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RESEARCH ARTICLE

Hepatoprotective Effect of *Zingiber Officinale* Roscoe against Anti-Tubercular Drug-Induced Liver Toxicity in Albino Rats

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Abstract

The present study was aimed to investigate the hepatoprotective effect of ethanolic extract of *Zingiber officinale* Roscoe against hepatotoxicity induced by antitubercular drugs in Wistar albino rats. The ethanolic extract of rhizomes of *Zingiber officinale* was prepared using a Soxhlet apparatus. 24 Wistar albino rats were randomly divided into four groups (n=6). Except Group 1, all the other groups were treated with antitubercular drugs [isoniazid (I) (7.5mg/kg), rifampicin (R) (10mg/kg) and pyrazinamide (P) (35mg/kg)]. Group 2 and group 4 were treated with Silymarin (100mg/kg) and *Zingiber officinale* (500mg/kg) respectively, one-hour prior administration of antitubercular drugs for 30 days. At the end of the study, blood was collected and the two animals from each group were sacrificed and the liver was sent for histopathological examination. Data calculated by mean ± standard error of the mean and by unpaired t-test. Any p-value less than 0.05 have been considered as statistically significant. Antitubercular drugs significantly increased the Serum glutamate oxaloacetate transaminase (SGOT), Serum glutamate pyruvate transaminase (SGPT), Alkaline Phosphate (ALP), Total bilirubin (TB), unconjugated bilirubin (UB) and Total protein (TP) levels as compared to the control group. Treatment with *Z. officinale* extract (500mg/kg) significantly ($P<0.05$-$P<0.001$) reduces the antitubercular drugs induced hepatic toxicity and biochemical elevations towards normal. *Zingiber officinale* extract was hepatoprotective against antitubercular drugs induced hepatotoxicity in albino rats.

Keywords: *Zingiber officinale extract, Isoniazid, Rifampicin, Pyrazinamide, Liver enzymes.*

Introduction

Tuberculosis (TB) continues to be a serious public health problem mostly in developing countries and is caused by various species of *Mycobacterium*. The common human *Mycobacterium* pathogens are *Mycobacterium tuberculosis* and *Mycobacterium bovis* [1]. About 1/3rd of the world's population is infected with tuberculosis (WHO). Maximum no. of TB cases in the world is found in India, China, South Africa, Nigeria, and Indonesia among them India ranked top on the list.

Due to the spread of the HIV, there is an increased prevalence of tuberculosis, infection with *Mycobacterium avium* complex and multidrug-resistant tuberculosis [2, 3]. The *Mycobacterium* developed resistance by using any single drug. To overcome resistance combinations of drugs are therefore needed during therapy [4].

Liver toxicity is the most common adverse effect of antitubercular drugs. The adverse effect increases in a synergistic manner when drugs are given in combination form [5].*Zingiber officinale* is commonly called Ginger or Saunth. It is about 1m high perennial herb with branching rhizome. Ginger consists of volatile oil (0.25-3%), which comprises α-zingiberene, ß-bisabolene, α-farnesene, ß sesquiphellandrene, and α-curcumin.

Aroma and flavour are the two important characters of ginger. The aroma of ginger is due to volatile oil while the flavour and pungency are due to phenolic ketones of oleo-resin. Gingerols, shogaols, zingerone, parasols, gingediol etc form the phenolic ketones of oleo-resin [6, 7]. Ginger is widely used as a spice and flavouring agent.

It is also used as an aromatic stimulant, stomachic, carminative agent [8] and anthelmintic action [9]. From the literature search; it has been found that *Zingiber officinale,* can produce hepatoprotective activity against many drugs induced hepatotoxicity. The rationale behind this study is to find out a new hepatoprotective drug against antitubercular drugs induced hepatotoxicity, which is potent, safe and cost-effective.

Materials and Methods

Collection and Authentication of Plant Material

Rhizomes of *Zingiber officinale* Roscoe were collected from katihar district, Bihar, India and authenticated by Botanical Survey of India, Howrah (W.B.) (Authentication no.CNH /Tech II/2016/73).

Place and Year of Work

The present study has been carried out at Department of Pharmaceutical Sciences, Kumaun University, Bhimtal Campus, Nainital (Uttarakhand), India during 2017 to 2018.

Preparation of Plant Extract

Rhizomes of *Zingiber officinale* were washed with water, sliced without unpeeling and shade dried till moisture was removed, powdered and stored in the separate airtight containers. The dried powder was defatted with petroleum ether in the soxhlet apparatus using the hot percolation method, the marc was extracted again with 95% ethanol by hot percolation method for 48 hrs. The extract was separated by filtration and concentrated on rota vapour at reduced pressure. The yield obtained was 12% (w/w). Ethanolic extracts of rhizomes were subjected to a preliminary phytochemical screening of various constituents [10, 11, 12].

Drugs and Chemicals

All the chemicals used were of analytical grade and were procured from Yarrow Chem Products, Mumbai, Merck's India, Mumbai and some from local suppliers.

