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Research Article

HEPATOPROTECTIVE ACTIVITY OF AQUEOUS EXTRACTS OF *CHRYSANTHEMUM INDICUM* FLOWERS ON PARACETAMOL INDUCED LIVER INJURY IN ALBINO RATS

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ABSTRACT

Objective: The present investigation was evaluated that protective activity of aqueous extract of flowers of *Chrysanthemum indicum* studied against paracetamol-induced hepatotoxicity in animal model.

Methods: Bioactive functional groups, such as alcohol, carboxylic acid, and amines, were present in the aqueous extract of flowers of *C. indicum* identified by Fourier transform infrared spectroscopy. The animals were grouped into 5 and each group has 6 animals and induced the hepatic failure. Silymarin was used as reference standard. Aqueous extract of flowers of *C. indicum* treated in a different dose which was compared with control group of animals.

Results: Aqueous extract of flowers of *C. indicum* reduced the level of aspartate transaminase (AST), alanine transaminase (ALT), serum bilirubin, protein, triglycerides, and cholesterol compared than paracetamol treated Group II animals. Histopathological studies were confirmed that reduction of necrosis and inflammation in the liver cells.

Conclusion: Thus, these results revealed that the aqueous extract of flowers of *C. indicum* shown very significant ($p<0.01$) hepatoprotection against paracetamol-induced hepatic failure in animal model by reducing AST, ALT, serum total bilirubin, protein, triglycerides, and cholesterol levels.

Keywords: Hepatoprotective activity, *Chrysanthemum indicum,* Paracetamol.

INTRODUCTION

Plants are major sources of our earth playing a vital role in pharmacological applications such as antidiabetic [1], hepatoprotective [2], cardioprotective [3], nephroprotective [4], and anticancer activities [5]. *Chrysanthemum indicum* Linn, a perennial herb of the family Compositae, is widely dispersed throughout China, north Russia, Southeast Asia and Europe. *C. indicum Linn* is traditionally used for many applications and now it is reported for various uses such as antimicrobial against bacteria (*Klebsiella pneumonia, Escherichia coli*, *Streptococcus mutans, Pseudomonas aeruginosa, Bacillus subtilis,* and *Staphylococcus aureus)* and fungi (*Trichoderma viride, Candida albicans, Penicillium chrysogenum, and Aspergillus niger)* [6], Larvicidal agents against dengue fever mosquito, *Aedes aegypti* L. [7,8], Antiarthritic [9], anti-inflammatory, and immunomodulatory activities [10].

The liver is one of the most vital organs in the human body that functions as a center of metabolism of nutrients, excretion, digestion, detoxification, etc. [11]. Liver damage was caused by the excess usage of drugs contains toxic chemicals, alcohol, and environmental pollutants. Liver damage involves cell necrosis, lipid peroxidation, and other oxidative damages [12]. In addition, the biochemical markers such as aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), protein, and bilirubin levels in serum were also increased [13].

Liver injury caused generation of free radicals by affecting the cellular membrane functions [14-17]. Many chemical drugs and radiation therapy were widely used for the treatment of liver damage. However, these were high expensive, poor compatibility, and cause many side effects [18]. To overcome these problems herbal derived drugs were formulated as hepatoprotective drug because of they are inexpensive, better cultural acceptability, better compatibility with the human body and minimal side effects. These herbal drugs have shown the ability to maintain the normal functional statues of the liver with or without fewer side effects [19].

The use of natural remedies for the treatment of liver diseases has a long history and medicinal plants and their derivatives are still used with better formulation by identifying their active chemical constituents. These curative properties of chemical constituents such as phenols, coumarins, essential oil, monoterpenes, carotenoids, glycosides, flavonoids, organic acids, lipids, alkaloids, and xanthenes were elevated and used to hepatoprotective drugs. In this study, biochemical markers (AST, ALT, bilirubin, lipid peroxidation, total proteins, etc.) in the injured liver was assessed that protective activity of aqueous extract of flowers of *C. indicum* studied against paracetamol-induced hepatotoxicity in animal model.

METHODS

Collection of plant

The plant *C. indicum* flowers were collected from Adhiparasakthi Agricultural College flower garden.

