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Hepatoprotective evaluation of *tinospora cordifolia* against drug induced hepatotoxicity

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Abstract---Purpose: Detoxification of several drugs and xenobiotics and also the metabolic and physiological processes happens in the liver. The liver is severely damaged by the reactive chemical intermediates by the detoxification process. There are various drugs available in the market and consumption of these drugs may lead to idiosyncratic drug reactions mediated hepatotoxicity. Various drugs are called off from the market because of its hepatotoxicity. Due to these reasons there has been search for the hepatoprotective drugs that has lead to the screening of the herbal drugs. Therapeutic efficiency of the herbal drugs are been evaluated by the pharmaceutical industries and scientific groups. Various herbal drugs present in different ethnopharmacological practices having hepatoprotective activity are used to treat some of the liver diseases. Method: The methanolic extract of *TinosporaCardifolia* has hepatoprotective properties was studied in rats by inducing toxicity with Paracetamol. Result: The plant extract possessed significant hepatoprotective activities that was confirmed by observing the lowered levels of AST, ALP, ALT, LDH and bilirubin. Conclusion: The goal of this study is to see if the methanolic extract of *TinosporaCardifolia* has any hepatoprotective properties in albino wistar rats.

Keywords---Hepatoprotective, Paracetamol, Methanolic extract, *Tinosporacordifolia*, etc.

Introduction

The liver is an essential organ because of its biochemical activities. It helps in detoxifying the toxic substances. As a result, liver damage caused by hepatotoxic substances is extremely harmful. Therefore, there has been increasing need of

medicines that will help in preventing such damages.[1] The liver is responsible for the detoxification of toxic substances, which leads to a variety of hepatic disorders. Hepatocarcinoma, alcoholism, Drug induced hepatotoxicity (DIHT), viral infections are also the factors that resulted in liver damage, which is a major concern. During DIHT, formation of proinflammatory cytokines and reactive free radicals from the hepatic neutrophils and Kupffer cells cause severe oxidative stress [1]. Due to the negative side effects of synthetic medications, a systematic study technique was required to examine herbal drugs that were claimed to have hepatoprotective properties. [2] *Tinosporacordifolia* is a tropical plant that is used in African and Asian traditional medicines that has worked in treating various diseases [3,4]. In other countries, in Cameroun, coffee is considered as a substitute for roasted seeds while other parts of the plants are used for the treatment of metabolic and CVS disorders. Phytochemical studies of *Tinosporacordifolia* leaves disclosed the presence of pharmacologically active families of a molecule, that included terpenoids, tannins, cardiac glycosides, terpenes, saponins, anthraquinones, inorganic elements [5,6]. Extracts of these plants were reported to have antifungal, antiviral [7,8] antibacterial, antihelminthic [9,10], antispasmodic, analgesic, antipyretic, anti-inflammatory [11,12] and hepatoprotective properties in humans and experimental models [13,14]. Verma, et al. (2010) reported the effect of methanolic extract of *Tinosporacordifolia* for to manage the alloxan-induced diabetic rats [15], anti-allergic, anti-inflammatory, and anti-lipid peroxidizing properties have been reported by Sreejith et al. [16].

The goal of this study is to see if the Methanolic extract of *Tinosporacordifolia* root has any hepatoprotective properties in albino wistar rats.

Materials and Method

Plant collection

The fresh plant of *Tinosporacordifolia* was collected from the region of Taluka Shirpur, District Dhule, India. Botanical survey of India Pune, Maharashtra certified the chosen plant. Barks were dried at R.T. so that loss of chemical constituents was avoided and milled with the help of grinding machines.

Extraction methodology [17]

The root of the plant was washed with tap water, dried at R.T. and transformed to a coarse powder. The extractions of the powdered drug was done with solvents like Petroleum ether (60-80°C), Methanol, chloroform, water-methanol and water separately by the Soxhlet extraction method. Then finally the obtained extract was evaporated and then dried under tray dryer and vacuum dryer to obtain thick extract.

Animals

Healthy adult Swiss albino wistar rats of either sex weighing between 160 to 180 gm were used for acute toxicity study and hepatoprotective activity.

