PROTECTIVE AND THERAPEUTIC EFFECTS OF THE INDIAN MEDICINAL PLANT FICUS GLOMERATA IN CCL4-INDUCED LIVER DAMAGE

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ABSTRACT

Ficus glomerata Roxb (Moraceae) is widely distributed medicinal plant, used in traditional system of medicine. The present work is carried out to evaluate the hepatoprotective activity of Methanolic extract of Ficus glomerata Roxb fruits. Fruit powder was defatted with petroleum ether and chloroform. Hepatotoxicity was induced by two dose of CCl₄ (2ml/kg). Methanolic extract at the dose level of 150 mg/kg, and 300 mg/kg body weight was administered orally for 7 days. The effect of extracts on liver marker enzymes, antiradical enzymes and architecture of hepatocytes was measured. There is a significant increase (P<0.05) in the levels of reduced liver marker enzymes and antiradical enzymes due to oxidative stress; the histopathological study reveals that the regeneration of damaged hepatic cells in the test animals. These results indicate that the F. glomerata is a potent source of hepatoprotection.

KEYWORDS: Ficus glomerata, Hepatoprotection, Antioxidant, Oxidative stress, CCl₄

INTRODUCTION

Imbalance in the production and detoxification of free radicals by the biological system causes oxidative stress in living organisms.[1] The oxidative stress in biological system results in the generation of free radicals or reactive oxygen species (ROS) like superoxide, hydroxyl and peroxyl.[2,3] The unstable free radicals are mediators of inflammation and through this they interact with platelets, granulocytes are also involved in production and activation of eicosinoids and release of cytokines, which results in the oxidative stress through spreading from organ to organ.[4] An antioxidant is a molecule capable of lowering or preventing the oxidation of other molecules.[5] The most common inbuilt reactive oxygen
species (ROS) of biological system include superoxide anion, hydrogen peroxide (H₂O₂), peroxyl radicals, reactive hydroxyl (OH⁻) radicals and nitrogen derived free radicals.[6] Production of high amount of reactive oxygen species overcomes inbuilt antioxidant system and damages the cells, tissues and organs.[7] Hence, there is a need to develop novel drugs from traditional medicine to protect and strengthen the biological system to avoid serious disorders of liver, cardiac and cancer diseases. As there is lack of satisfactory hepatoprotective and anti oxidant drugs available in the market, there is a need to explore the medicinal plants for the search of potent drug which has less side effect and more potent to cure the disease.[8,9]

*Ficus glomerata* Roxb (Syn. *Ficus racemosa*) belongs to Moraceae family is an evergreen, moderate to large sized spreading, lactiferous, deciduous tree popularly known as the Cluster Fig.[10,11] It is widely distributed all over India, Northern Australia and other parts of Asia; fruits are edible and used in various dishes.[12] *Ficus glomerata* has been used in traditional system widely due to its rich medicinal properties.[13] Recent medical practices have explored the pharmacological actions of different parts of *Ficus glomerata* such as Stem, bark and leaves and showed antioxidant, anti diabetic, antibacterial, anti-inflammatory and antipyretic effects. [14-18] In view of its wide range of pharmacological activities, the present study is undertaken to study the effect of *Ficus glomerata* fruits on carbon tetrachloride induced liver damage and its anti-oxidant property.

**MATERIALS AND METHODS**

**Collection of Plant material and Extraction**

The *Ficus glomerata* Roxb fruits were collected from botanical garden of Gulbarga University, Gulbarga, and authenticated from Department of Botany, Gulbarga University, Gulbarga. The fruits were washed and dried under shade, grinded to coarse powder. Powder is subjected to soxhlet extraction using petroleum ether, chloroform and methanol solvents successively. The solvent free Methanolic extract dissolved in 1% DMSO is used for the present study.

**Chemicals**

Liv- 52 was purchased from Himalaya health care pvt. Ltd. All other Chemicals CCl₄, Olive oil, DMSO, including Solvents used were of high purity and of analytical grade marketed by Hi- Media Laboratories, Mumbai and Sigma Aldrich, Mumbai.
Experimental Animals
Healthy Swiss albino rats of Wister strain were divided into five groups, each group containing six animals. Animals were maintained under standard laboratory conditions as described by CFTRI Mysore, India. Animals were allowed free accesses to food and water. Experimental protocols were approved by Institutional Animal ethical committee, Gulbarga University, Gulbarga (1994).