Preparation of plant extract

Fresh flowers were collected and were dried under shade. The dried flower was powdered by mixer grinder. About 5 g of *C. indicum* powder was taken into a beaker and added 100 ml of distilled water and boiled for 10 minutes after that they were filtered with Whatmann no 1 filter paper. The extracts were allowed to store and used for experimental animals.

Animals

Adult male Wistar albino rats maintained at the college weighing 170-200 g were used for the hepatoprotective studies. The rats were housed in polypropylene cages and kept under standard laboratory

conditions (temperature 25 ± 2°C; natural light-dark cycle). The rats had free access to drinking water and commercial standard pellet diet (Lipton India Ltd, Mumbai, India). The commercial rat feed contained 5% fat, 21% protein, 55% nitrogen free extract and 4% fiber (w/w) with adequate minerals and vitamin contents. The laboratory animal protocol used for this study was approved by the Institutional Animals Ethics Committee.

Hepatoprotective studies of *C. indicum* flowers

Experimental design

The rats were randomly divided into five groups of 6 animals each.

Group I (Control): Control rats, received orally distilled water.

Group II (Induced): Induced rats, orally received paracetamol (2 g/kg body weight) dissolved in water for 7 days.

Group III (Paracetamol + Silymarin): Standard rats, orally received paracetamol (2 g/kg body weight), followed by silymarin (100 mg/kg body weight) dissolved in water for 7 days.

Group IV (Paracetamol + ASN): Treated rats, orally received paracetamol (2 g/kg body weight), and followed by aqueous extract of *C. indicum* flowers (300 mg/kg body weight) dissolved in water for 7 days.

Group V (Paracetamol + ASN): Treated rats, orally received paracetamol (2 g/kg body weight), and followed by aqueous extract of *C. indicum* flowers (600 mg/kg body weight) dissolved in water for 7 days.

Collection of blood

Animals of all the groups were sacrificed by cervical decapitation on the 8th day. Blood samples of each group were collected separately into sterilized dry centrifuge tubes and allowed to coagulate for 30 minutes at 37°C. The clear serum obtained after centrifugation was used for the estimation of serum ALT, serum AST, serum bilirubin, serum protein, cholesterol, and triglycerides.

Assay of serum AST (E.C.2.6.1.1) and ALT (E.C.2.6.12)

Assay of AST and ALT was performed according to the method of Reitman and Frankel [20]. One ml of substrate was incubated for few minutes at 37°C. Then, 0.2 ml of serum was added and incubated for 1 hr in the case of AST and 30 minutes for ALT. To the control, serum was added after incubation. The reaction was arrested using 1.0 ml of 2,4-Dinitrophenylhydrazine solution and the tubes were kept at room temperature for 20 minutes. About 10 ml of 0.4 N sodium hydroxide was added to all the tubes. A set of standards was also treated in a similar manner. The development of color was measured calorimetrically at 520 nm. The enzyme activity was expressed as IU/L.

Estimation of serum bilirubin

The serum total bilirubin was estimated according to the method of Malloy and Evelyn [21]. About 0.2 ml of serum was taken in a tube and to this 1.0 ml of water was added. A 0.5 ml of HCl was added to the blank tube. To the sample tube, 2.5 ml of methanol and 0.5 ml of diazo reagent were added. The tubes were incubated at room temperature for 30 minutes. The development of blue color in the reaction mixture was read at 540 nm in a Shimadzu spectrophotometer, along with bilirubin calibrator solutions. The values were expressed as mg/dl.

Estimation of serum total protein

The serum total protein was estimated as per the method of Lowry *et al.* [22]. Typically, 0.5 ml of serum was mixed with 4.5 ml of alkaline copper reagent and then allowed to stand at room temperature for 10 minutes. Then, 0.5 ml of Folin's phenol reagent was added. The formation of blue color was read at 640 nm in a Shimadzu spectrophotometer after 10 minutes. A standard graph was obtained with different concentrations of bovine serum albumin solution. The values were expressed as g/dl.

Estimation of cholesterol

Serum total cholesterol was determined in serum by the method of Parekh and Jung [23]. A value of 10 ml of ferric - uranyl acetate reagent was added to 0.1 ml of serum sample, mixed well, allowed to stand for 5 minutes and centrifuged. 3.0 ml of the supernatant was taken for analysis. Similarly, 0.1 ml of standard cholesterol was treated, and 3.0 ml aliquots were taken. Blank tube contained 3.0 ml of ferric - uranyl acetate reagent. About 2.0 ml of sulfuric acid - ferrous sulfate reagent was added to all the tubes and mixed well. After 20 minutes, the color intensity was measured at 560 nm in a Shimadzu spectrophotometer. The values were expressed as mg/dl.

