



International Journal of Medicine and Pharmaceutical Research

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Research Article

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Protective Effect of *Terminalia Arjuna* Leaves against CCl₄ Induced Hepatic Damage in Mice

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ABSTRACT

In the present study ethanolic extract of the leaves of *Terminalia arjuna* was evaluated for hepatoprotective activity in carbon tetrachloride liver-damaged rats. Hepatotoxicity was induced via intraperitoneal injection of ccl₄ (1:1) in olive oil, at a dose of 0.5 ml/kg body wt. Liv 52 syrup (0.5 ml/kg) was given as reference standard and exhibited significant hepatoprotection against carbon tetrachloride induced liver-damaged rats. The animals received ethanolic extract of leaves of *terminalia arjuna*, orally, at a dose levels of 200 and 300 mg/kg b.wt. for two weeks. *terminalia arjuna* has shown significant hepatoprotection against ccl₄ induced hepatotoxicity in albino rats in reducing alkaline phosphatase (ALP), alanine amino transferase (ALT), aspartate amino transferase (AST) and levels of total protein were investigated. The extracts of the test plant exhibited significant ($p < 0.05$) hepatoprotective activity against the ccl₄ induced liver models by improving liver function which was indicated by reduction in the levels of SGOT, SGPT, ALP, GGT, total bilirubin and total protein.

Keywords: *Terminalia arjuna*, ccl₄, liv 52, ethanolic extract, hepatoprotective

ARTICLE INFO

CONTENTS

1. Introduction	50
2. Materials and Methods	51
3. Results and discussion	52
4. Conclusion	54
5. References	54

Article History: Received 31 January 2017, Accepted 05 March 2017, Available Online 10 April 2017

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Manuscript ID: IJMPR3346



PAPER-QR CODE

Citation: B. Aruna, et al. Protective Effect of *Terminalia Arjuna* Leaves against CCl₄ Induced Hepatic Damage in Mice. *Int. J. Med. Pharm. Res.*, 2017, 5(2): 50-54.

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1. Introduction

Liver is a vital organ play a major role in metabolism and excretion of xenobiotics from the body. Liver injury or liver dysfunction is a major health problem that challenges not International Journal of Medicine and Pharmaceutical Research

only health care professionals but also the pharmaceutical industry and drug regulatory agencies. Liver cell injury caused by various toxic chemicals (certain anti-biotic,

chemotherapeutic agents, carbon tetrachloride (CCl₄), thioacetamide (TAA) etc.), excessive alcohol consumption and microbes is well-studied. Herbal medicines have been used in the treatment of liver diseases for a long time. A number of herbal preparations are available in the market. The present review is aimed at compiling data on promising phytochemicals from medicinal plants that have been tested in hepatotoxicity models using modern scientific system.

The liver plays an astonishing array of vital functions in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction. And it functions as a centre of metabolism of nutrients such as carbohydrates, proteins, lipids and excretion of waste metabolites. The bile secreted by the liver has, among other things, plays an important role in digestion. Therefore, maintenance of a healthy liver is essential for the overall well being of an individual. Liver cell injury caused by various toxicants such as certain chemotherapeutic agents, carbon tetrachloride, thioacetamide, chronic alcohol consumption and microbes are common. Enhanced lipid peroxidation during metabolism of ethanol may result in development of hepatitis leading to cirrhosis.

Herbal drugs have gained importance and popularity in recent years because of their safety, efficacy and cost effectiveness. One of the important and well documented uses of plant products is their use as hepatoprotective agents. Hence, there is an ever increasing need for safe hepatoprotective agent. In spite of tremendous strides in modern medicine, there are hardly any drugs that stimulate liver function, offer protection to the liver from damage or help regeneration of hepatic cell. Many formulations containing herbal extracts are sold in the Indian market for liver disorders. But management of liver disorders by a simple and precise herbal drug is still an intriguing problem. Several Indian medicinal plants have been extensively used in the Indian traditional system of medicine for the management of liver disorder. Some of these plants have already been reported to possess strong antioxidant activity.

Terminalia arjuna is a tree bark that is used medicinally in Ayurveda for the purposes of cardiovascular health pertaining to the heart itself. It has a large variety of bioactives, with the water extract showing promise at improving left ventricle function of the heart without any observable toxicity of side effects when taken at 500mg thrice a day (every 8 hours). There are numerous human studies conducted on arjuna bark, although many of them are low in sample size. Nevertheless, the water extract appears to be effective in improving cardiac function in persons who have recently undergone cardiac trauma or injury. Myocardial Infarction is the most commonly researched ailment in this regard. Only one study exists on otherwise healthy persons, but arjuna showed benefit in improving left ventricle function in an exercise test and the benefits may affect a person regardless of health state.

