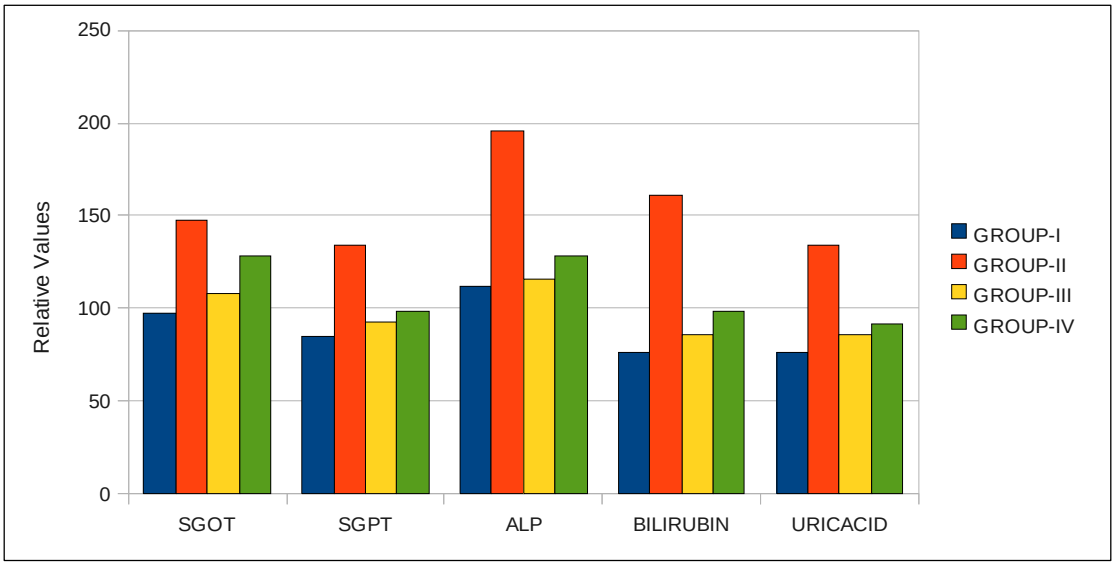
3. RESULTS AND DISCUSSION

The hepatoprotective effect of *Aegle marmelos* to the CCl_4-induced hepatic damage in albino rats are depicted in the Table 1. The concentrations of hepatic enzymes such as ALT, AST, ALP, total bilirubin and uric acid in serum significantly increased in CCl_4-treated rats (Group-II) when compared to normal control (Group-I). After *Aegle marmelos* leaf powder suspension treatment (1000 mg/kg) to the rats of Group-IV, the levels of the hepatic markers were significantly reversed close quarters to that of normal control. The treatment for the rats of group III was as identical as the treatment for the rats of Group IV were subjected, except that Liv.52 standard drug was used instead of *Aegle marmelos* leaf powder against the induced-hepatotoxicity. From the quick inspection to Fig. 1, it could be obviously inferred that the *Aegle marmelos* leaf powder must possesses hepatoprotective constituents, which may be similar in structures and functions to that of active constituents of Liv. 52, a standard drug against hepatotoxicity.

Table 1: Analysis of SGOT, SGPT, ALP, bilirubin and uric acid in the serum of normal rats, CCl_4-induced hepatic rats, hepatic rats treated with standard drug and hepatic rats treated with leaf powder of *Aegle marmelos*.

Animal Groups	*Particulars*	*SGOT (U/L)*	*SGPT (U/L)*	*ALP (U/L)*	*Bilirubin (mg/dl)*	*Uricacid (mg/dl)*
GROUP - I	Control	97 ± 6	85 ± 5	112 ± 7	76 ± 3	76 ± 4
GROUP - II	Toxic control	148 ± 3	134 ± 10	196 ± 4	161 ± 4	134 ± 10
GROUP - III	Standard drug(Liv.52)	108 ± 2	93 ± 3	116 ± 4	86 ± 5	86 ± 2
GROUP - IV	Aegle marmelos (leaf powder)	128 ± 7	98 ± 3	128 ± 6	98 ± 6	92 ± 5

Figure 1: Bar diagram representing average concentration of SGOT (U/L), SGPT (U/L), ALP (U/L), Bilirubin (mg/dl) and uricacid (mg/dl) in rats belonging to four different groups (please refer materials and methods).



