International Journal of Scientific Research and Engineering Development-– Volume 5 Issue 6, Nov- Dec 2022

Available at www.ijsred.com

RESEARCH ARTICLE OPEN ACCESS

Hepatoprotective Activity of Phyllanthus Emblica Against CCl_4 Induced Hepatotoxicity in Rats

S.Anitha Kumari[1] and P. Madhusudhanachary[2]

[1]Department of Zoology, University college for women, Koti,Hyderabad,India.

E-mail I.D annithaashinde@gmail.com

[2]Ultrastructure unit,National Institute of Nutrition, Tarnaka, Hyderabad,India.

E-mail I.D madhunin123@gmail.com

ABSTRACT

The present study was performed to assess the hepatoprotective activity of Phyllanthus emblica fruit extract against CCl_4 induced hepatotoxicity in male wistar rats. The rats were divided into 5 groups with each group consisting of 6 rats. All the rats were maintained for a period of 21 days. The group I rats served as control. Group II rats received CCl_4 at a dose of 2ml/kg body weight/day. Group III rats received aqueous fruit extract of Phyllanthus emblica (200 mg/kg body.wt/day. Group IV received aqueous fruit extract of Phyllanthus emblica and CCl_4 while group V received silymarin at a dose of 100mg/kg body weight. The hepatic enzymes such as ALT, AST, ALP, LDH and the total protein and bilirubin were estimated.

All the hepatic enzymes i.e. ALT, AST,ALPand LDH were found to be increased in group II when compared to control (group I). However, the enzyme levels were significantly decreased when treated with Phyllanthus emblica and silymarin (group III, IV and V).Further, there was a significant reduction in total protein levels in group II when compared to control(Group I). The total protein levels were found to be increased in rats treated with Phyllanthus emblica and silymarin(Group III,IV & V). In group II animals, the bilirubin levels were found to be increased whereas, there was a significant decrease in the bilirubin levels in rats treated with Phyllanthus emblica and silymarin (group III, IV & V) when compared to control rats. The study indicates that the animals treated with CCl_4 alone showed higher hepatoxicity, while the animals treated with CCl_4 and Phyllanthus emblica showed protective effect in the liver suggesting the ability of Phyllanthus emblica fruit extract to rectify the hepatic damage and serve as a potential hepatoprotective agent.

Keywords:- Phyllanthus emblica, rats, hepatoprotective activity, CCl_4.

INTRODUCTION:

Liver is an important metabolic organ. It regulates several important metabolic functions including detoxification and secretory functions in the body. Any abnormality or distortion of these metabolic functions leads to hepatic injury[1]. Hence, the liver diseases remain to be one of the serious health problem with numerous disorders. Management of the liver disease is of major concern in themedical science. It is a challenge to the modern medicine. Despite considerable progress in the treatment of liver diseases by the oral hepatoprotective agents, search for new drugs is continuous due to its limitations [2,3]. There is a need for a drug that stimulates the liver function or offers protection to the liver from the damage or helps in regenerating the hepatic cells[4]. Liver disorder is considered to be one of the top priority disease in the world for which an effective alternative therapy is required. Further, the use of

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modern medicine is associated with risk of relapses as well as danger of side effects [5], whereas on the other hand, the natural herbal drugs used in the liver diseases are reported to be more effective and safe. Further, the phytochemicals derived from the plant extracts possess multiple activities. It is also believed that the natural formulated compound is more active than the isolated form [6].

The genus Phyllanthus belonging to Eurphorbiaceae is one of the most important plant group with high medicinal value[7]. In India, Phyllanthus species which are traded as raw drugs[8], have been extensively used for the treatment of liver disorders, intestinal infections and diabetes[9,10,11].

About 53 species of Phyllanthus are found in India, out of which 23 sps are endemic [12], and only a very few of these are investigated for their medicinal value. Among the various Phyllanthus sps, Phyllanthus emblica or Emblica officinalis occupies a pivotal position in the Ayurvedic system of medicine. According to the ancient Indian mythology, it is the first tree which is created in the world. The fruit of Phyllanthus emblica is referred to as the sustainer by Ayurvedic physicians. Further, it is reported to possess, anticancer, antiulcer, antioxidant, antipyretic and analgesic activity, immunomodulatory effect, antimicrobial activity, antilipedemic activity, cardio-protective activity, antidiabetic activity as well as hepatoprotective activity[13-22].