Estimation of triglycerides

Serum triglycerides were estimated by the method of Foster and Dunn [24]. To an aliquot of serum, 0.1 ml of methanol was added, followed by 4.0 ml of isopropanol. Then, 0.4 g of alumina was added to all the tubes, shaken well for 15 minutes, centrifuged and then 2.0 ml of the supernatant fluid was transferred to labeled tubes. Exactly 0.6 ml of saponification reagent was added to the tubes and then placed in a water bath at 65°C for 15 minutes for saponification. Then, 1 ml of metaperiodate reagent and 0.5 ml of acetylacetone reagent were added to all the tubes, mixed well and incubated in a water bath at 65°C for 1 hr. A series of standards of 8- 40 µg triolein was treated similarly along with a blank containing only the reagents. All the tubes were cooled, and the color intensity was measured at 405 nm in a Shimadzu spectrophotometer. The values were expressed as mg/dl.

Histopathology studies

A portion of liver tissue in each group was fixed in 10% formalin (formalin diluted to 10% with normal saline) and processed for histopathology. After paraffin embedding, and block marking, serial section of 5 µm thickness were made, stained with hematoxylin and eosin and examined under microscope.

Statistical analysis

The statistical significance was assessed using one-way analysis of variance (ANOVA) using SPSS 16 software. The values are expressed as mean ± standard deviation and $p<0.05$ was considered significant.

RESULTS AND DISCUSSION

Phytochemical analysis using Fourier transform infrared (FTIR)

The presence of functional molecules in the aqueous extracts of *C. Indicum* flower was identified using FTIR spectroscopy spectrum (Fig. 1). The broadband was observed at 3292.70/cm corresponding to O-H stretching, H-bonded alcohols and phenols. A low absorption peak was observed at 2919.01/cm indicates the presence of O-H stretching carboxylic acids. Weak peaks were formed at 1733.58/cm and 1602.36/cm due to the presence of C=O stretching carboxylic acid and N-H bend primary amines, respectively. The presence of aromatic amines was identified by showing peak at 1286.99/cm. A strong narrow band was noted at 1013.11/cm assigned to C-O stretching esters and ethers. However, it concluded that the aqueous extract contained more phytochemicals such as carboxylic acids, primary amines, aromatic amines, esters, and ethers could be actively involved in the hepatoprotective activity.

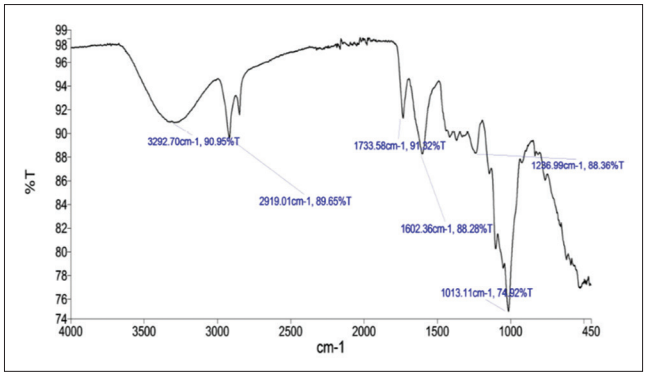

Fig. 1: Fourier transform infrared spectroscopy spectrum of aqueous extract *Chrysanthemum indicum* flowers

Hepatoprotective activity of *C. indicum* flowers

The drug paracetamol has been used in this study to induce the cell necrosis in experimental model animals. This hepatotoxicants increase the level of enzymes such as transaminases, lipid peroxidation, and ALP was the clear indication of cellular leakage and affects the functional integrity of the cell membrane [25].

