Research Article

HEPATOPROTECTIVE EFFECT OF ETHANOLIC EXTRACT OF *CURCUMA LONGA* LINN ON ANTITUBERCULAR DRUGS INDUCED HEPATOTOXICITY IN ALBINO RATS

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Article Received on: 21/08/18 Approved for publication: 20/09/18

DOI: 10.7897/2230-8407.0910235

ABSTRACT

Objective: The aim of this study was to screen the hepatoprotective effect of ethanolic extract of *Curcuma longa* on antitubercular drugs induced hepatotoxicity in rats. **Methods:** Hepatotoxicity was induced by a combination of three antitubercular drugs [isoniazid (I) 7.5mg/kg, Rifampin (R) 10 mg/kg and Pyrazinamide (P) 35mg/kg] given orally as a suspension for 30 days in rats. 95% ethanolic extract of *Curcuma longa* (500mg/kg) was given orally for 30 days. The hepatoprotective activity was assessed using various biochemical parameters like SGOT, SGPT, ALP, Total bilirubin, unconjugated bilirubin, and total protein. At the end of study histological examinations were also carried out. **Results:** Treatment with *Curcuma longa* significantly ($P<0.05$ - $P<0.001$) prevented the drug- induced an increase in serum levels of hepatic enzymes. Histopathology of liver tissue showed that *Curcuma longa* preserved the normal hepatic cell architecture. **Conclusion:** The results of this study indicate the protective effect of *Curcuma longa* against liver injury which may be attributed to its hepatoprotective activity, and thereby scientifically support its traditional uses.

Keywords: *Curcuma longa* Isoniazid, Rifampin, Pyrazinamide, liver function test.

INTRODUCTION

Tuberculosis (TB) is the common infectious killer in the world and is also the important causes of death due to HIV infected and in antimicrobial resistance person. Based on the Global TB report 2016 India has the highest burden of TB. In India annually 28 lakh cases of TB estimated in which 1.3 lakh incidents is of multi-drug resistant.¹

The 1st line drugs like isoniazid, rifampicin, pyrazinamide, ethambutol, and streptomycin were used for the treatment of TB. These drugs were used in the combination as per WHO recommendation. But their combination causes hepatotoxicity as a major side effect. As a result treatment effectiveness is reduced. If hepatotoxicity is moderate or severe treatment should be stopped reintroduced after the hepatotoxicity has resolved.²

Curcuma longa commonly known as Turmeric was used as a medicinal herb in India from ancient times. It has played a great role in the day-to-day life of ancient Indians as a wound –healer, as a medicine for stomach ache, cough, poison, etc, for dyeing clothes and for worshipping their god and goddesses. This plant has acquired great importance in the modern world with its anti-inflammatory, anticancer, antioxidant, and a variety of other medicinal properties.³

Curcuma longa is a perennial herb which grows 2-3 ft. high with a short stem and tufted leaves. Rhizomes are short and thick. *Curcuma longa* rhizome contains Volatile oil (1-6.5%) mainly composed of α and β termerone, monoterpene, 5% curcuminoids mainly curcumin, demethoxy curcumin and bis demethoxy curcumin, resin, and abundant zingiberaceous starch grains. The main active constituent of *Curcuma longa* is curcumin (50-60%) which is the yellow substance and is responsible for the therapeutics activities.⁴

MATERIALS AND METHODS

Collection and Identification of Plant

The rhizomes of *Curcuma longa* were collected from the Katihar district, Bihar, India and authenticated by Botanical Survey of India, Howrah. (CNH/TechII/2016/73).

Extract preparation

Collected rhizomes were washed with water, sliced without unpeeling, shade dried till moisture is removed, powdered and stored in the airtight container.

Powdered rhizomes of *Curcuma longa* were defatted with petroleum ether in Soxhlet apparatus for 24 hrs using hot percolation method. The extract was filtered and the marc was extracted again with 95% ethanol by hot percolation method by using a Soxhlet apparatus for 48 hrs. The extract was filtered and the solvent was evaporated at reduced pressure by using rota-evaporator apparatus. The yield of the extract obtained was 7 %.

Phytochemical Screening

Preliminary phytochemical screening of the ethanolic extracts was done according to Standard methods. (Table 1)^{5,6}

Experimental animals

Healthy Wistar albino rats (150-250g), aged 3 months of either sex were obtained from Departmental Animal house and were kept at $25\pm 2^\circ\text{C}$, relative humidity 44-56% with 12 h dark and light cycles. Animals were provided with standard rodent pellet diet and water *ad-libitum* throughout the experiment. The study was approved by the Institutional Animal Ethics Committee (KUDOPS/47).

Induction of hepatotoxicity

Isoniazid (7.5mg/kg), Rifampin (10mg/kg) and Pyrazinamide (35mg/kg) were used for induction of hepatotoxicity.⁷⁻⁹ All the drugs were dissolved in sterile distilled water. All three drugs were obtained in powder form from Yarrow Chem Products, Mumbai. *Curcuma longa* (500mg/kg) was used as a hepatoprotective agent.¹⁰

Study design

24 Wistar albino rats of either sex were used for the study. They were randomly divided into 4 groups with six (6) rats in each group.