Carbon tetrachloride (CCl_4) is a hepatoxic agent and is widely used in many experimental animals to investigate the hepatoprotective activity of medicinal plants[23].

Keeping all the above facts in view, the present study was undertaken to investigate the hepatoprotective activity of aqueous fruit extract of Phyllanthus emblica on CCl_4 induced hepatic injury in male wistar Rats.

MATERIALSAND METHODS:

All the chemicals used were of Analytical grade.

Preparation of Aqueous fruit extract of Phyllanthus emblica:

Fresh fruits of Phyllanthus emblica were purchased from the local market. The fruits were then washed, deseeded, air dried and powdered with a mechanical grinder passing through a sieve and stored in a tight container. Then about 25gms of the air dried fruit powder is refluxed with ethanol at 45^{o}C for 3 hours using the soxhlet apparatus. The mixture is then filtered and the filtrate is evaporated using vacuum evaporator and then air dried at 40^{o}C. The stock solution of the crude ethanolic extract (Aqueous extract) was prepared by diluting the dried extract with 0.25% dimethyl sulphoxide (DMSO) solution to obtain a final concentration of 100mg/ml.

Experimental procedure:

Male healthy wistar rats weighting about 150-200gms each were used for the present study.The rats were divided into 5 groups with each group consisting of 6 rats. All the groups were maintained for a period of 21days. The group I rats received the normal saline and served as the control. Group II rats received CCl_4 (Hepatotoxic agent) at a dose of 2ml/Kg/body weight/day. Group III rats received aqueous fruit extract of Phyllanthus emblica at a dose of 200mg/kg/body weight/day. Group IV received aqueous fruit extract of Phyllanthus emblica (200mg/ Kg body weight/day) and CCl_4 (2ml/kg/body weight / day) while group V received silymarin (standard drug) at a dose of 100mg/kg body weight/day.

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The animals were sacrificed on the 21st day of the experiment, blood was collected and the serum separated. The hepatic enzymes, such as Alanine transaminase (ALT), Aspartate Transaminase (AST), Alkaline phosphatase (ALP), Lactate dehydrogenase (LDH), total protein and bilirubin were estimated.

ALT and AST were determined following the method of Reitman and Frankel [24], ALP by King and Kind [25], LDH by King and Waind[26], Total protein by Lowry et al [27] and bilirubin by Malloy and Evelyn[28].

STATISTICAL ANALYSIS: The statistical analysis was performed using one- way analysis of variance(ANOVA) followed by NewmannKeul's multiple range tests. The values are expressed as mean± SEM.The probability value i.e $p< 0.01$ was found to be statistically significant.

RESULTS AND DISCUSSION: The results of the effect of CCl_4 on aqueous fruit extract of Phyllanthus emblica are shown in Table I and II.

TABLE-I

Effect of Phyllanthus emblica on the hepatic enzymes in CCl_4 induced hepatotoxicity in male wistar Rats.

Group	ALT(IU/ml)	AST(IU/ml)	ALP(IU/ml)	LDH(U/L)
I-Normal control	48.32±2.30	85.18±4.14	66.15±2.12	312.25±9.40
II- CCl_4 Treated	154.85±4.95*	215.41±7.38*	173.16±5.68*	453.20±25.15*
III-Phyllanthus emblica Treated	60.15±2.39***	88.40±2.52***	70.15±3.65***	316.05±12.24***
IV-Phyllanthus emblica + CCl_4 Treated	90.60±3.65**	135.05±5.20**	114.20±4.15**	406.10±15.48**
V-Silymarin Treated	61.30±3.05***	90.54±2.76***	69.40±3.13***	320.41±15.45***

Values are expressed as Mean±S.E.M of 6 Rats/Treatment
* Values are significantly different from Normal control at $p<0.01$
**Values are significantly different from CCl_4 treated at $p<0.01$
***Values are significantly different from CCl_4 treated at $p<0.001$

TABLE-II

Effect of Phyllanthus emblica on Total Protein and Bilirubin in CCl_4 induced hepatotoxicity in male wistar Rats.

