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Hepatoprotective Activity of *Terminalia arjuna* Leaf Against Paracetamol-Induced Liver Damage in Rats

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In present study, the methanolic extract of *Terminalia arjuna* was evaluated for its protective effect on paracetamol-induced liver damage in Wistar rats. Serum biochemical parameters *viz*., serum glutamine oxaloacetate transaminase, serum glutamine pyruvate transaminase, serum alkaline phosphatase (SALP), total protein, bilirubin, cholesterol, triglyceroides and liver biochemical parameters such as lipid peroxidation, reduced glutathione (GSH) content and catalase (CAT) activity were estimated. Serum and liver biochemical observations indicated that methanolic extract of *Terminalia arjuna* exerted remarkable hepatoprotective efficacy against paracetamol-induced hepatic damage in Wistar rats that is may be due to its augmenting endogenous antioxidant mechanisms.

Key Words: Lipid peroxidation, Glutathione, Biochemical, Silymarin.

INTRODUCTION

Liver, the key organ of metabolism and excretion is an important organ for detoxification of xenobiotics, environmental toxicants and liver damage is associated with distortion of several metabolic functions. Hence liver diseases are of serious health problem. In the absence of reliable liver protective drugs in allopathic medical practices, naturally occurring compounds have been found to have major role in the management of various liver diseases. Numerous medicinal plants and their formulations are used for liver disorders in ethnomedical practices and in traditional systems of medicine in India. However a satisfactory remedy for serious liver diseases is not still available, so search for effective hepatoprotective drugs are continued.

Terminalia arjuna Roxb. (Combretaceae), commonly known as Arjuna, is a large tree grown on the banks of rivers, streams and dry watercourses throughout in Indian peninsula. The fruits of the plant are used as tonic[1]. Externally its leaf paste is used as a cover on sores and ulcer. The bark is antidysenteric, antipyretic, astringent, cardiotonic, lithotriptic and tonic and powder of the bark acts as diuretic in cirrhosis of liver and gives relief in symptomatic hypertension[2,3]. A decoction of bark made with milk is given every morning on an empty stomach or its powder with milk as a cardiotonic[4]. The powder of the bark is also given with honey in fractures and contusions with echymosis. Besides this, the extract of the bark as astringent is used for cleaning sores, ulcers and cancers *etc*.[5]. The extract of the bark are prescribed in scorpion-stings and lowering blood glucose[6]. No pharmacological investigation is still reported on *T. arjuna* leaf. Present investigation was aimed to evaluate the hepatoprotective potential of *T. arjuna* leaf against paracetamol-induced hepatic damage in Wistar rats in pursuit of newer liver protectants.

EXPERIMENTAL

The leaves of *T. arjuna* were collected during January 2008 from Nadia, West Bengal, India. The plant species was authenticated by Dr. Lakshmi Narashimhan, Scientist, Botanical Survey of India, Central National Herbarium, Howrah, West Bengal, India. The voucher specimen [CNH/I-I/(216)/2008/Tech.II/216] was maintained in our laboratory for future reference. The leaves were shade-dried with occasional shifting and then powdered with mechanical grinder passing through sieve No. 40 and stored in an air-tight container.

Bovine serum albumin from Sigma Chemical Co., St. Louis, Mo, USA; trichloroacetic acid (TCA) from Merck Ltd., Mumbai, India; thiobarbituric acid (TBA), paracetamol, 5,5'-dithio-*bis*-2-nitro benzoic acid (DTNB), phenazonium methosulphate (PMS), nicotinamide adenine dinucleotide

(NADH) and reduced glutathione (GSH) from SISCO Research Laboratory, Mumbai, India. Silymarin, potassium dichromate and glacial acetic acid from Ranbaxy, Mumbai. All the other reagents used were of analytical reagent grade obtained commercially.

Preparation of extract: The powdered plant material (400 g) was macerated at room temperature (24-26 ºC) with methanol (850 mL) for 4 days with occasional shaking, followed by re-maceration with the same solvent for 3 more days. The macerates were combined, filtered and distilled off in reduced pressure. The resulting concentrate was vacuum dried at 40 ºC to yield the dry extract [methanolic extract of *Terminalia arjuna* (META), yield 21.45 % w/w]. The dry extract was kept in a vacuum desiccator until use. Preliminary phytochemical studies of META revealed the presence of alkaloids, triterpenoids, tannins and flavonoids[7].

