

HEPATOPROTECTIVE POTENTIAL OF *GREWIA UMBELLIFERA* AGAINST ACETAMINOPHEN-INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

Hepatoprotective potential of aqueous ethanolic extract of *Grewia umbellifera* against acetaminophen induced hepatic damage in albino rats was observed. In the present study the effect of aqueous ethanolic extract of *Grewia umbellifera* on blood and liver glutathione, Na⁺ K⁺- ATPase activity, serum marker enzymes, serum bilirubin, glycogen and thiobarbutiric acid reactive substances against acetaminophen induced damage in rats have been studied to find out the possible mechanism of hepatoprotection. It was observed that extract of *Grewia umbellifera* has reversal effects on the levels of above-mentioned parameters in acetaminophen hepatotoxicity. The

extract of *Grewia umbellifera* functions as a hepatoprotective agent and this hepatoprotective activity of *Grewia umbellifera* may be due normalization of impaired membrane function activity.

KEY WORDS: *Grewia umbellifera*, glutathione, hepatoprotection, lipid peroxidation, Na⁺K⁺-ATPase, paracetamol, thiobarbutiric acid reactive substances, serum marker enzymes

INTRODUCTION

Liver is the vital organ of metabolism and excretion. About 20,000 deaths found every year due to liver disorders. Hepatocellular carcinoma is one of the ten most common tumors in the world with over 2,50,000 new cases each year.

Acetaminophen(Paracetamol) hepatotoxicity is caused by the reaction metabolite N-acetyl-p-benzo quinoneimine (NAPQI), which causes oxidative stress and glutathione (GSH) depletion. It is a well-known antipyretic and analgesic agent, which produces hepatic necrosis at higher doses.^[1] Paracetamol toxicity is due to the formation of toxic metabolites when a part of its metabolized by cytochrome P-450. Introduction of cytochrome^[2] or depletion of hepatic glutathione is a prerequisite for paracetamol-induced hepatotoxicity.^[3,4]

In India, about 40 polyherbal commercial formulations reputed to have hepatoprotective action are being used. It has been reported that 160 phyto- constituents from 101 plants have hepatoprotective activity.^[5] Liver protective herbal drugs contain a variety of chemical constituents like phenols, coumarins, lignans, essential oil, monoterpenes, carotinoids, glycosides, flavanoids, organic acids, lipids, alkaloids and xanthines. Plant extracts of many crude drugs are also used for the treatment of liver disorders. Extracts of different plants of about 25 plants have been reported to cure liver disorders.^[6]

In spite of tremendous strides in modern medicine, there are hardly any drugs that stimulate liver function, offer protection to the liver from damage or help regeneration of hepatic cell.^[7] There are however, members of drugs employed in traditional system of medicine for liver affections.^[8] Many formulations containing herbal extracts are sold in the Indian market for liver disorders. But management of liver disorders by a simple and precise herbal drug is still an intriguing problem. Several Indian medicinal plants have been extensively used in the Indian traditional system of medicine for the management of liver disorder. Some of these plants have already been reported to posses strong antioxidant activity.^[9-11]

Grewia umbelliferea (Tiliaceae) (GU) is herbaceous medicinal plant that has been distributed in Kanniyakumari district, Tamilnadu, India. The aerial plant part extract is much more useful in treatment like spleen damage, liver complications and cardio disorders.^[12-14]

But still no scientific and methodical investigation has so far been reported in literature regarding its action on liver. Therefore, the present investigation has been designed to study the possible mechanism of aqueous ethanolic extract of *Grewia umbelliferea* on the biochemical parameter against paracetamol induced hepatic damage in albino rats.

MATERIALS AND METHODS

Plant material

Aerial part of *Grewia umbellifera* (Tiliaceae) was collected from Tirunelveli district, Tamil Nadu, India in the month of March and dried in a shade. The plant material was taxonomically identified and authenticated by V.Chelladurai (Research Officer) Botany (C.C.R.A.S) Government of India. Voucher specimen (JCAS-10/2014-2015) has been retained in the Dept of Biochemistry, Jaya College of Arts & Science, Chennai.

Extraction

Shade-dried powder (1kg of *Grewia umbellifera*) was extracted by percolation at r.t. with 70% ETOH. Aqueous ethanolic extract of *Grewia umbellifera* was concentrated under reduced pressure and dried in a vacuum desiccator (50°C). The residue (30.9 gm) was dissolved in 70% ETOH and filtered.

Animals

Studies were carried out using Wistar albino rats of either sex (150-200g), obtained from Indian Veterinary Preventive medicine (IVPM), Ranipet, Tamilnadu, India. The animals were grouped and housed in polyacrylic cages (38 x 23 x10 cm) with not more than six animals per cage and maintained under standard laboratory conditions (temperature $25 \pm 2^{\circ}\text{C}$) with dark and light cycle (12/12 h). The animals were fed with standard pellet diet supplied by Poultry Research Station, Nandhanam, India and fresh water *ad libitum*. All the animals were acclimatized to laboratory condition for a week before commencement of experiment.