Toxicity study: (OECD 423). [18]

Acute toxicity tests were carried out in accordance with the standards No. 423 of the Organisation for Economic Cooperation and Development. The Albino wistar

rats, male or female, were separated into six groups, each with six animals. Plant extract was administered orally as a single dose to rats at different dose levels of 50, 250, 500, 1000, 1500 and 2000mg/kg b.w. Each animal was observed during 30 min and periodically during 24hrs and special attention was given for first 4hrs and thereafter 14 days daily.

Experimental procedure

Paracetamol induced liver damage:[19]

Procedure:

Albino wistar rats (150-250g) were used for the study. All of them were randomly divided into the six groups, with each group having six animals and the treatment given to them is as follows:

Group I: Normal (Distilled water p.o.)

Group II: Toxicant (On 5th day Paracetamol 3g/kg, 1% CMC p.o.)

Group III: Standard (Silymarin 50mg/kg p.o. + on 5th day Paracetamol 3g/kg, p.o.).

Group IV: Extract treated (Methanolic extract of *Tinosporacordifolia* 50 mg/kg p.o. + On 5th day Paracetamol 3g/kg, p.o.).

Group V: Extract treated (Methanolic extract of *Tinosporacordifolia* 100 mg/kg p.o. + On 5th day Paracetamol 3g/kg, p.o.).

Group VI: Extract treated (Methanolic extract of *Tinosporacordifolia* 200 mg/kg p.o. + On 5th day Paracetamol 3g/kg, p.o.).

Distilled water as a vehicle or extract of *Tinosporacordifolia* were administered orally for 7 days. On 5th day, Paracetamol suspension (1% CMC) dose of 3g/kg p.o. was administered (19) Blood was taken from all groups of animals after 48 hours of paracetamol administration by puncturing the retro-orbital plexus. At R.T., the blood samples were left to coagulate for 45 minutes. Centrifugation was done at 300 r.p.m. at R.T. for 20 minutes, the serum thus obtained after centrifugation was subjected to biochemical investigations viz. ALT, AST, ALP, LDH, TB, and DB. All of the animal's livers were removed and processed for histological studies.

Histopathological studies

Small portions of liver were preserved in 10% buffered formalin saline from each of the six animals in each of the groups (pH 7.4). The liver damage was subsequently observed by preparing paraffin sections and staining them with haematoxylin-eosin dye.

Statistical analysis

The data represent mean S.E.M. Results were statistically analysed by one-way ANOVA followed by Dunnett's test. The minimum level of significance was set at $p < 0.005$.

Results

Paracetamol induce Hepatotoxicity

A significant increase was seen in serum levels of ALT, AST, LDH, ALP, total bilirubin after paracetamol was administered. Table 1 and Table 2, has summarized the hepatoprotective actions of roots of *Tinosporacordifolia* on the hepatotoxicity caused by Paracetamol. Maximum hepatoprotective actions was seen at 200mg/kg dose level of *Tinosporacordifolia* and then it was compared to Silymarin.

Table 1. Physical Parameter
Effect of Methanolic extract of *Tinosporacordifolia* on liver weight and liver volume in Paracetamol induced hepatotoxicity

Group	Body weight (gm)	Liver weight (gm)	Liver volume(ml)
Normal	264	9	8.5
Paracetamol treated	221	11	10
STD+ Paracetamol	262	10	8.5
50 mg/kgMethanolic extract of <i>Tinosporacordifolia</i> + Paracetamol3g/kg	235	10.5	11
100 mg/kgMethanolic extract of <i>Tinosporacordifolia</i> + Paracetamol3g/kg	256	10.5	10.5
200 mg/kgMethanolic extract of <i>Tinosporacordifolia</i> + Paracetamol3g/kg	267	9	9.5

Table 2. Biochemical parameter Effect of Methanolic extract of *Tinosporacordifolia* on various biochemical parameters in Paracetamol induced hepatotoxicity