2. Materials and Methods

2.1. Chemicals

2,2-diphenyl-1-picrylhydrazyl (DPPH), Ethanol, ammonium molybdate, sodium phosphate, sulphuric acid, anhydrous disodium hydrogen phosphate, potassium dihydrogen phosphate, and sodium chloride, aspartate amino transferase (AST), alanine amino transferase (ALT), Alkaline phosphatase (ALP), LIV 52, carbon tetra chloride (CCl₄). All other chemicals and reagents were of analytical grade.

2.2. Preparation of plant extract

The leaves of *Terminalia arjuna* (combretaceae) plant were collected from local areas of Kurnool. The whole plant leaves were cleaned and dried in the shade, and the dried powder placed into the container. Extract was prepared by using Soxhlet extractor. The extract is evaporated for dryness, and stored in a cool place. The percentage yield of extract was 17.7 % (w/w) of the initial raw material.

2.3. Phytochemical analysis

The qualitative phytochemical analysis of ethanolic extract of *Terminalia arjuna* was carried out to determine the active phytochemical constituents which are responsible for the anti oxidant and hepatoprotective property.

2.4. Animals

Wistar albino rats weighing between 150-250 g were obtained from the national institute of health, Hyderabad. Rats were kept in plastic cages at animal house under a standard condition of temperature 23±2°C, relative humidity of 50% and light and dark cycles of 12h:12h, and fed with standard diet ad libitum. All the experimental procedures were carried out in accordance with the guidance of Institutional Animal Ethical Committee (IAEC). The animals were observed daily for any signs of toxicity. Body weight was recorded at regular intervals throughout the experimental period.

2.5. Acute toxicity studies:

Acute toxicity study of fresh leaf extract of *T.arjuna* was carried out in rat according to OECD 423 guidelines. Different doses of extract was administered up to 5 mg/kg and the rats were observed for a period of 72 hrs for behavioural changes, toxic symptoms and mortality.

2.6. Experimental design

Animals were randomly divided into 5 groups, each group of 6 animals. And the animals are tested for 2 weeks

- Control group (received only vehicle i.e. olive oil, 1ml/kg of rat body weight, orally): instead of plant extract and ccl₄)
- Standard group (liv 52 syrup- 1ml/kg of the rat body weight, i.p and ccl₄ 0.5 ml/kg, i.p).
- Ccl₄ control group(All the rats in this group were given ccl₄ 0.5 ml/kg , i.p)
- Extract treated group (low dose 200mg/kg, orally and ccl₄ 0.5 ml/kg , i.p)
- Extract treated group (high dose 300mg/kg and ccl₄ 0.5 ml/kg , i.p)

2.7. Estimation of biochemical parameters:

Each animal was anaesthetized with diethyl ether. Heart puncture was done with a 5ml disposable syringe and 2ml blood was drawn very gently and slowly. The blood collected was shifted immediately to clean dried

centrifugation tubes, allowed to clot and serum was separated by centrifugation at 3000 rpm for 15 min. Serum was separated and then preserved in the cuvettes at 20°C in the freezer until analysis. Biochemical estimations were made the following day. Serum samples collected from different groups were analyzed for aspartate transaminase (AST), Alanine transaminase (ALT) and alkaline phosphate (ALP) using procedure and packed kits made by bicone, Germany. The absorption was recorded using the 4010 Spectrophotometer.

2.8. Histopathological studies:

At the end of the experimental period, animals were sacrificed by cervical decapitation. Anatomy of the liver was studied immediately after sacrificing the animals. Liver sections taken immediately after dissection from the liver, fixed in 10% buffered formalin dehydrated in gradual ethanol (50-100%) cleared in xylene and embedded in paraffin sections (4-5 µm thick) were prepared and then stained with haematoxylin and eosin (H and E) dye for degeneration, infiltration of kupffer cells and lymphocytes were studied under power 40x. the extent of ccl₄ induced liver damage was evaluated based on the pathological lesions in liver sections. hepatocyte necrosis, fatty change, hyaline degeneration, ballooning degeneration, and infiltration of inflammatory cells were prominent in the histological findings. The liver pathology was scored as described by French et al. 2000 as follows.

Score 0 = no visible cell damage

Score 1 = focal hepatocyte damage on less than 25 % of the tissue

Score 2 = focal hepatocyte damage on 25-50 % of the tissue

Score 3 = extensive, but focal, hepatocyte lesions

The morphology of any lesions observed was classified and registered (Gray, 1958). Statistical analysis was performed using the SPSS for Windows statistical package, version 10.0 (SPSS Inc. Chicago, IL, USA). Data were expressed as means ± S.E.M. The effects of drug treatments were evaluated statistically using the one-way analysis of variance (one-way ANOVA) followed by the Dunnett post-hoc test to correct for multiple comparison treatments. Statistical significance was set at the p <0.05 level.