Paracetamol toxicity

The ethanolic extract of *Grewia umbellifera*, paracetamol, saline were given with the help of feeding cannels. Three groups (Group I, Group II and III) of rats, six rats in each group were taken. The capitula extract at a fixed dose (400 mg kg^{-1} , P.O.) that was daily fed for seven days to one group (Group III) of rats and paracetamol (200 mg kg^{-1} P.O.) was administered on 5th day after 5th administration of the extract. The paracetamol treated group (Group II) received normal saline in place of *Grewia umbellifera* extract. After 48h of paracetamol feeding rats were sacrificed by cervical dislocation for estimation of blood glutathione, reduced liver glutathione, liver $\text{Na}^{+}\text{K}^{+}$ -ATPase activity, serum marker enzymes, serum bilirubin and liver thiobarbutiric acid reactive substances using standard methods.

Assay of liver glutathione and blood glutathione

Blood was collected, allowed to clot and serum separated. Liver was dissected out and used for biochemical studies. Freshly collected livers were washed with 0.9% NaCl, weighed and homogenates were made in a ratio of 1g of wet tissue to 9ml of 1.25% KCl by using motor driven Teflon-pestle. Reduced glutathione (GSH) was estimated using DTNB.^[14] The blood glutathione was estimated by the method of Beutler.^[15] The absorbance was read at 412 nm.

Liver Na⁺ K⁺-ATPase activity

To measure the liver Na⁺K⁺ -ATPase activity the liver was dissected out quickly, rinsed with cold phosphate buffer, liver plasma membranes were isolated and subjected for the estimation of Na⁺ K⁺-ATPase activity.^[16]

Thiobarbituric acid reactive substances (TBARS)

The concentration of TBARS was measured in liver using the method of Ohkawa *et al.*^[17] The concentration of TBARS was expressed as “ moles of malondialdehyde per mg of protein using 1,1,3,3, -tetra- ethoxypropane as the standard.

Serum marker enzymes

The serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP)^[18,19], total serum protein (TSP)^[20] and bilirubin^[21] were measured. Glycogen (GLY)^[22] and protein^[20] were estimated in liver homogenate.

Statistical analysis

The data were expressed as mean = S.E.M. and statistically assessed by one-way analysis of variance (ANOVA). The difference between drug treated animals and controls was evaluated by student's t-test.

RESULTS AND DISCUSSION

Table 1: Effect of AET of Capitula of *Grewia umbellifera* on blood and liver glutathione (GSH), liver Na⁺K⁺-ATPase, serum marker enzymes (ALT, AST, ALP) and liver thiobarbituric acid reactive substances (TBARS) in Paracetamol intoxicated albino rats

Parameters	Group 1	Group2	Group3
Blood GSH (mg%)	1.58± 0.09 ^a	0.40 ± 0.05	1.40 ± 0.04 ^c
Liver GSH (moles g ⁻¹ liver)	11.01± 0.32 ^c	6.32 ± 0.34	10.02±0.21 ^b
Na ⁺ K ⁺ -ATPase (U mg ⁻¹ protein)	7.26 ± 0.54 ^a	6.21 ± 0.19	8.06±0.26 ^c
TBARS (n mol of MDA g ⁻¹ of wet tissue h ⁻¹)	59.2±1.06 ^c	567.4 ^a ± 9.5	282.7±0.21 ^c
ALT (U mg ⁻¹ protein)	58±0.13	167±1.01	61±0.54
AST (U mg ⁻¹ protein)	92.7 ± 10.3	159.4±10.9	98.61±1.89 ^c
ALP (KA unit)	69.4±2.01 ^a	172.8±18.1	44.5±1.01 ^b
Bilirubin (mg%) (Total)	58.9±7.60 ^b	97.1±6.84	54.9±2.4
TSP (mg protein mL ⁻¹ serum)	1.43±0.12 ^a	2.89±0.10	1.33±0.16 ^b
GLY (mg g ⁻¹ wet tissue)	59.89±2.69 ^a	56.12±2.16	61.5±1.99 ^c
Body weight	32.10 ± 2.94 ^a	24.45 ± 1.10	31.49±2.54 ^c
Before treatment (g)	142±0.63 ^a	143.6±0.20	141±0.44 ^c
After treatment (g)	153±0.15 ^b	142.3±30	156±0.07 ^b
Liver weight (g)	6.89±0.03	5.78±0.60	6.45±0.05 ^b

Results are mean of six observations ± S.E.M.

^a p<0.001 when compared with normal control (Group 1).

^b p<0.001 when compared with normal control (Group 1).

^c p<0.001 when compared with paracetamol treated control (Group 2).