Group	SGOT (I.U./L)	SGPT (I.U./L)	ALP (I.U./L)	LDH (I.U./L)	T.B. (%mg)	D.B. (%mg)
Normal	66.10± 1.2*	47.09± 0.6*	187.2± 1.7*	141.2 ± 1.3*	0.16± 0.006	0.17± 0.004**
Paracetamol Treated3g/kg	171.1± 1.4**	131.7 ±3.2	351.3± 3.2*	270.4 ± 1.4*	1.04± 0.1*	0.6± 0.016
STD + Paracetamol3g/kg	97.12 ±1.1	75.12 ±0.8	212.2± 0.9*	180.2 ± 3.5*	0.36± 0.01*	0.24± 0.004
50 mg/kgMethanolic extract of <i>Tinosporacordifolia</i> + Paracetamol3g/kg	138.5 ±1*	112.2 ±0.6	315.2± 1.2*	232.8 ± 1.6*	0.6± 0.03**	0.51 ± 0.01*
100 mg/kgMethanolic extract of <i>Tinosporacordifolia</i> + Paracetamol3g/kg	117.2 ±0.9	98.1 ±0.7**	280.1± 2.4*	212.7 ± 1.8*	0.6 ±0.02	0.44± 0.01*

200 mg/kgMethanolic extract of <i>Tinosporacordifolia</i> + Paracetamol3g/kg	102.6 ±1	88.19 ±0.3	241.1 ±2.6*	200.1 ± 0.4*	0.46 ±0.01	0.32± 0.01*
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Values are expressed as mean±S.E.M. (n=6)

*P<0.05, **P<0.01, when compared with the Paracetamol treated group (one-way ANOVA followed by Dunnett test)

Control animals had normal hepatocytes with a well-preserved nucleolus, conspicuous nucleus, central vein, and cytoplasm on histopathology. No signs of necrosis, fatty change, inflammation, were seen in these animals (Fig. 1). Liver sections of the animals administered only paracetamol exhibited severe necrosis and inflammatory cell infiltration in the centrilobular region. Inflammatory cells were found in the portal triad as well. (Fig. 2). Pretreatment with *Tinosporacordifolia* at 50 and 100mg/kg dose showed a reduction of necrosed area and inflammatory infiltrates in the centrilobular area with the disappearance of inflammatory infiltrate around portal triad (Figs. 4 and 5). *Tinosporacordifolia* at 200mg/kg dose showed a greater reduction of the necrosed area and sparse inflammatory cell infiltration around the central vein (Fig. 6) as compared to 50 and 100mg/kg dose.

Histopathological changes of Paracetamol induced hepatotoxicity in albino wistar rat liver (H and E × 100)

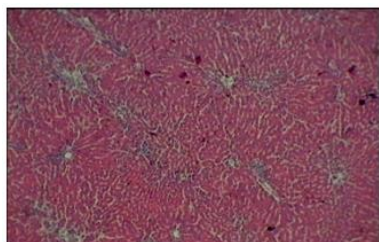


Figure: 1
Normal liver
Normal histology showing
prominent central vein, portal
triads and normal hepatocytes.
(H and E X 100)

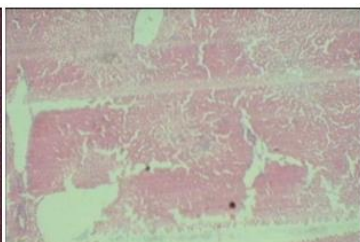


Figure: 2
Toxicant control Paracetamol
(3g /kg) Focal areas of liver cell
degeneration, lymphocytic
infiltration, fatty degeneration
(H and E X 100)

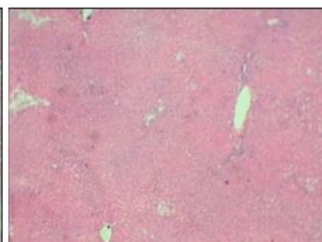
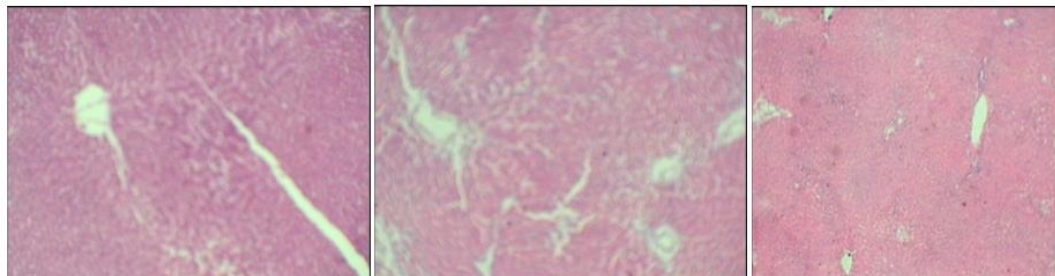