3. Results and Discussion

3.1. Preliminary phytochemical screening

The extraction of powdered leaf (17gm) of *Terminalia arjuna* was carried out by soxhlation using ethanol as solvent.

3.2. Colour, nature and yield of extract

Qualitative phytochemical investigation of crude plant extract of *Terminalia arjuna* (greenish black colour) revealed, the presence of alkaloids, carbohydrates, starch, protein, amino-acid, steroids, flavanoids, and absence of starch.

Table 1

S.no	Extract	Colour	Consistency	Yield (gm)
1	Ethanol	Greenish black	Sticky	4.2

3.3. In-vivo Hepatoprotective Study

Body weight and weight of liver

No animals died during CCl₄ administration period. The administration of CCl₄ caused a significant decrease in the body weight of rats as compared with the control rats. The animals co-treated with *Terminalia arjuna* extract (200mg and 300mg/kg body wt) for two weeks also gained weight during the experimental period. Liver weights and liver index (the ratio of liver weight to body weight) were higher in CCl₄-treated animals than in control animals. Co-treatment with *Terminalia arjuna* extract (200mg and 300mg/kg body wt.) resulted in both liver weights and liver index that were significantly reduced compared to those of CCl₄-treated rats.

Biochemical analysis

CCl₄ is activated by phase-II detoxifying enzymes in liver cell endoplasmic reticulum to form trichloromethyl and peroxytrichloromethyl free radicals. These can react covalently with several biomolecules such as protein, nucleic acid and lipid, resulting in cellular membrane degeneration, increased permeability, and leakage of cytoplasmic ALT, AST and ALP. Serum levels of ALT, AST and ALP should serve as hepatotoxicity indexes. Indeed, CCl₄ administration produced significant elevations of serum ALT and AST compared to the normal control group. However, pretreatment of rats with *Terminalia arjuna* extract (200mg and 300mg/kg body wt) significantly decreased these serum biochemical indices as compared with the CCl₄ treatment group. Hepatic SOD and CAT activities in the CCl₄ treatment group were reduced compared to the normal control group. These antioxidant enzyme activities were all statistically significantly greater in the group treated with *Terminalia arjuna* extract (200mg and 300mg/kg body wt) compared with the CCl₄ treatment group.

Histopathological findings

The central vein, hepatocyte and portal space were observed to be normal in the control group. CCl₄ is a hepatotoxicant known to produce a characteristic centrilobular pattern of degeneration and necrosis. In the present study, CCl₄ application constituted histopathological changes in the liver. Severe hyperemia was observed in the area surrounding the central veins. Wide vacuolar degeneration of hepatocytes and lymphocyte infiltration were observed. Derangement of the hepatocyte cord and necrosis at the periphery of central vein were also determined in CCl₄ group. The histological appearance of the *Terminalia arjuna* extract-treated groups was quite similar to that of the control group, and tissue damage and necrosis were of less extent in this group than the CCl₄ group. Minimal tissue degeneration was observed at the periphery of the central vein. No derangement was observed at hepatocyte cords. Lymphocyte infiltration was not detected in the *Terminalia arjuna* extract treated group. Hydropic and vacuolar degeneration were found only at the periphery of the central vein. Moderate degenerative changes, vacuolar degeneration of hepatocytes, and lymphocyte infiltration at the periphery of the central vein were determined in this group.

4. Discussion

The present study demonstrates the hepatoprotective and anti oxidant effects of ethanolic extract *T. arjuna* leaves against CCl₄ induced liver injury in rats. The liver is one of the vital organ in our body responsible for detoxification of toxic chemicals and drugs. Thus it is the target organ for all toxic chemicals. Numerous studies noted that CCl₄ is widely used to induce liver damage because it is metabolised in hepatocytes by CYP 450, generating a highly reactive carbontrichloromethyl radicle, leading to initiating a chain of lipid peroxidation and thereby causing liver fibrosis. CCl₄ not only initiates lipid peroxidation but also reduces tissue CAT and SOD activities and this depletion results from oxidative modification of these proteins. In the present study ethanolic extract of leaves of arjuna were evaluated for the hepatoprotective activity using hepatotoxicity induced by CCl₄ in rat model and find out the therapeutically better efficacious extract. An attempt was made to find out the correlation between anti oxidant and hepatoprotective activity. CCl₄ is being used extensively to investigate hepatoprotective activity on various experimental animals. A major defence mechanism involves the anti oxidant enzymes, including SOD, catalase and glutathione peroxidase, which convert active oxygen molecules into non toxic compounds. Liver damage was assessed by biochemical studies (SGOT, SGPT, ALT and Total bilirubin) and by histopathological