The glutathione level in liver homogenate and in blood, liver Na⁺K⁺-ATPase, serum marker enzymes and liver thiobarbituric acid reactive substances are given in Table 1. The concentration of GSH in animals treated with paracetamol was significantly (p<0.001) reduced in homogenate of liver and so was the level of glutathione in blood and Na⁺K⁺-ATPase level (p<0.001) as compared with saline control animals. While thiobarbituric acid reactive substances of paracetamol treated animals was significantly higher (p<0.001) than the saline treated control animals. Administration of capitula of *Grewia umbellifera* extract increased the concentration of glutathione in liver (p<0.01) and glutathione in blood (p<0.001) and liver Na⁺K⁺-ATPase activity significantly (p<0.001) when compared to its paracetamol treated control group. On the other hand, the increased level of liver thiobarbituric acid reactive substances of paracetamol treated animals was significantly (p<0.001) reduced in group of animals receiving both capitula extract and paracetamol.

The abnormal high level ($p < 0.001$) of serum ALT, AST, ALP and bilirubin observed (Table 1) in paracetamol induced liver toxicity. Treatment with AET of *Grewia umbellifera* reduced the enhanced level of serum ALT, AST, ALP and bilirubin. On the other hand, the level of liver glycogen of paracetamol treated animals was significantly ($p < 0.001$) reduced. Paracetamol is a common antipyretic agent, which is safe in therapeutic doses but can produce fatal hepatic necrosis in man, rats and mice with toxic doses.^[23-25] Protection against paracetamol-induced toxicity has been used as a test for potential hepatoprotective activity by several investigations.^[26-30]

Liver is the most important and main part of the animal body. It is highly affected primarily by toxic agents and that why the above-mentioned parameters have been found to be of great importance in the assessment of liver damage. From our results, it can be speculated that (i) decreasing effect GSH, blood glutathione, liver Na^+K^+ -ATPase activity and increasing effect of liver thiobarbituric acid reactive substance level in rat treated with paracetamol were due to hepatocellular damage and (ii) *Grewia umbellifera* extract afforded protection from such paracetamol induced liver damage. Possible mechanism that may be responsible for the protection of paracetamol induced the following capitula extract by if self-act as a free radical scavenger intercepting those radicals involved in paracetamol metabolism by microsomal enzymes. Its ability is to inhibit rat hepatic microsomal membrane lipid peroxidation and to scavenge on radicals, as well as to interact with 1, 1-di phenyl-2-picrylhydrazyl radical (DPPH). Thus, by trapping oxygen related free radicals capitula extract could hinder their interaction with polyester fatty acids and would abolish the enhancement of lipids peroxidative processes leading to MDA formation^[4,11] *Grewia umbellifera* extract pretreatment exhibited a normal effect on the glutathione of the blood and liver cells. The extract significantly increased the hepatic and blood glutathione. Then results suggest that a significantly higher content glutathione in blood and liver would offer the tissue a better protection against an oxidative stress, thus contributing to the abolishment of paracetamol infused hepatotoxicity. (c) The activities of Na^+K^+ -ATPase are decreased in paracetamol-induced animals; capitula extract prevented this effect of paracetamol. Therefore, capitula extract may be useful agent for the normalization of paracetamol induced impaired membrane function.^[4]

The abnormal high level of serum ALT, AST, ALP and bilirubin observed in our study (Table 1) are the consequence of paracetamol induced liver dysfunction and denotes the damage to the hepatic cells. Treatment with the extract of *Grewia umbellifera* reduced the enhanced level of serum ALT, AST, ALP and bilirubin, which seem to offer the protection and maintain the functional integrity of hepatic cells. A reduction in total serum protein (TSP) (Table 1) and liver glycogen (GLY) observed in the paracetamol treated rats may be associated with the decrease in the number of hepatocytes which in turn may result into decreased hepatic capacity to synthesize protein and GLY and consequently decrease in the liver weight (Table 1). But, when the AET was given along with paracetamol, the significant increase in TSP and liver GLY was observed indicating the hepatoprotection activity of extract and also accounting for the increase in the liver weight most probably through the hepatic cell regeneration.

From our results, it can be concluded that decreased the levels of GSH, blood glutathione, serum marker enzymes, liver Na⁺K⁺-ATPase activity and increased liver thiobarbutiric acid reactive substance level in albino rates treated- paracetamol was due to hepatocellular damage. Extract of *Grewia umbellifera* afforded protection from such paracetamol induced liver damage. Possible mechanism that may be responsible for the protection of paracetamol induced liver amage by capitula extract include the following- (a) *Grewia umbellifera* extract could act as a free radical scavenger intercepting those radicals involved in paracetamol metabolism by microsomal enzymes. (b) A significantly higher content GSH in blood and liver would afford the tissue a better protection against antioxidative stress, thus contributing to the abolishment of paracetamol induced hepatotoxicity. (c) Therefore, *Grewia umbellifera* extract is a promising hepatoprotective agent. The hepatoprotective action combined with antioxidant activity has a synergistic effect to prevent the process of initiation and progress of hepatocellular damage.^[4]

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