Acute Toxicity studies
This study is carried out according to OECD guidelines for testing acute oral toxicity Test No.423.[19] The animals were fasted overnight prior to the dosing and plant extract was administered orally up to 4000 mg/kg. No mortality is seen at the dose of 150 mg/kg of body weight and 300 mg/kg body weight. Hence, the above tested dose is selected to conduct the experiment.

CCl₄ induced oxidative stress
Oxidative Stress was induced in test animals by treating with 30% of CCl₄ (2 ml/kg of body weight) intraperitoneally. Group I received single dose of saline at the dose level 2 ml/kg body weight for seven days and served as control. Group II received CCl₄ 2 ml/kg of body weight on first day and last day of dosing, Group III, IV, V received CCl₄ 2 ml/kg of body weight for First day and last day of dosing, along with single doses of Liv-52, 150 mg/kg FGME, 300 mg/kg FGME respectively for seven days.[20]

Drug Administration: CCl₄ was given intraperitoneally and Saline, Live-52 and plant extracts were given by oral dosing method.

Group-I: This group received normal saline.
Group-II: This group received 2ml/kg of CCl₄ (30% in olive oil)
Group-III: This group received 2ml of CCl₄+2ml of Liv-52
Group -IV: This group received 2ml of CCl₄ +150mg/kg of FGME.
Group -V: This group received 2ml of CCl₄ + 300mg/kg of FGME.

On eighth day of experiment all animals were sacrificed and blood was collected, allowed to clot for 30min and centrifuged at 2500 rpm for 15 min, serum was separated and used for the estimation of biochemical markers. Liver was dissected, washed in ice-cold saline, used for histopathological studies.
Estimation of Liver marker Enzymes
The serum was used to estimate functional state of liver enzymes and other biochemical markers. Serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALP) and Lactate dehydrogenase (LDH) was estimated using Bioanalyzer instrument.[23]

Estimation of Anti–radical Enzymes
Liver tissue homogenate was prepared in 0.5 M Tris hydrochloric acid buffer (pH 7.4) at 4°C. The homogenate was centrifuged at 8000rpm for 10min and the supernatant was again spun at 12000 rpm for 15min, the obtained mitochondrial fractions were used for the assay of enzymes namely Catalase, peroxidase, superoxide dismutase and glutathione reductase.[22-24]

Histopathological Studies
The liver was dissected out and washed in ice-cold saline and fixed in 10% formalin, after 24 hours embedded in paraffin wax.3μ-5μ thin sections were taken with the help of microsection instrument and stained with Haematoxylin and Eosin. Mounted sections were observed and captured under photomicroscope (Lieca). Observation of change in the central vein, sinusoids, hepatocyte, kappa cells and ballooning was done.[25-27]

Statistical Analysis
The results are expressed as the mean ± SEM of six animals in each group. The data was analyzed by one-way ANOVA followed by student’s t-test. The values of P< 0.05 were considered statistically significant.

RESULTS
Effect of Ficus glomerata fruit extract on liver marker Enzymes
The activity of liver marker enzymes, SGPT, SGOT, ALP, and LDH as shown in “Fig. 1” were observed in the CCl4 treated group. Enzymes concentration was found to be elevated, as compare to the control group (P< 0.05), the FGME treatment at the dose 150mg/kg has moderately decreased the elevated enzyme concentration; whereas 300mg/kg of FGME treated group has recovered significantly.
Anti-Radical Enzyme Estimation

Estimation of antiradical enzymes in liver like, GSH, CAT, POD and SOD was carried out represented in “Fig. 2”. The level of GSH, CAT, POD and SOD enzymes were found to be reduced in stress-induced group. Test dose of FGME at 150mg/kg and 300mg/kg body weight treated groups increased the level of GSH, CAT, POD and SOD significantly when compared to Liv-52 treated groups. The dose of FGME at 300mg/kg body weight treated group was found to be more effective than 150mg/kg of FGME treatment.

