



Effect of n-hexane extract of *Tinospora crispa* on reduced glutathione and malondialdehyde level in paracetamol induced hepatotoxicity in rats

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ABSTRACT

The present study demonstrates that, treatment with the n-hexane extract of *Tinospora crispa* was able to ameliorate chronic hepatotoxicity due to oxidative stress e.g. Paracetamol induced hepatotoxicity. Considering the biochemical and histological parameters together, it was observed that hepatic reduced glutathione (GSH) concentrations of the n-hexane extract post treated group was increased to significant level and hepatic malondialdehyde (MDA) concentration was reduced to significantly lower level compared to the paracetamol treated groups. This study suggests near normal alleviation of paracetamol induced chronic hepatotoxicity in rats.

KEY WORDS:

Tinospora crispa, Paracetamol induced hepatotoxicity

INTRODUCTION-

Metabolism in the liver is always associated with the generation of reactive oxygen (ROS) and nitrogen species (RNS)¹. These toxic products of metabolism are nullified by endogenous detoxicating machineries e.g. enzymatic antioxidants (superoxide dismutase, catalase) and non enzymatic antioxidants (glutathione, selenium, α -tocopherol, β -carotene). Nature maintains a critical balance between the toxic and detoxifying mechanisms which the liver encounters each day. Imbalance may however occur by several conditions such as viral hepatitis², alcohol abuse³, cirrhosis of liver⁴, hepatocellular carcinoma⁵ and paracetamol induced liver damage⁶ which may lead to cause liver damage.

In the past, researchers studied the mechanism of toxic action of paracetamol (Acetaminophen) focused on metabolic activation of the drug, depletion of glutathione and covalent binding of the reactive metabolite n-acetyl-p-benzoquinone imine (NAPBQI) to cellular protein as the main cause of hepatic cell death⁷. In addition to covalent binding of the metabolite, oxidative stress appeared as another mechanism in the development acetaminophen toxicity⁶. Under conditions of NAPBQI overproduction, reduced glutathione (GSH) concentrations may be very low in the centrilobular cells and the major peroxide detoxification enzyme GSH peroxidase becomes inhibited⁸. Nake⁹ reported that the administration of encapsulated superoxide dismutase decreased the

paracetamol toxicity in rat. Jaeschke and Bajt¹⁰ also demonstrated that oxidant stress causes mitochondrial membrane permeability transition pore, loss of membrane potentials of mitochondria, depletion of ATP might be responsible for the typical nuclear DNA fragmentation of paracetamol induced cell death.

Metabolism of various endogenous and exogenous compounds generate reactive oxygen and nitrogen species (ROS and RNS) which are involved in the pathogenesis of liver disease^{11,12,13}. ROS rapidly react with a variety of cellular molecules and thereby interfere with cellular function¹⁴. Infection with HCV is associated with increased levels of ROS/RNS and decreased antioxidant levels in patients. Patients infected with HCV show increase in lipid peroxidation levels in liver samples, serum, and peripheral blood mononuclear cells². Adachi and Ishii¹⁵ reported that long term ethanol fed rats showed an increase ROS production, hepatocytes damage and apoptosis; this reaction was prevented by antioxidants. Most hepatocellular carcinoma occur in cirrhotic livers, and the common mechanism for carcinogenesis is chronic inflammation associated with oxidative stress¹⁶. Amimoto¹⁷ reported that treatment of fasted mice with paracetamol lead to hepatic injury. Paracetamol caused an increased amount of Thiobarbituric acid reactive substance (TBARS), which was accompanied by a loss of reduced form of coenzyme Q9 and coenzyme Q10 functioning as antioxidants. Paracetamol also markedly decreased hepatic glutathione levels. These results suggested that oxidative stress followed by lipid peroxidation might play a role in the pathogenesis of paracetamol induced hepatic injury.