CCl_4 is one of the most commonly used hepatotoxin to induce hepatotoxicity in animal models. It has been reported that hepatotoxic chemicals, in general, release free radicals (ROS, RNS), which act on hepatic cells, especially in the liver parenchyma cells [24]. The hepatic parenchyma cells are the production site for various enzymes, bile salts and bile pigments etc. The CCl_4 used as hepatotoxin in the present study is metabolically activated by the cytochrome-P450 in the endoplasmicreticulum to form a trichloromethyl free radical ($CCl_3^{\bullet}$), which combines with cellular lipids and proteins in the presence of oxygen to induce lipid-peroxidation [25]. When the parenchyma cells are severely damaged by the free radicals generated from the hepatotoxin, structural and functional alterations takes place in the cells and consequently concentrations of SGPT, SGOT ALP, total bilirubin and uric acid in serum would be drastically elevated. SGPT, SGOT and ALP are the liver specific enzymes and are considered to be very sensitive and reliable parameters for assessing hepatotoxicity as well as hepatoprotective activity of various compounds. The enzymes SGPT and SGOT catalyze the interconversion of amino acids and α-keto acids through transformation of amino groups between them and ALP regulates the activities of proteins participating in phosphorylation/dephosphorylation processes [26]. The liver plays a significant role in the conjugation of glucoronic acid with bilirubin to make water soluble complex, which is easily excreted from the system. These formations occur in liver because only the liver is having the specific enzyme glucoronyltransferase [27]. Treatment with *Aegle marmelos* leaf powder reduces the increased level of SGPT, SGOT, ALP and bilirubin in the serum of rats, unrevealing the remarkable protection of hepatic cells by the leaf powder. Uric acid is the end product of purine metabolism in humans and increased level of uric acid excretion is called as hyperuricemia [28]. This condition may be an indication of damages in the tissues of liver and kidney. In the present study, we show that uric acid level is increased in toxic control, which should be probably due to hepatic cell damage by $CCl_3^{\bullet}$ radical. After treatment with *Aegle marmelos* leaf powder to the rats of Group IV, level of uric acid in the rats could be brought to the near serum level of healthy rats (Table 1 & Fig.1). This observation makes us to speculate that certain phytochemicals of the leaf powder are capable of restoring the tissues of liver and kidney to their original shapes. The implications of the present study is many-folds: (i) the *Aegle marmelos* is commonly spread all over south India (ii) the leaves of the plant can be obtained at free of cost (iii) our preliminary data on animal studies suggest that the crude leaf powder has no side effect, when the dose of 1500 mg/kg is administrated to rats for 14 days (data not shown). The crude powder can be prepared and stored for long-lasting use [29] (iv) different forms of the leaves of *Aegle marmelos* are being used in

traditional medicines to cure ailments such as fever, jaundice and constipation. In these backgrounds, we strongly believe that the phytochemicals of the leaf powder are with multiple targets and will be very useful on designing *de novo* drugs for a few numbers of liver diseases/disorders. At present, we are working on the isolation of the active compounds present in the leaf powder of *Aegle marmelos* using various solvent extraction techniques.

4. CONCLUSION

Using the animal models, we have shown that crude leaf powder of *Aegle marmelos* has potential to act against CCl_4-induced hepatic damage in albino rats. Moreover, the extent of hepatoprotective effect of the leaf powder is comparable to that of standard drug, Liv.52 being used against hepatotoxicity, in general. This observation unambiguously suggests that the leaf powder of *Aegle marmelos* must contain lead compounds that may provide profound implications on designing *de novo* anti hepatic drugs. We are presently working on identifying and elucidating the three-dimensional structures of a few lead compounds from the crude leaf powder. We strongly believe that the outcomes of the study will trigger exciting research on addressing liver diseases in a cost effective manner.

ACKNOWLEDGEMENT

Mr.K.Jayachandra is thankful to Prof. A. Maheshwaran, Principal, Jaya College of pharmacy, Thiruninravur, Chennai for the facilities he generously provided to execute some of the research work presented in the article. Dr.T.Sivaraman appreciates Mr. S. Sivarajesh, research scholar, SASTRA University for his fine details on the animal studies of the work.

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