Group I

Normal control (n=6, the animals were given normal saline only).

Group II

Hepatotoxic control (n=6, the animals were given antitubercular drugs for 30 days)

Group III

Treatment group (n=6, the animals were given antitubercular drugs + *Curcuma longa* extract for 30 days).

Group IV

Standard group (n=6, the animals were given antitubercular drugs + Silymarin for 30 days).

At the end of the experiment, the blood was collected from all groups of animals by retro-orbital plexus method under light ketamine anaesthesia. From each group, two animals were sacrificed by overdose anaesthesia. The liver was removed and kept in 10% formalin solution for histopathological studies.

Biochemical study

Serum was separated from the blood by centrifugation at 6,000 rpm for 15 min and analysed for Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT), Serum Alkaline Phosphatase (ALP), Serum unconjugated bilirubin, Total bilirubin and Total protein by diagnostic kits ERBA using semi autoanalyzer (Transasia-Model ERBA,CHEM 5V2).

Histopathological analysis

Small sections of liver tissues were fixed in 10% formalin solution and processed for embedding in paraffin. 5 microns sections were cut and stained with hematoxylin and eosin and examined for histopathological changes under the microscope.

Statistical analysis

The statistical analysis was done by using Graph pad version 3.10. The results were reported as the mean±SEM (standard error of mean). Statistical significance $p<0.05$ (*), $p<0.01$ (**), or $p<0.001$ (***) was determined by using the Turkey t-test or ANOVA.

RESULT

Phytochemical results

The result of the preliminary phytochemical analysis of *Curcuma longa* is shown in table 1.

Table 1: Preliminary Phytochemical Analysis

Phytochemical constituents	Status of the compound
1.Carbohydrate	Present
2.Reducing sugar	Present
3.Protein	Present
4.Tannin	Absent
5. Alkaloids	Absent
6.Flavonoids	Present
7.Diterpenes	Absent
8.Glycosides	Present
9. Saponin	Present

Biochemical Result

The hepatoprotective effects of ethanolic extract of *Curcuma longa* on antitubercular drugs induced rats are shown in Table 2 and Figures (1 to 6). Administration of Isoniazid (7.5mg/kg), Rifampin (10mg/kg) and pyrazinamide (35mg/kg) significantly elevated ($p<0.001$) each of SGPT, SGOT, ALP, Serum Total Bilirubin and Unconjugated Bilirubin levels and TP significantly decreased ($P<0.001$) when compared to control group. Treatment of ethanolic extract of *Curcuma longa* at a dose of 500mg/kg, 1 hr prior to antitubercular drugs administration significantly reversed the elevation of Serum AST, ALT, ALP, Total and unconjugated bilirubin as compared to hepatotoxic group II rats.

Table 2: Effect of *C.longa* on serum AST(IU/L),ALT(IU/L),ALP(IU/L), TB(mg/dl), UB(mg/dl) and TP(g/dl) against I+R+P induced hepatotoxicity in rats (Mean±SEM)(n=6)

Tests	Control group	Hepatotoxic group	Treatment group	Standard group
1.AST(SGOT)	33.7±1.756	152.11±5.155	89.45±7.10	44.566±1.588
2. ALT(SGPT)	79.75±11.75	188.76±5.68	134.73±15.05	91.166±11.586
3. ALP	136.43±2.719	376.2±30.141	209.783±7.667	157.93±3.204
4.Total Bilirubin	0.3383±0.019	0.765±0.030	0.65±0.018	0.481±0.03825
5.UnBilirubin	0.2533±0.0197	0.6116±0.018	0.5116±0.011	0.4033±0.03765
6.Total protein	7.588±0.2383	5.945±0.1983	6.91±0.1386	7.4116±0.2773

Fig 1: AST Hepatotoxic group Vs AST Treatment group ($***P<0.001$), AST Control group Vs AST Treatment group ($***P<0.001$), AST Hepatotoxic group Vs AST Standard group ($***P<0.001$)

Fig 2: ALT Hepatotoxic group Vs ALT Treatment group ($*P<0.05$), ALT Control group Vs ALT Treatment group ($*P<0.05$), ALT Hepatotoxic group Vs ALT Standard group ($***P<0.001$).

Fig 3: ALP Hepatotoxic group Vs ALP Treatment group ($***P<0.001$), ALP Control group Vs ALP Treatment group ($*P<0.05$), ALP Hepatotoxic group Vs ALP Standard group ($***P<0.001$)

Fig 4: Total bilirubin Hepatotoxic group Vs Total bilirubin Treatment group ($**P<0.01$), Total bilirubin Control group Vs Total bilirubin Treatment group ($***P<0.001$), Total bilirubin Hepatotoxic group Vs Total bilirubin Standard group ($***P<0.001$)

Fig 5: Unconjugated bilirubin Hepatotoxic group Vs Unconjugated bilirubin Treatment group ($**P<0.01$), Unconjugated bilirubin Control group Vs Unconjugated bilirubin Treatment group ($***P<0.001$), Unconjugated bilirubin Hepatotoxic group Vs Unconjugated bilirubin Standard group ($***P<0.001$)

Fig 6: Total Protein Hepatotoxic group Vs Total Protein Treatment group ($**P<0.01$), Total Protein Control group Vs Total Protein Treatment group (ns $P>0.05$), Total Protein Hepatotoxic group Vs Total Protein Standard group ($**P<0.01$).