Group	Total Protein(mg/ml)	Bilirubin(mg/ml)
I-Normal control	6.14±0.55	0.54±0.14
II- CCl_4 Treated	3.28±0.41*	0.98±0.22*
III-Phyllanthus emblica Treated	5.98±0.66***	0.58±0.16***
IV-Phyllanthus emblica + CCl_4 Treated	4.32±0.15**	0.70±0.12**
V-Silymarin Treated	6.02±0.74***	0.59±0.17***

Values are expressed as Mean±S.E.M of 6 Rats/Treatment
*Values are significantly different from Normal control at $p<0.01$
**Values are significantly different from CCl_4 treated at $p<0.01$
***Values are significantly different from CCl_4 treated at $p<0.001$

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It is observed that the liver enzymes such as ALT, AST, ALP and LDH were found to be increased in group II i.e. CCl_4 treated rats when compared to the control rats. However, the enzyme levels were significantly decreased when treated with Phyllanthus emblica and Silymarin i.e. (Group III, IV and V). There was a significant reduction in the total protein levels in group II animals due to CCl_4 toxicity compared to the control animals (group I) as shown in Table II. However, the total protein level was found to be increased in animals when treated with Phyllanthus emblica and Silymarin (Group II, IV and V). In group II animals, the bilirubin levels were found to be increased whereas there was a significant decrease in the bilirubin levels in animals when treated with Phyllanthus emblica and Silymarin (Group III, IV and V).

In the present study, the hepatoprotective activity of aqueous fruit extract of Phyllanthus emblica was evaluated by inducing the hepatotoxicity using CCl_4.CCl_4 induced the liver damage and cause the changes that are similar to that of viral hepatitis as reportedby[29,30,31].

CCl_4 affects the cytochrome P_{450} and yields trichloromethyl radicals which react with polysaturated fatty acids and leads to lipid peroxidation. These free radicals further initiate the other cellular targets to form another set of free radicals thus affecting almost all types of cellular molecules[32].

The free radical scavenging effect is initiated by the antioxidants as advocated by several authors. Phyllanthus emblica consists of bioactive molecules such as flavonoids, tannins etc which are responsible for the antioxidant scavenging activities as reported by[21,33].

In the present study, CCl_4 treated rats (group II) showed elevated levels of ALT, AST, ALP and LDH which are similar to the results as reported earlier [34,35].ALT and AST are the indicators of liver damage as advocated by several authors[36-40].

The pathological alteration of the liver in the biliary flow reflects in an increase in the alkaline phosphatase levels in serum[41]. Further, the histopathological changes in the liver also leads to an increase in the LDH levels in the serum[42]. All the above results thus bears a testimony to our present findings. However, the enzyme levels were significantly decreased when they were treated with Phyllanthus emblica and silymarin. The decline or reductionin ALT, AST, ALP and LDH in the treated animals (Group III, IV and V) may be due to healing of the hepatic tissues and regeneration of hepatocytes [43]. Oxidative damage is considered to be the major cause of metabolic dysfunction during pathogenesis that affects the proteins, lipids and nucleic acids [44]. In the present study, there was a significant decrease intheprotein levels in group II (CCl_4 treated) animals compared to the control (group I). However, the total protein level was found to be increased in animals treated with Phyllanthus emblica and silymarin (Group III, IV and V).

In CCl_4 treated animals (group II), the bilirubin levels were found to be increased, whereas in animals treated with Phyllanthus emblica and silymarin (group III, IV and V), it was significantly decreased. The decrease in the protein levels in the present study may be due to the hepatotoxicity of CCl_4.

CCl_4significantly affects the proteins thus decreasing its level in the serum. Further, the biliary dysfunction in rats due to the hepatotoxicity of CCl_4 increases the bilirubinlevel as reported by Uday Bandyopadhyay[44] in concomitant with our present findings.

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The tannins and flavonoids present in aqueous fruit extract of Phyllanthus emblica contains very powerful antioxidant as well hepatoprotective properties[45,46].

Our present study also indicates that the animals treated with CCl_4 alone showed higher hepatotoxicity while the animals treated with CCl_4 and Phyllanthus emblica showed protective effects in the liver. These findings suggest that Phyllanthus emblica fruit extract have the ability to rectify the hepatic damage and can be used as a potential hepato-protective agent.

CONCLUSION: The aqueous fruit extract of Phyllanthus emblica shows promising hepatoprotective activity. However, characterization of the active biomolecules, their mode of action and other in –vivo experiments are needed to further evaluate their therapeutic value as natural hepatoprotective agents.

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