Adult male Wistar albino rats weighing 170-200 g were used for the present investigation. They were housed in a clean polypropylene cage and maintained under standard laboratory conditions (temperature 25 ± 2 ºC with dark/light cycle 12/12 h). They were fed with standard pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum*. The animals were acclimatized to laboratory conditions for 1 week prior to experiment. All procedures described were reviewed and approved by the University Animal Ethics Committee, Jadavpur University.

Acute toxicity: The acute oral toxicity of methanolic extract of *Terminalia arjuna* in male Swiss albino mice was studied as per reported method[8].

Treatment schedule: The rats were divided into five groups (n = 8). A single dose of 650 mg/kg paracetamol in 2 % methyl cellulose was administered orally to each animals in group II, III, IV and V. After administration of paracetamol suspension, methanolic extract of *Terminalia arjuna* (META) was administered orally (p.o.) at the doses of 100 and 200 mg/kg body weight (b.w.) to groups III and IV, respectively daily for 14 days. Group V received reference drug silymarin (25 mg/kg b.w.; p. o.) daily for 14 days[9]. Group I served as normal (vehicle) control and group II served as paracetamol control and received normal saline (5 mL/kg b.w., p.o.) for 14 days. After 24 h of last dose, blood was collected from overnight fasted rats of each group by cardiac puncture for estimation of serum biochemical parameters. Then the rats were sacrificed by cervical dislocation for the study of liver biochemical and histopathological parameters.

Body weight, liver and kidney weight: The body weight of rats of each group were measured just before and 14 days after methanolic extract of *Terminalia arjuna* treatment. Liver and kidney weights of all rats were measured after post treatment sacrifice.

Serum biochemical estimations: Collected blood was used for the estimation of serum biochemical parameters *viz*., serum glutamine oxaloacetate transaminase (SGOT), serum glutamine pyruvate transaminase (SGPT), serum alkaline phosphatase (SALP), total bilirubin, total cholesterol and triglycerides content were estimated by using commercially available kits (Span Diagnostic Ltd., Surat, India). Serum total protein was estimated according to the reported method[10].

Liver biochemical estimations: Lipid peroxidation *i.e.*, thiobarbituric acid reactive substances (TBARS) was estimated by the method of Fraga *et al*.,[11] and expressed as mM/100 g of liver tissue. Reduced glutathione (GSH) was determined by the method of Ellman[12] and was expressed as mg/100 g of liver tissue. Catalase (CAT) activity was assayed according the method described by Sinha[13] and expressed as µ moles of H_2O_2 consumed/min/mg of liver tissue.

Statistical analysis: All results were expressed as the mean ± standard error of mean (SEM). The results were analyzed for statistical significance by one-way ANOVA followed by Dunnett's post hoc test of significance. $p < 0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

Acute toxicity: The oral LD_{50} value of the methanolic extract of *Terminalia arjuna* (META) in mice was 900 mg/kg body weight.

Body weight, liver and kidney weight: The body weight, liver and kidney weights of rats from paracetamol control group (after 14 days) were significantly ($p < 0.001$) decreased when compared with normal control group. Methanolic extract of *Terminalia arjuna* at 100 and 200 mg/kg b.w. significantly ($p < 0.001$) maintained the body weight, liver and kidney weights towards normal in a dose related manner as compared to STZ control (Table-1).

Serum biochemical parameters: Biochemical parameters like SGOT, SGPT, SALP, bilirubin, total cholesterol and triglycerides in the paracetamol control group were significantly ($p < 0.001$) elevated as compared to the normal saline group. Treatment with META at the dose of 100 and 200 mg/kg significantly ($p < 0.001$) reduced their levels towards the normal values in a dose dependent manner. The total protein content was found to be significantly decreased in the paracetamol control group as compared with the normal saline group ($p < 0.001$). Administration of META in paracetamol-intoxicated rats at the both doses significantly ($p < 0.001$) increased the

TABLE-1
INFLUENCE OF METHANOLIC EXTRACT OF *Terminalia arjuna* (META) ON BODY WEIGHT AND WEIGHT OF KIDNEY AND LIVER IN NORMAL AND PARACETAMOL-INTOXICATED RATS

Group	Dose	Initial body wt. (g)	Final body wt. (g)	Final liver wt. (g)	Final kidney wt. (g)
I (Normal saline)	5 mL/kg	169.76 ± 7.8	173.54 ± 5.2	6.54 ± 2.9	1.36 ± 1.3
II (Paracetamol)	650 mg/kg	170.68 ± 7.2	165.54 ± 4.5*	3.15 ± 2.3*	0.95 ± 1.1*
III (Paracetamol + META)	100 mg/kg	165.54 ± 4.2	151.72 ± 2.9**	5.81 ± 3.3**	1.21 ± 1.5**
IV (Paracetamol + META)	200 mg/kg	166.41 ± 5.2	155.94 ± 1.8**	5.94 ± 3.6**	1.25 ± 1.2**
V (Paracetamol + silymarin)	25 mg/kg	177.53 ± 4.5	169.76 ± 3.3**	6.36 ± 3.1**	1.29 ± 1.6**

Data are expressed as mean ± SEM (n = 6); *$p < 0.001$ compared with normal control; **$p < 0.001$ compared with PCM control group. PCM: Paracetamol.