Cellular GSH (reduced glutathione) concentration is decreased markedly in response to protein malnutrition, oxidative stress and many pathological conditions¹⁸. Levels of GSH are regarded as an index of toxicity, since its depletion in parenchymal organs leads to binding of toxic chemicals to cellular structure and eventually cell death¹⁹.

The traditionally available remedies for hepatic disorders from natural sources also are worth mentioning at this point. The herbal agents are cheap, easily obtainable, environment friendly and expected to lack organ-specific significant adverse effects. In this subcontinent, stem, leaves and whole plant of *Tinospora crispa* known in Bengal as 'guloncha' has been used widely for the treatment of bodyache, rheumatic pain, jaundice, pyrexia, tetanus, leprosy, diabetes, malaria, syphilis, sprain, eczema, sedative, loss of appetite, cold for a long period apparently without any scientific basis by traditional medical practitioners²⁰. *Tinospora crispa* belongs to the Menispermaceae family. Several plants belonging to this family have also been scientifically recognized as to contain phytochemical constituents having significant pharmacological activities²⁰. The *Tinospora crispa* stem extract possess high antioxidative properties and the activities is comparable to the established antioxidants, such as BHT and

vitamin C²¹. The flavonoids detected in *Tinospora crispa* are Catechin, Luteolin, Morin, and Rutin²¹. Catechin could prevent cardiovascular disease by protecting LDL from oxidative damage through its free radical quenching and metal chelating abilities²². Luteolin has strong scavenging properties for superoxide radicals²³. The present study decided to explore the curative role of the n-hexane extract of *Tinospora crispa* (Tc) in chronic hepatotoxicity induced by paracetamol in the rat model.

MATERIALS AND METHODS

The study was carried out in the laboratory of the Department of Pharmacology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, during the period from July 2010 to March 2011. 48 adult rats of the Long-Evans Norwegian strain aged between 3-4 months (weighing between 160-180 g) were obtained from the animal house of BSMMU. The rats were housed in standard sized metallic cages (3-4 rats / cage) in a well ventilated room. They were allowed to live at room temperature, fed normal rat diet.

Procedure for obtaining n-hexane extract of *Tinospora crispa* stem:

About 7 Kg of the herb (*Tinospora crispa*) was collected from suburbs of Dhaka, Tongi and Gazipur. The stems of the herb were cleaned, cut into small pieces and shed dried for several days. They were grinded by clean sterile grinding machine. About 1500 g powder of *Tinospora crispa* stem were obtained. The dried powdered herb was suspended in 6 litre of ethanol for 3 days. The whole was then filtered with filter paper. The filtrate was concentrated by rotary vacuum evaporator at 40-50°C and reduced pressure. About 600 ml of crude ethanol extract of *Tinospora crispa* was obtained. An equal amount of n-hexane was added to the crude ethanol extract and left for 24 hours. 2 layers were formed : n-hexane soluble nonpolar upper portion and ethanol soluble aqueous lower portion. The n-hexane soluble layer was separated by a separator funnel and was concentrated with rotary vacuum evaporator at 40°C. About 113.28 ml (14.5 g) light green n-hexane extract was obtained which was preserved in an amber coloured air tight glass bottle at 0-4°C until preparation of suspension.

Description of groups treated with drugs and extracts:

The experiment was designed to demonstrate the effect of post-treatment of the n-hexane extracts of *Tinospora crispa* on hepatic GSH, MDA concentrations as well as liver histology in paracetamol induced hepatotoxicity. For this purpose 48 rats were taken and grouped as follows-

Group C (control group) - 6 rats received normal rat diet and water *ad libitum* for 21 days and were sacrificed on the 22nd day.

Group P₂₂ (paracetamol control group) – 6 rats received daily a single dose of paracetamol at a dose of 150 mg/Kg body weight orally by ryle's tube from day 1 to day 21 and were sacrificed on the 22nd day.

Group P₃₆ (paracetamol treated group) - 6 rats received daily a single dose of paracetamol at a dose of 150 mg/Kg body weight orally by ryle's tube from day 1 to day 21 and were sacrificed on the 36th day.