Fig (1 to 6) Comparison of liver biochemical concentrations in different experimental groups.

Histopathological results

The histopathological results are as follows:

1. Control group

1. Central vein; 2. Portal triad; 3. Sinusoids; 4. Hepatocytes all are normal

2. Hepatotoxic(ATD) group

1. Portal vein (congested), 2. Infiltration of Macrophages around the bile duct, 3-congestion of the hepatic artery, 4. Sinusoid dilated 5- hepatocytes degenerate

3. Treatment (Curcumin) group

1-Central vein (Normal), 2-Sinusoids (Normal), 3-Hepatocytes (Normal),

4. Standard (Silymarin) group
Sinusoids (Normal).

Histopathological results showed that in the control group liver cells are normal. In hepatotoxic group Central vein becomes enlarged, RBCs in CV, Portal vein and hepatic artery congested, Infiltration of Macrophages, Sinusoid dilated and hepatocytes degenerate. In the curcumin group, liver cells are like to control group.

DISCUSSION

In the present study, we have observed the hepatoprotective effect of *Curcuma longa* against antitubercular drugs induced hepatotoxicity in rats. In the combination of antitubercular drugs, we have observed that their toxicity is increased in a synergistic manner. All antitubercular drugs are metabolized in the liver.^{11, 12} Isoniazid is metabolised to Acetyl hydrazine which is further metabolised by CYP450 to toxic metabolites that leads to hepatotoxicity.¹³

Rifampicin is a potent inducer of cytochrome P450 enzyme as it increases the production of toxic metabolites isonicotinic acid and hydrazine from Isoniazid. Rifampicin also increases the

production of toxic metabolites by increasing metabolism of acetyl hydrazine (AcHz). It shortened the plasma half-life of AcHz due to its oxidative elimination rate of AcHz increases and it is quickly converted to its active metabolites. Pyrazinamide increases the incidence of hepatotoxicity when given in combination with isoniazid and rifampicin¹⁴

Oxidative stress - induced hepatic injury is another important mechanism of hepatotoxicity produced by antitubercular drugs. The present study revealed an increased level of SGPT, SGOT, ALP, TB, UNB while decreased in TP level in the serum of the hepatotoxic group on exposure to antitubercular drugs indicate liver damage. An increase in the levels of these marker enzymes in serum was due to the leakage of the enzyme from the liver as a result of hepatocellular damage. Increases in AST and ALT levels are sensitive indicators of acute hepatic necrosis and Increases in ALP level indicate hepatobiliary disease.¹⁵

Curcuminoids in turmeric ranged from 2.5 to 6% of which curcumin accounted for about 49% of the total pigments, demethoxycurcumin about 29% and *bis*-demethoxycurcumin

about 22%. Curcumin possesses excellent antioxidant properties. A combination of the three curcuminoids was more powerful antioxidant than each of the single components — curcumin, demethoxycurcumin, or bisdemethoxycurcumin — used alone. Molecular structures that contribute for the biological activity of curcuminoids are: (1) *p*-hydroxyl groups — antioxidant activity, (2) keto groups — anti-inflammatory, anticancer, antimutagen, and (3) double bonds — anti-inflammatory, anticancer, antimutagen.

The antioxidant activities of curcuminoids may include one or more of the following mechanism: (1) Scavenging or neutralizing of free radicals, (2) Interacting with oxidative reactions and preventing it. (3) Scavenging of free oxygen and preventing oxidative reactions, (4) inhibiting cytochrome P-450 oxidative enzymes, and (5) chelating with metal ions like iron (Fe) and preventing its oxidative properties.³

On treatment with an ethanolic extract of *C. longa* at a dose of 500 mg/kg, 1 hr prior to antitubercular drugs the hepatic histopathology as well as the serum marker enzyme levels were near to normal indicating reversal of antitubercular drugs induced hepatotoxicity and confirming the free radical scavenging property of *C. longa*.

CONCLUSION

The present study shows that *Curcuma longa* treatment mitigates antitubercular drugs induced hepatotoxicity, which could be due to its antioxidant nature along with the free radical scavenging property.

ACKNOWLEDGMENT

The author is thankful to the staffs of the Department of Pharmaceutical Sciences, Kumaun University, Bhimtal campus, Bhimtal, Nainital for good support during the research work.

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Cite this article as:

Babul Kumari et al. Hepatoprotective effect of ethanolic extract of *Curcuma longa* Linn on antitubercular drugs induced hepatotoxicity in albino rats. *Int. Res. J. Pharm.* 2018; 9(10):106-110 <http://dx.doi.org/10.7897/2230-8407.0910235>

Source of support: Nil, Conflict of interest: None Declared

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