examinations. CCl₄ produces an experimental damage that histologically resembles viral hepatitis. Toxicity begins with the change in endoplasmic reticulum, which results in the loss of metabolic enzymes located in the intracellular structures. The toxic metabolite CCl₃ radicle produced which further reacts with oxygen to give trichloromethyl peroxy radicle. Cytochrome p450 2E1 is the enzyme responsible for this conversion. This radicle binds covalently to the macro molecules and causes peroxidative degradation of lipid membrane of the adipose tissue. In this view, the reduction in levels of SGOT and SGPT by the extracts is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by CCl₄. This effect is agreement with the commonly accepted view that serum levels of transaminases returns to normal with the healing of hepatic parenchyma and regeneration of hepatocytes. Alkaline phosphate is the prototype of these enzymes that reflects the pathological alterations in biliary flow. CCl₄ induced elevation of this enzymatic activity in the serum is in line with high level of serum bilirubin content. The alcohol induced suppression of the increased ALP activity with the concurrent depletion of raised bilirubin suggest the possibility of the extracts to have ability to stabilize biliary dysfunction in rat liver during hepatic injury with CCl₄. Thus, administration of alcoholic extract of *T. Arjuna* leaves were reevaluated for hepatoprotective activity against the toxic effect of CCl₄.

Table 2: Preliminary Photochemical Tests

S.No	Phytoconstituent	Test	Results
1	Carbohydrate	Molish test	+ve
2	Starch	Iodine test	-ve
3	Protein	Millon's test	+ve
4	Amino-acid	Cysteine test	+ve
5	Steroid	Salkowski test	+ve
6	Flavanoids	Ferric chloride test	+ve
7	Alkaloids	Mayer's test	+ve
8	Tannins and phenolic compounds	5% FeCl ₃ test	+ve
9	Oxalic acid	Calcium chloride	+ve
10	Inorganic acid	Sulphate test	+ve

+ve = positive, ve = Negative

Table 3: Biochemical analysis

Parameters	Control	Standard	Ccl4 control	Extract low dose	Extract high dose
Initial body weight (g)	224 ± 10.4	216 ± 9.8	223 ± 13.2	219 ± 12.5	220 ± 11.4
Final body weight (g)	256 ± 15.3	249 ± 17.2	226 ± 10.5 +++	232 ± 8.5	245 ± 15.2 **
Weight gain (g)	32 ± 4.9	33 ± 3.7	3 ± 0.7 +++	13 ± 3.4	25 ± 4.1 **
Liver weight (g)	5.3 ± 0.34	5.2 ± 0.41	8.5 ± 1.2 +++	6.9 ± 0.64	5.9 ± 0.83 ***
Liver index	2.07 ± 0.04	2.09 ± 0.03	3.76 ± 0.07 +++	2.97 ± 0.05	2.41 ± 0.03 ***

Table 4

Parameters	Control (Olive oil)	Ccl4 control	Standard (liv.52 syrup) +ccl ₄	extract (low dose)+ccl ₄	extract (high dose)+ccl ₄
AST(IU/L)	40.81 ± 3.28	40.02 ± 2.14	30.20±1.05	25.21±1.15	20.02±2.12
ALT(IU/L)	121.81 ± 9.28	35.35 ± 0.18	25.02±1.15	19.05±0.14	34.21±2.22
ALP(IU/L)	270.45 ± 12.44	221.02 ± 6.92	133.02±2.12	66.02±1.05	59.15±1.22
Total protein	62.64±2.4	26.27 ± 0.81	86.12±2.14	44.05±1.02	64.21±2.05

Values are mean ± S.E.M. (n= 6 rats per each group).

*P Values are mean \pm S.E.M. (n = 6 rats per each group). The liver index was calculated as liver weight / body weight \times 100, *P Serum levels of alkaline phosphatase (ALP), aspartate transaminase (AST) and alanine transaminase (ALT) activities, loss/gain in body weight, gross and microscopic appearance of liver were studied and results are presented

4. Conclusion

The present study has established the hepatoprotective activity of ethanolic extract of the leaves of *Terminalia arjuna* by *in vivo* method. It was prepared based on plenty literature search. Presently *in-vitro* and *in-vivo* methods are being used for antioxidant evaluation purpose. DPPH method is the most frequently used one for *in vitro* anti oxidant activity evaluation. In this study the phytochemicals present in *T.arjuna* leaves showed hepatoprotective activity comparable to Liv 52 in CCL4 induced hepatotoxicity in rats. The protective action was improved further by increasing the dose of the extract. Apart from the anti lipid peroxidative and antioxidant actions, these active phytochemicals might have played a role in restoring the cytochrome P450 enzyme systems or promoted the liver regenerative activity, in future the derivatives of these phytochemicals or their combinations may show efficacy in various experimental toxic models. They may be developed as future drugs for using human liver diseases with antioxidant, antifibrotic, immunomodulatory, antiviral, and regenerative properties.

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