Experimental Animals

Healthy Wistar albino rats (150-250g), aged 3 months of either sex were obtained from Departmental Animal House and were kept in polypropylene cages, 3 rats per cages at 25±2°C, with 12 h dark and light cycles. Animals were provided standard rodent pellet diet and water *ad-libitum* 15 days before and throughout the experiment. The study was approved by the Institutional Animal Ethics Committee of the department (KUDOPS/47).

Study Protocol

Hepatotoxicity was induced by using Isoniazid (7.5mg/kg, p.o), Rifampicin (10mg/kg, p.o) and Pyrazinamide (35mg/kg, p.o) for 30 days and Silymarin (100 mg/kg, p.o) were used as the standard drug. The doses were taken from previous literature [13, 14, 15].

Experimental Design

The rats were randomly divided into **4 groups** of six rats in each group.

Group I was the Control group and received a normal diet. **Group II** Standard group and received Silymarin + one-hour prior administration of antitubercular drugs, **Group III** Disease group and received antitubercular drugs, **Group IV** *Z .officinale* (500mg/kg, p.o) [14] + one-hour prior administration of antitubercular drugs for 30 days.

Biochemical Studies

After the end of the experiment, blood was collected from retro-orbital plexus under light ketamine anesthesia and two animals from each group were sacrificed. The liver was removed and kept in 10% formalin solution for histopathological studies. Serum was separated from the blood by centrifugation at 6,000 rpm for 10 min and analysed for Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT), Serum Alkaline Phosphatase (ALP), Serum unconjugated bilirubin (UB), Total bilirubin (TB) and Total protein (TP) by diagnostic kits ERBA using semi auto analyser (Transasia-Model ERBA,CHEM 5V2).

Histopathological Studies

For the histopathological study, the liver tissues were fixed in 10% formalin solution, dehydrated in gradual ethanol (50-100%v/v) cleaned in xylene and embedded in paraffin. 5µm thickness sections were cut, stained with hematoxylin-eosin dye and observed under the microscope (100 xs).

Statistical Analysis

The data were statistically analysed by using SPSS version 20 software. All variables were expressed as mean ±SEM. The data were also calculated by unpaired t-test. An alpha level of 5% has been taken, i.e. if any p-value is less than 0.05 it has been considered as significant.

Result

Phytochemical Results

Table 1: Results of the preliminary phytochemical analysis of *Z. officinale*

Phytochemical constituents	*Z. officinale* ethanolic extract
Carbohydrate	+
Reducing sugar	+
Protein	+
Tannin	+
Alkaloids	--
Flavanoids	+
Steroid / Terpenoids	--
Glycosides	+
Saponin	+
Phenol	--
Coumarin	+

(Present = +, Absent = --)

Biochemical Result

The hepatoprotective effects of ethanolic extract of *Z. officinale,* on antitubercular drugs induced rats are shown in Table 2. Administration of Isoniazid (7.5mg/kg), Rifampin (10mg/kg) and pyrazinamide (35mg/kg) significantly elevated (p<.001) each of SGOT, SGPT, ALP, Serum Total Bilirubin and Unconjugated Bilirubin levels and TP significantly decreased (P<.001) when compared to control group. Treatment of ethanolic extract of *Z. officinale* at a dose of 500mg/kg, 1 hr before antitubercular drug administration significantly reversed the elevation of Serum SGOT, SGPT, ALP, Total and unconjugated bilirubin and TP increased as compared to Disease group rats.

Table 2: Effect of *Z. officinale* on serum SGOT (IU/L), SGPT (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)

Parameters	Control group	Standard group	Disease group	*Z. officinale* group
SGOT (IU/L)	33.7 ± 1.76	44.47± 1.58***	152.12± 5.15#	123.33±7.6*
SGPT (IU/L)	79.75±11.76	91.17±11.59***	188.77±5.69#	118.4±16.52**
ALP (IU/L)	136.43±2.72	157.93±3.2***	376.2±30.14#	265.65±9.83 **
TB (mg/dl)	0.34±0.02	0.48±0.04***	0.93±0.09#	0.72±0.01*
UB (mg/dl)	0.25±0.02	0.4 ±0.04**	0.76±0.07#	0.57±0.02*
TP (g/dl)	7.59±0.24	7.41±0.28**	5.95±0.2#	6.89±0.11**

Data expressed as mean ± SEM, n=6 in each group. Significance at #: P<0.001 compared with the respective control group I. *: P<0.05, **: P<0.01, ***: P<0.001 compared with group 3 (Disease group)

Histopathological Study

The histopathological results are as follows:

Figure 1: Control group Sinusoids (Normal), hepatocytes (Normal)

Figure 2: Standard (Silymarin) group Sinusoids (Normal)