Group PH₃₆ (paracetamol + n-hexane extract treated group) - 6 rats received daily a single dose of paracetamol at a dose of 150 mg/Kg body weight orally by ryle's tube from day 1 to day 21 and n-hexane extract solution of *Tinospora crispa* at a dose of 200 mg/Kg body weight orally by ryle's tube from day 22 to day 35 & were sacrificed on the 36th day.

Group P₄₃ (paracetamol treated group) - 6 rats received daily a single dose of paracetamol at a dose of 150 mg/Kg body weight orally by ryle's tube from day 1 to day 21 and were sacrificed on the 43rd day.

Group PH₄₃ (paracetamol + n-hexane extract treated group) - 6 rats received daily a single dose of paracetamol at a dose of 150 mg/Kg body weight orally by ryle's tube from day 1 to day 21 and n-hexane extract solution of *Tinospora crispa* at a dose of 200 mg/Kg body weight orally by ryle's tube from day 22 to day 42 & were sacrificed on the 43rd day.

Sacrifice of animals and collection of samples:

After inducing chloroform anaesthesia on the animal, The liver was dissected out after opening the abdomen by median incision. A small part (approximately 500 mg) of liver tissue was immersed immediately into Tyrode's solution contained in a separate beaker placed on an ice bath, weighed (after being blotted on filter paper) and kept in deep freeze until homogenized. The rest of the portion was preserved in 10% formalin for subsequent histological processing.

Estimation of hepatic reduced glutathione (GSH) concentrations

2-nitrobenzoic acid is reduced by SH group to form 2-nitro-5-mercaptobenzoic acid which has an intense yellow colour & can be used to measure SH groups by spectrophotometer.

Estimation of hepatic malondialdehyde (MDA) concentrations

Malondialdehyde (MDA) is formed as a result of lipid peroxidation. It reacts with thiobarbituric acid (TBA). The reaction yields a pink MDA-TBA adduct. The coloured complex can be measured by spectrophotometer.

Statistical analysis:

Data obtained from the findings of the above experiments have been expressed as mean \pm Standard deviation (mean \pm SD). The results were presented by tables. Significance of difference between groups were assessed by using 'One way Analysis of Variance' (ANOVA) followed by Bonferroni 't' test. The difference between groups were considered highly significant at $P < 0.001$, moderately significant at $P < 0.01$, and significant at $P < 0.5$.

RESULTS

Table I. Effect of *Tinospora crispa* extract on hepatic reduced glutathione(GSH) level (mg/ g of tissue) in paracetamol induced hepatotoxicity in rats.

Group	GSH (mg/g) (mean \pm SD)	Increased %	F value	P - value
C	5.28 \pm 0.28		60.467	< 0.001 ***
P ₂₂	2.30 \pm 0.40			
P ₃₆	2.61 \pm 0.08	13.48 %		
PH ₃₆	3.87 \pm 0.24	68.26 %		
P ₄₃	3.09 \pm 0.25	34.35 %		
PH ₄₃	4.01 \pm 0.15	74.35 %		

Anova show significant difference in GSH level among groups [F (7,47) = 60.467, $P < 0.001$] (Table I). Hepatic GSH level in control(C), paracetamol treated(P₂₂) groups were 5.28 \pm 0.28 mg/g & 2.30 \pm 0.40 mg/g, showing evidences of paracetamol induced hepatotoxicity. When n-hexane extract was administered from day 22 to day 35 (PH₃₆) & from day 22 to day 42 (PH₄₃) in paracetamol induced hepatotoxic group, GSH level markedly increased from 2.61 \pm 0.08 mg/g (P₃₆) to 3.87 \pm 0.24 mg/g (PH₃₆) (by 68.26%) & from 3.09 \pm 0.25 mg/g (P₄₃) to 4.01 \pm 0.15 mg/g (PH₄₃) (by 74.35%) showing hepatoprotective effect of n-hexane extract.