Fig. 1: Effect of FGME on Liver Marker Enzymes

Fig. 2: Effect of FGME on Anti-radical Enzymes
Histopathological studies
The histopathological scores of liver sections in control group have shown the presence of normal cellular architecture, cytoplasm and visible central veins (“Fig. 3A”), whereas in stress and hepatotoxicity induced animals there is an extensive liver injuries, characterized by moderate to severe hepato-cellular degeneration and necrosis around the central vein, fatty changes, ballooning degeneration are noticed (“Fig. 3B”). Liv-52 treated group has shown more significant recovery compared to control animals treated with saline (“Fig. 3C”) The FGME treatment at the dose of 150 mg/kg b/w has showed recovery and protection of hepatocytes degradation, local necrosis and vacuolization (“Fig. 3D”) whereas FGME at the dose of 300 mg/kg b/w treatment has showed more significant protection (“Fig. 3E”) than 150 mg/kg b/w FGME treatment, all the structural damages are reserved to normal.

Fig. 3A: Liver histology of Rat from Saline treated Control group.

Fig. 3B: Liver histology of Rat from CCl4 treated group
Fig. 3C: Liver histology of CCl4+Liv-52 treated group

Fig. 3D: Liver histology of Rat from CCl4+FGME 150mg/kg treated test group

Fig. 3E: Liver histology of Rat from CCl4+ FGME 300mg/kg treated test group

Fig. 3: Effect of Ficus glomerata on test rat liver architecture
DISCUSSION

Carbon tetrachloride induced hepatotoxicity is the most commonly used model system for evaluating hepato protective potency of plant extracts.[4] In the present study, methanolic extract from Ficus glomerata fruits were used for the in vivo validation. The hepatotoxic effect of CCl4 is due to its metabolite trichloromethyl radicals, which binds to the biological macromolecules and results in the peroxidative degradation of endoplasmic reticulum membrane.[24] Increase in the level of SGOT and SGPT in the serum of toxicity induced animals is observed after cellular leakage, and loss of functional integrity of cell membrane which is due to the free radicals generated by CCl4.[8] Increased levels of SGOT, SGPT, ALP and LDH were significantly reduced by the treatment of FGME, when compare to the control in a dose dependent manner, this may be due to the action of phenolics and flavonoids present in the FGME of Ficus glomerata. This effect may be either by preventing the generation of free radicals or by strengthening hepatocytes activities. Earlier studies on different parts of Ficus glomerata have reported the presence of antioxidant flavonoids such as ficusin, rutin and favonoid glycosides.[13,14,16]

Bors et al., 2011 have observed the role of flavonoids in diminishing the effect of superoxide anion by its dismutation and detoxification of free radicals generated by the metabolism of carbon tetra chloride by the coordinated actions of cellular antioxidants.[28] Studies conducted by Niki, 2004 [3] and Cao, 1996 [5] reveals that the GSH conjugation plays a vital role in detoxification of metabolites, which are the major cause of liver injury. Catalase (CAT) is an enzymatic antioxidant widely distributed in all animal tissues. CAT decomposes hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals. Hepatotoxicity induced animals in this experiment has showed decrease in the level of GSH and CAT due to the toxicity of trichloromethyl radicals. Whereas the test animals treated with FGME at the dose of 150mg/kg of body weight and 300mg/kg of body weight has showed enhancement in GSH and CAT antioxidants significantly in dose dependent manner. This was supported by the report of kubsad et al., 2008 [25], who have reported the role of plant polyphenols in retaining antioxidant status in hepatotoxicity induced animals. Standard drug Liv-52 has also showed an effective increase in the CAT and GSH levels.

CCl4 treatment produces effects in liver like loss of hepatocytes arrangement, necrosis, and degeneration.[6] FGME (150mg/kg b/wand 300mg/kg b/w) treated groups have retained the normal cellular architecture when compared to standard hepato protective drug Liv-52 treated
animals. Previous studies on *Ficus* have shown the presence of antioxidant flavonoids such as ficusin, rutin and flavonoid glycosides. \[^{29}\] Several investigations have reported the presence of active compounds in *Ficus glomerata* for the hepatoprotective and antioxidant activities. \[^{10,14,30}\] Based on the above observations it can be concluded that the *Ficus glomerata* is having a potential antioxidant and hepatoprotective components that can be used for further studies.

**CONCLUSION**

Methanolic extract from *Ficus glomerata* exhibited strong hepatoprotective action when administered at a daily dose of 150 and 300 mg kg\(^{-1}\) for 1 week in CCl\(_4\) stress-induced rats. Our findings suggest that the extract could be used as supplements in healthcare foods and drugs. However, the possible mechanisms of the hepatoprotective effect of *Ficus glomerata* warrant further investigation.

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