TABLE-2
EFFECTS OF METHANOLIC EXTRACT OF *Terminalia arjuna* (META) ON SERUM BIOCHEMICAL PARAMETERS IN NORMAL AND PARACETAMOL-INTOXICATED RATS

Group	Dose	SGOT (IU/L)	SGPT (IU/L)	SALP (U/L)	Total protein (g/dL)	Total cholesterol (mg/dL)
I (Normal control)	5 mL/kg	19.17 ± 1.05	21.83 ± 1.66	76.17 ± 2.71	7.95 ± 0.18	121.67 ± 2.09
II (Paracetamol)	650 mg/kg	36.33 ± 1.31*	38.81 ± 1.14*	133.81 ± 3.88*	4.05 ± 0.27*	161.21 ± 1.88*
III (Paracetamol + META)	100 mg/kg	25.83 ± 1.54**	29.17 ± 2.33**	103.0 ± 3.53**	6.05 ± 0.16**	142.00 ± 2.99**
IV (Paracetamol + META)	200 mg/kg	20.00 ± 1.57**	22.83 ± 0.79**	83.50 ± 4.01**	7.45 ± 0.47**	129.18 ± 4.53**
V (Paracetamol + slymarin)	25 mg/kg	19.58 ± 0.68**	21.91 ± 0.72**	79.33 ± 1.43**	7.93 ± 0.13**	124.31 ± 1.65**

Data are expressed as mean ± SEM (n = 6); *$p < 0.001$ compared with normal control; **$p < 0.001$ compared with PCM control group.

total protein content as compared with the paracetamol control (Table-2).

Liver biochemical parameters: The levels of thiobarbituric acid reactive substances (TBARS) were significantly ($p < 0.001$) increased in paracetamol control animals as compared to normal control group. Treatment with META at 100 and 200 mg/kg b.w. significantly ($p < 0.001$) reduced the TBARS levels when compared with paracetamol control animals in dose related manner. The level of reduced glutathione (GSH) was significantly ($p < 0.001$) depleted in paracetamol control group as compared with normal control group. Reduced GSH level was found to be significantly and dose dependently ($p < 0.001$) elevated towards normal level on administration of META as compared with paracetamol control group. There was significant ($p < 0.001$) reduction in catalase activity in paracetamol control group compared with normal group. Administration of META significantly ($p < 0.001$) recovered catalase activity towards normal when compared with paracetamol control animals (Table-3).

Paracetamol is a widely used antipyretic and analgesic drug which is safe in therapeutic doses but can cause fatal hepatic damage in human and animals at higher toxic doses[14]. Bioactivation of paracetamol by hepatic cytochrome P-450 leads to formation of a highly reactive and toxic metabolite N-acetyl-*p*-benzoquinone imine. N-Acetyl-*p*-benzoquinone imine (NAPQI) is normally detoxified by conjugation with reduced glutathione (GSH) to form mercapturic acid which is excreted in urine. Toxic overdose of paracetamol depletes hepatic reduced glutathione (GSH) content so that free NAPQI binds covalently to cellular macromolecules causing acute hepatocellular necrosis. The NAPQI then causes acylation or oxidation of cytosolic and membrane proteins and generation of reactive oxygen species (ROS). This leads to further oxidation of protein thiols, lipid peroxidation, DNA fragmentation and ultimately cell necrosis[15-17].

It has been well established that elevated levels of SGOT, SGPT and SALP are indicative of cellular leakage and loss of functional integrity of the hepatic cell membranes implying hepatocellular damage. Serum total protein and bilirubin levels on the other hand are related to the function of the hepatic cells revealing the functional status of the hepatic cell[9]. Elevated serum cholesterol and triglyceride levels in paracetamol challenged rats indicated impaired fat metabolism due to hepatic damage. The META decreased the elevated serum enzyme activities, bilirubin and lipid contents with elevation of total protein content in the paracetamol treated rats which are comparable to the normal control group. It appears that the META preserved the structural integrity of the hepatocellular membrane which is evident from the significant reduction in paracetamol-induced rise in serum marker enzymes in rats. Methanolic extract of *Terminalia arjuna* also showed marked effect in controlling the loss of body weight, liver and kidney weights of paracetamol-intoxicated rats.