Table II. Effect of *Tinospora crispa* extract on hepatic malondialdehyde (MDA) level (pmol/ mg of protein) in paracetamol induced hepatotoxicity in rats.

Group	GSH (pmol/mg) (mean \pm SD)	Reduction %	F value	P - value
C	71.83 \pm 4.71		293.960	< 0.001 ***
P ₂₂	205.51 \pm 8.47			
P ₃₆	192.74 \pm 12.67	6.21 %		
PH ₃₆	110.21 \pm 6.69	46.37 %		
P ₄₃	172.71 \pm 6.71	15.96 %		
PH ₄₃	94.66 \pm 4.18	53.90 %		

Anova show significant difference in MDA level among groups [F (7,47) = 293.960, $P < 0.001$] (Table II). Hepatic MDA level in control(C), paracetamol treated(P₂₂) groups were 71.83 \pm 4.71 pmol/mg & 205.51 \pm 8.47 mg/g, showing evidences of paracetamol induced hepatotoxicity. When n-hexane extract was administered from day 22 to day 35 (PH₃₆) & from day 22 to day 42 (PH₄₃) in paracetamol induced hepatotoxic group MDA level markedly decreased from 192.74 \pm 12.67 pmol/mg (P₃₆) to 110.21 \pm 6.69 pmol/mg (PH₃₆) (by 46.37%) & from 172.71 \pm 6.71 pmol/mg (P₄₃) to 94.66 \pm 0.15 pmol/mg (PH₄₃) (by 53.90%) showing hepatoprotective effect of n-hexane extract.

Histological examination of the liver sections post treated with extracts of *Tinospora crispa* showed remarkable reduction in necrosis and degenerative changes against paracetamol induced toxicity.

DISCUSSION

The reputation of *Tinospora crispa* (guloncha) to the rural physicians regarding alleviation of hepatitis encouraged the present researchers in their attempts to evaluate the curative effect of its n-hexane extract in chronic hepatotoxicity. This study was designed to observe the post treatment effect of the n-hexane extract of *Tinospora crispa* upon hepatotoxic adult rats. Rats were made hepatotoxic by administration of paracetamol (150 mg / Kg body weight) orally daily for 21 days. Paracetamol induced hepatotoxic rats were sacrificed on 22nd, 36th and 43rd day. The paracetamol treated group sacrificed on 22nd day (P₂₂) demonstrated significant decrease in hepatic GSH & increase in MDA concentrations (P < 0.001), indicative of hepatocellular damage. Histology of liver showed centrilobular necrosis, inflammatory cell infiltrate and pyknotic nuclei suggestive of hepatic damage.

In the paracetamol treated rats sacrificed on day 36 and day 43 (P₃₆, P₄₃) the lowered hepatic GSH & increased MDA concentrations demonstrated a tendency to increase & decrease respectively, but significant recovery did not occur. Histological observations suggested that damages to the hepatic lobules persisted upto the mentioned dates. So the effect of extract administered following paracetamol intoxication in PH₃₆ & PH₄₃ were compared with those of P₃₆ & P₄₃ respectively.

The rats of groups PH₃₆ & PH₄₃ demonstrated significantly (P < 0.001) higher concentrations of GSH & lower concentrations of MDA in comparison to group P₃₆ & P₄₃ respectively. Compilation of biochemical and histological parameters together suggested alleviation of hepatic damage.

CONCLUSION

Tinospora crispa stem are rich in flavonoid compounds like Catechin, Luteolin, Morin, and Rutin which all have antioxidant activities²¹. These findings are in accordance with the findings of the present study where the n-hexane extract of *Tinospora crispa* stem administration suggest less lipid peroxidation or less oxidative stress.

Cavin²⁴ earlier reported that, *Tinospora Crispa* stem also contains antioxidant compounds n-cis feruloyltyramine, n-trans feruloyltyramine & secoisolaricireresinol; perhaps these may counter the oxidative damage induced by paracetamol (and its metabolite NAPBQI).

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