Figure 3: Disease group Hepatocytes degenerate; Sinusoids dilated

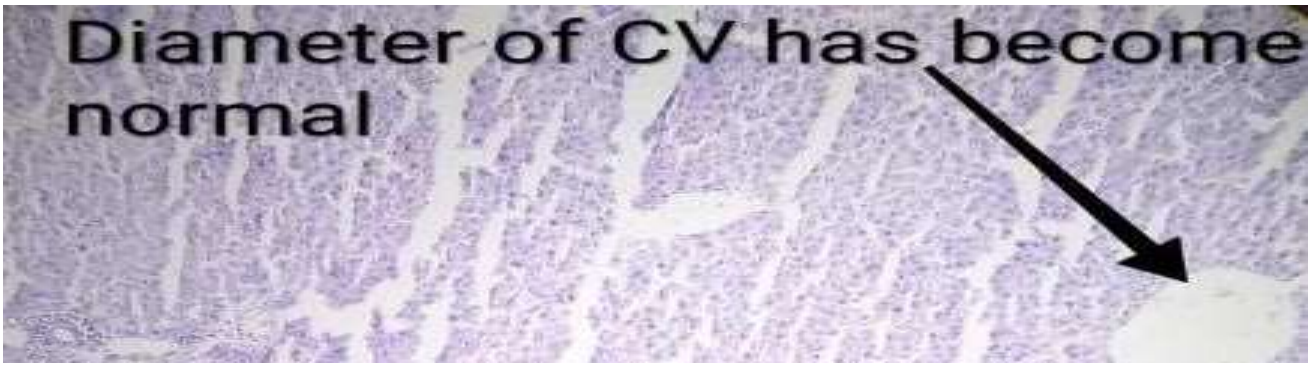

Figure 4: *Zingiber officinale* group Sinusoids (Normal), Hepatocytes (Normal)

Discussion

The present study was made to investigate the Hepatoprotective activity of *Zingiber officinale* Roscoe against antitubercular drug-induced liver toxicity in albino rats. Isoniazid is metabolised into acetyl diazine. Acetyl diazine is in itself a toxic metabolite or may break down to reactive ions and radicals that bind with hepatic cells and causes hepatic injury [16].

Rifampicin is a powerful inducer of several metabolic enzymatic pathways specially Cyt P450. Cyt P450 increases the metabolism of isoniazid yielding more toxic metabolites. Thus it explains rifampicin potentiates the hepatotoxicity of other anti TB drugs. Rifampicin interferes with the bilirubin uptake that results in transient hyperbilirubinemia [17]. PZA or PZA metabolites are also responsible for hepatotoxicity.

In comparison to Isoniazid and Rifampicin, the $t_{1/2}$ of Pyrazinamide is longer, dose-dependent and it is further increased in the presence of liver injury [18].Antitubercular drugs also produced hepatotoxicity by other important mechanisms i.e oxidative stress-induced hepatic injury. It increases the liver cell lipid peroxidation [19].SGOT, SGPT, ALP, Total bilirubin, unconjugated bilirubin are mainly present in the liver when there is hepatic injury these enzymes leak into the bloodstream.

On administration of antitubercular drugs these enzymes level increases in the blood. This condition becomes reversed to normal after treatment with *Zingiber officinale* [20]. On Phytochemical screening *Zingiber officinale* shows the presence of flavanoids, glycosides, reducing sugar, tannin and saponin. Hence, the mechanism of hepatoprotection of *Zingiber officinale may*

be due to its antioxidant properties present in these phytochemicals and another anti-inflammatory property which may inhibit inflammatory hepatic injury.

These results were in agreement with Tasduq *et al.,* 2006 [21]. The findings were in agreement with the previous work done which were as follows Ajith *et al*., studied that a single dose of aqueous extract of ginger (200, 400 mg/kg before acetaminophen) administration prevents the acetaminophen-induced hepatotoxicity in rats. The cohorts' administration of ginger had decreased the levels of SGOT, SGPT, and ALP in serum and increased levels of SOD, GSH, CAT and GST in the liver [22].

Akinloye OA *et al.,* reported that the ethanolic extract of *Zingiber officinale* (400mg/kg) was effective in reducing the nitrobenzene induced hepatotoxicity. Pre-treatment with ginger decreased the nitrobenzene-induced increase in the biochemical levels of SGOT, SGPT, ALP, gamma-glutamyl transferase (GGT), TB, TP and restored the altered levels of plasma cholesterol and triglycerides [23].

Helal *et al*., with Wistar rats have shown that when compared to the cohorts receiving the only tetracycline, the group also receiving aqueous extract of ginger had reduced levels of SGOT, SGPT, GGT, Lactate dehydrogenase, blood glucose, total lipids, triglycerides, cholesterol, low-density lipoprotein (LDL) cholesterol, urea and creatinine [24]. The active constituents of ginger are Gingerols that have been shown to inhibit cyclooxygenase activity in platelets, therefore, platelets release and aggregation is affected [25, 26]. Gingerols were found to affect both COX -1 and COX -2 [27]. 8-Paradol and shogaol, the constituents of ginger, showed strong inhibitory effects on the COX-2 enzyme [28].Modern anti-inflammatory drugs (eg: NSAIDS) produces ulcers in the digestive system as a common side effect. But ginger not only prevents the inflammation reactions, but it also prevents ulcers in the digestive tract [29].

Conclusion

Zingiber officinale extract was hepatoprotective against antitubercular drugs induced hepatotoxicity in albino rats.

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