Table 1 shows the effect of aqueous extract *C. indicum* flowers (300 mg/kg and 600 mg/kg) on serum biochemical markers like AST, and ALT in paracetamol induced liver toxicity. Fig. 2 and Table 1 show that serum AST, and ALT levels were significantly higher in animals receiving paracetamol ($p<0.05$) and decreased significantly in Groups IV and V which they received 300 mg/kg and 600 mg/kg of aqueous extract ($p<0.01$), respectively. Hepatic damage causes elevated level of liver enzymes such as serum AST, and ALT. Treatment with *C. indicum* flowers at 600 mg/kg revealed comparable activity with reference standard silymarin (25 mg/kg). Aqueous extract of *C. indicum* flowers decreased the liver markers AST (103.35±4.23 U/l), and ALT (45.93±1.94 U/l).

The level of total bilirubin, protein, triglycerides and cholesterol in paracetamol intoxicated animals were significantly increased when compared to control (Figs. 3-5 and Tables 2 and 3). The paracetamol treated showed increased levels of serum bilirubin, protein, triglycerides and cholesterol are due to the damage caused by paracetamol. These levels were restored significantly in flower extract treated animal groups Groups IV and V.

Histopathology analysis

Histopathological studies in control rat showed normal physiological cells (Fig. 6a). Fig. 6b showed focal necrosis and inflammation in paracetamol treated animals (Group II). Groups III, IV, and V showed minimum necrosis with swelling which were treated with standard silymarin, 300 mg/kg and 600 mg/kg of aqueous extract, respectively. The animals treated with aqueous extract of *C. indicum* flowers appeared to be normal as in Group I (Fig. 6a-e). These histopathological studies confirmed a hepatoprotective activity of *C. indicum* flower extract against paracetamol toxicated liver damage.

CONCLUSION

This present work evaluated that the hepatoprotective potential of aqueous extract of flowers of *C. indicum* against paracetamol induced hepatotoxicity. Aqueous extract of flowers of *C. indicum* was significantly reduced the

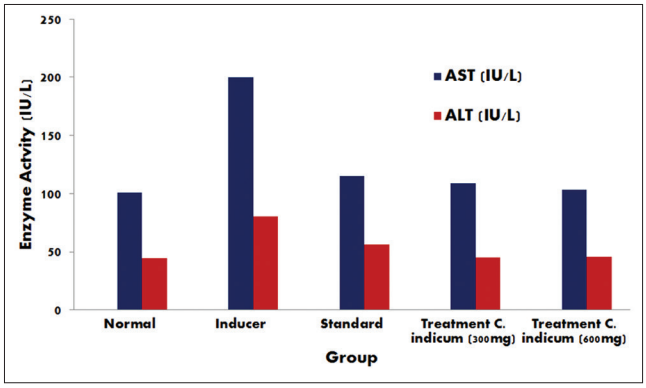

Fig. 2: Changes in the activity of aspartate transaminase and ALT using *Chrysanthemum indicum* flower extract

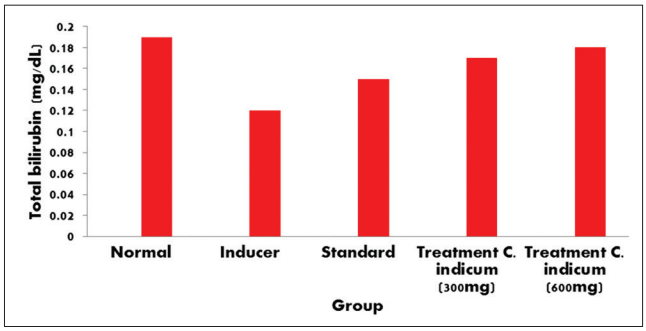

Fig. 3: Changes in the activity of bilirubin using *Chrysanthemum indicum* flower extract

Table 1: Changes in the activity of AST and ALT in experimental animals

Groups	AST	ALT	Level of significance (p)
Group I (control)	100.67±4.33	44.83±1.80	<0.01
Group II (paracetamol)	200.50±10.17	80.67±4.82	<0.05
Group III (paracetamol+silymarin)	115.67±4.51	52.33±1.76	<0.05
Group IV (paracetamol+300 mg aqueous extract)	109.33±4.91	50.33±2.21	<0.01
Group V (paracetamol+600 mg aqueous extract)	103.34±4.23	45.93±1.94	<0.01

AST: Aspartate transaminase, ALT: Alanine transaminase

Table 2: Changes in the levels of serum bilirubin and total protein in control and experimental rats