Lipid peroxidation is a phenomenon involved in peroxidative loss at unsaturated lipids, thus bringing about cellular lipid degradation and membrane disordering. Reactive oxygen species (ROS) results in lipid peroxidation and subsequently increase in thiobarbituric acid reactive substances (TBARS) levels. Elevated lipid perixodation causes degradation of cellular macromolecules leading to tissue damage[18]. A marked increase in the concentration of TBARS in paracetamol -intoxicated rats indicated enhanced lipid peroxidation leading to tissue injury and failure of the antioxidant defense mechanisms to prevent overproduction of ROS. Methanolic extract of *Terminalia arjuna* showed ability to prevent paracetamol induced elevation of TBARS level, suggesting that META inhibited hepatic lipid peroxidation in paracetamol intoxicated rats. This implies the reduction in free radical production and subsequent decrease in damage to the hepatocellular membranes.

Glutathione is the endogenous non-enzymatic antioxidant in human body system and it is protective against chemically induced hepatic damage and oxidative stress[19]. Depleted GSH level with elevated level of lipid peroxidation in paracetamol-

TABLE-3
INFLUENCE OF METHANOLIC EXTRACT OF *Terminalia arjuna* (META) ON LIVER BIOCHEMICAL PARAMETERS IN NORMAL AND PARACETAMOL-INTOXICATED RATS

Group	Dose	TBARS (mmol/100 g of wet liver tissue)	GSH (mg/ 100 g of wet liver tissue)	CAT (μmoles of H_2O_2 consumed/min/mg of wet liver tissue)
I (Normal control)	5 mL/kg	0.85 ± 0.06	104.15 ± 4.60	10.55 ± 0.58
II (Paracetamol)	650 mg/kg	6.45 ± 0.08*	36.42 ± 2.15*	1.97 ± 0.41*
III (Paracetamol + META)	100 mg/kg	2.91 ± 0.09**	94.33 ± 6.29**	6.33 ± 0.74**
IV (Paracetamol + META)	200 mg/kg	1.15 ± 0.06**	102.91 ± 7.15**	9.96 ± 0.62**
V (Paracetamol + silymarin)	25 mg/kg	0.92 ± 0.04**	103.33 ± 5.83**	10.13 ± 0.77**

Data are expressed as mean ± SEM (n = 6); *$p < 0.001$ compared with normal control; **$p < 0.001$ compared with PCM control group. PCM: Paracetamol.

induced rats indicated that the experimental dose of paracetamol 650 mg/kg was highly hepatotoxic. It was confirmed from present study that the META dose dependently and significantly restored hepatic GSH content towards normal in paracetamol intoxicated rats indicating decreased free NAPQI level in the blood.

Enzymatic antioxidant mechanisms play an important role in the elimination of free radicals (ROS). Catalase (CAT) is a haem containing enzyme catalyzing the detoxification of H_2O_2 to water and oxygen[20]. The suppression of CAT activities as a result of liver damage was reported[21]. Similar findings were observed in present results in paracetamol treated mice. The administration of META significantly recovered the CAT activity towards normal in a dose dependent manner.

Preliminary phytochemical studies showed the presence of alkaloids, flavonoids, tannins and triterpenoids in META. Flavonoids and tannins are well known polyphenolic natural antioxidants due to their electron donating property which either scavenge the principal propagating free radicals or halt the radical chain[22]. Thus META, because of the presence of natural antioxidants, must have exerted protective action against paracetamol-induced hepatic damage, plausibly by increasing the hepatic reduced glutathione content, which would protect the tissue from NAPQI and free radicals, by modulating hepatic lipid peroxidation, by augmenting the activities of cellular antioxidant enzymes *viz.*, CAT thereby ameliorating the extent of oxidative stress mediated hepatocellular damage caused by paracetamol.

Conclusion

Therefore, from present study it can be concluded that the methanolic extract of *Terminalia arjuna* leaf dose dependently offered potential hepatoprotection against paracetamol-induced hepatic damage, normalizing biochemical parameters in Wistar rats plausibly by modulating lipid peroxidation and augmenting endogenous non-enzymatic and enzymatic antioxidant defense mechanisms.

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