Figure: 3
Standard silymarin (50mg/kg)
Small areas of cell degeneration
And almost normal histology
(H and E X 100)

**Figure: 4**

Methanolic extract of
Tinospora cordifolia 50 mg/kg
Small areas of cell
degeneration
(H and E X 100)

Figure: 5

Methanolic extract of
Tinospora cordifolia 100 mg/kg
Small areas of cell
degeneration and Lymphocytic
infiltration
(H and E X 100)

Figure: 6

Methanolic extract of *Tinospora
cordifolia* 200 mg/kg
Normal central vein Portal triad
Occasional areas of cell
degeneration
(H and E X 100)

Discussion

When there is structural damage to the liver, then the serum transaminase and bilirubin level rises and gets released into the circulation after the cellular damage [20]. In addition, the *Vivo* hepatoprotective potential against paracetamol-induced liver injury was revealed in this study. When provided at high dosages, paracetamol, a commonly used analgesic and antipyretic medication, has been reported to cause hepatotoxicity in experimental animals and people. [21] It's mostly metabolized into excretable glucuronide and sulphate conjugates in the liver. However, the hepatotoxic effect caused due to Paracetamol has led to the development of toxic metabolites. This occurs when liver Cytochrome p 450 converts a portion of paracetamol to N-acetyl-p-benzoquinoneimine, a highly reactive metabolite that is ordinarily conjugated with GSH and eliminated in the urine as conjugates. Paracetamol overdose causes mitochondrial malfunction and acute liver necrosis. [22]

In this study, rats were administered a dose of paracetamol that had hepatotoxic effects, as evidenced by significant increases in SGOT, SGPT, LDH, ALP, and Bilirubin levels. Prior to the administration of paracetamol alone, the rats were given a Methanolic extract of *Tinospora cordifolia*, which helped to reduce the above specified serum enzyme levels. The increased levels of SGOT and SGPT in serum showed cellular leakage as well as a lack of cell membrane functional integrity in the liver. The extract helped in reduction of SGOT and SGPT levels, which indicated the stabilization of plasma membrane and also the liver damage was repaired, that was caused due to Paracetamol.

Alkaline phosphatase (ALP) is the prototype of these enzymes that reflect the pathological alteration in biliary flow. The use of ALP in chemical induced liver dysfunction has been investigated in our study. Paracetamol induced elevation of this enzymatic activity in serum is in line with high level of serum bilirubin content. The extract's ability to reduce enhanced ALP activity while simultaneously depleting elevated bilirubin levels implies that it may be able to

stabilise biliary dysfunction in the rat liver during chronic hepatic damage caused by paracetamol.[23]

The present study discovered a significant rise in levels of SGOT, SGPT, ALP, LDH and serum bilirubin levels when exposed to Paracetamol that indicated hepatocellular damage. When Methanolic extract of *Tinospora cordifolia* at 50 mg/kg, 100 mg/kg and 200mg/kg dose was administered, the increased serum levels of above mentioned enzyme had significantly reduced and had come to normal levels same as that of Silymarin treatment.

Conclusion

Based on the preceding findings, it can be concluded that the active constituents present in the root callus extract of *Tinospora cardifolia* showed better activity for the treatment of hepatotoxicity when compared with active constituents present in natural root extract against the hepatic damage due to Paracetamol. However, more research is needed to characterise the efficacy of root extract by isolating and identifying the active elements responsible for antihepatotoxic action in natural root and root callus extracts.

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Conflicts Of Interest

The authors declare no conflict of interest.

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