Groups	Bilirubin (mg/dL)	Total protein (g/dL)	Level of significance (p)
Group I (control)	0.19±0.06	7.49±0.29	<0.01
Group II (paracetamol)	0.12±0.15	7.19±0.23	<0.05
Group III (paracetamol+silymarin)	0.15±0.06	7.33±0.28	<0.01
Group IV (Paracetamol+300 mg aqueous extract)	0.171±0.02	7.38±0.33	<0.05
Group V (paracetamol+600 mg aqueous extract)	0.182±0.03	7.44±0.38	<0.01

Table 3: Changes in the level of total cholesterol and triglycerides in control and experimental rats

Groups	Cholesterol (mg/dL)	Triglycerides (mg/dL)	Level of significance (p)
Group I (control)	121.67±5.62	108.50±5.14	<0.01
Group II (paracetamol)	349.50±15.86	219.83±10.20	<0.05
Group III (paracetamol+silymarin)	115.00±4.23	112.33±5.40	<0.01
Group IV (paracetamol+300 mg aqueous extract)	130.00±5.24	125.00±6.02	<0.05
Group V (paracetamol+600 mg aqueous extract)	122.87±5.52	110.17±5.62	<0.01

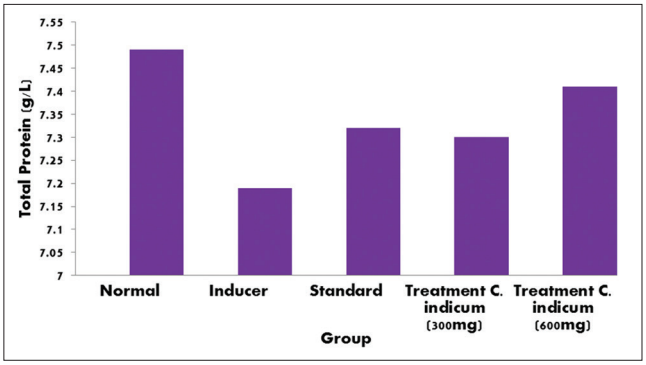

Fig. 4: Changes in the activity of serum total protein using *Chrysanthemum indicum* flower extract

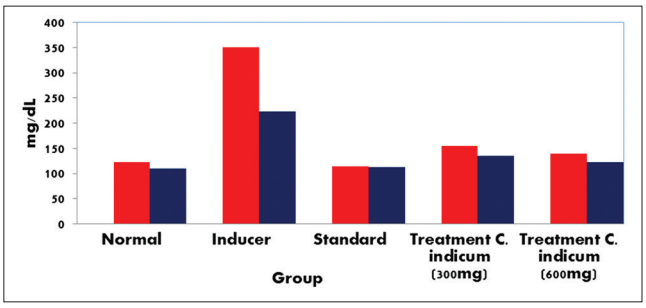

Fig. 5: Changes in the level of serum total cholesterol and triglycerides using *Chrysanthemum indicum* flower extract

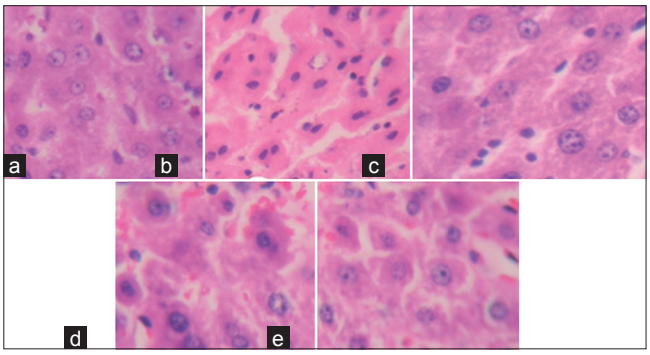

Fig. 6: Histopathological analysis of liver in (a) normal (b) paracetamol treated (c) paracetamol with silymarin (d) paracetamol with extract (300 mg) and (e) paracetamol with extract (600 mg)

levels of AST, ALT, bilirubin, protein, triglycerides and cholesterol was confirmed by biochemical analysis. The necrosis and inflammation caused by paracetamol in liver was restored using aqueous extract of *C. indicum* flower analyzed by histopathological studies. Phytochemical constituents such as phenol, alcohol, amine, and ester are strongly strengthen the use as hepatoprotective plant. Further studies were required to characterize the active principle for proper drug formulations.

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