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# Effect of *Indigofera tinctoria* Linn on liver antioxidant defense system during D-galactosamine / endotoxin - induced acute hepatitis in rodents

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Effects of pre-treatment with the alcoholic extract of *I. tinctoria* (500 mg/kg body wt/day, po for 21 days) on liver antioxidant defense system during acute hepatitis induced by D-galactosamine (D-GalN)/ endotoxin (LPS extracted by phenol water method from *E. coli* serotype 0111.$B_4$; 300 mg and 30 μg/kg body wt/day, ip, 18 hr before the assay) were investigated on the activities of enzymic antioxidants such as superoxide dismutase, catalase, glutathione peroxidase and glutathione-s-transferase, and levels of total reduced glutathione in the liver of normal and experimental groups of male albino rats. Since lipid peroxidation and associated membrane damage is a key feature of D-galN/LPS-induced liver injury, the levels of lipid peroxides, was estimated and used as an index of oxidative stress. D-GalN/endotoxin-induced hepatic damage was manifested by a significant decrease in the activities of antioxidant enzymes, decreased glutathione levels and increased levels of lipid peroxides. *I. tinctoria* pre-treated rats showed considerable protection against D-galN/endotoxin, induced oxidative stress as evidenced by a significant increase in the activities of all the antioxidant enzymes studied and significant decrease in the levels of lipid peroxides. Results indicate that pretreatment with *I. tinctoria* extract in rats is very effective in reducing D-GalN/endotoxin-induced oxidative stress suggesting an antioxidant effect.

Oxidative damage to crucial bimolecules due to excess generation of reactive oxygen species has been implicated as a major cause of organ damage and hence compounds capable of negating such damage have potential benefits[1]. The excess generation of reactive oxygen species during various pathophysiological states can lead to alterations of the cellular constituents resulting in diseased conditions[2]. In the recent years there has been considerable interest in natural products with antioxidant property in human diet. One of the areas which had attracted a great deal of attention is the possible use of antioxidant supplements in the prevention of diseases caused by oxidative damage.[3,4]

*Indigofera tinctoria* Linn (Leguminosae) a small erect medicinal shrub has been a part of the traditional Indian and Chinese medicinal system since time immemorial[5,6]. The plant has been used in the treatment of several nervous and hepatic disorders including hepatitis[7]. Indirubin, a component isolated from the plant has proved to be a very effective anticancer agent in both pharmacological and clinical trials[8,9]. Earlier studies with the alcoholic extract of the plant have confirmed its hepatoprotective effects against carbon tetra chloride-induced liver injury in rats, rabbits and mice[10,11]. Hepatoprotective effects of *I. tinctoria* (IT) extract against D-galactosamine/endotoxin-induced hepatitis in rats have been reported[12].

D-Galatcosamine given at the time of endotoxin challenge markedly sensitizes mice and other species to the lethal effects of endotoxin[13]. This amino sugar is known to selectively block hepatic transcription, and indirectly hepatic protein synthesis,[14] and as a consequence of endotoxin toxicity to result in liver failure.[15] Sakaguchi *et al.*[16] showed that endotoxin injection results in lipid peroxide formation and membrane damage in experimental animals, causing decreased levels of scavengers or quenchers of free radicals. D-Galactosamine highly sensitizes the host response of experimental animals to endotoxin and causes fulminant hepatitis within 8 hr after administration[17]. This immunological liver injury model has been used to evaluate the efficacy of several hepatoprotective agents[18] and hence selected as a model for inducing hepatotoxicity in the present investigation.

The effects of pre-administration with *Indigofera tinctoria* extract during D-galactosamine/endotoxin-induced liver injury, with respect to antiperoxidative

enzymes, glutathione dependent antioxidant enzymes, and non enzymic antioxidants have not been studied earlier. Hence the present study has been undertaken to examine if pre-treatment with *I. tinctoria* extract is capable of reducing free radical generation in the liver of rats treated with toxic doses of D-Galactosamine/endotoxin. Antioxidant enzyme activities and the levels of lipid peroxides were used as indicators of oxidative stress.

*Plant material*—The plant was collected from the gardens of the Central Research Institute for Siddha, Arumbakkam, Chennai during October 1998. It was authenticated by Dr.S.Usman Ali (Drug Research Scheme - Multi Disciplinary), from the above institute where a voucher specimen of the plant was deposited.

*Preparation of the extract*—Shade dried and coarsely powdered plant material (whole plant) was extracted with methanol in cold (48 hr). The extract was filtered, concentrated on a water bath, and then dried in vacuum (yield 10%).

*Chemicals*—D-Galactosamine and endotoxin (bacterial lipopolysaccharide from *E. coli* serotype $0111.B_4$ extracted by phenol water method) were obtained from Sigma Chemical Company St. Louis, MO, USA.

*Animals*—Adult male albino rats of Wistar strain weighing about 140-180 g obtained from the Fredrick Institute for plant protection and Toxicology, Padappai, Chennai were used. They were acclimatized to animal house conditions and were fed on a commercial pelleted rat chow (Hindustan Lever Limited, Bangalore, India) and water *ad libitum*.

The animals (24) were divided into 4 groups of 6 each according to the following experimental regimen. Group 1 comprised normal control rats. Group 2 rats were given two ip injections of D-galactosamine (300 mg/kg body wt/day) and LPS (30 µg/kg body wt/day). Group 3 rats were given IT extract (500mg/kg body wt. in olive oil/day, po for 21 days). Group 4 rats were given pre-treatment with the plant extract prior to D-galactosamine/ endotoxin challenge. After the experimental period, the animals were sacrificed by cervical decapitation. The liver was excised immediately after the sacrifice and washed with ice-cold saline. A portion of the liver was then homogenised in 0.1M Tris HCl buffer and the homogenate was used for assaying the activities of antiperoxidative enzymes superoxide dismutase (SOD; EC 1.15.1.1)[19], catalase (CAT; EC 1.11.1.6 J)[20], glutathione dependent antioxidant enzymes glutathione peroxidase (GPx; EC 1.11.1.19)[21], glutathione-s-transferase (GST; 2.5.1.18)[22], and for estimating the levels of total reduced glutathione (GSH)[23] and lipid peroxides (LPO)[24].

*Statistics*—The values were expressed as mean ± SD. statistical difference was analysed by Student's *t* test and *P* values were determined.

D-Galactosamine is a hepatotoxin that, induces liver damage *in vivo,* similar to human viral hepatitis, via the depletion of uridine nucleotides and subsequently diminishes the synthesis of RNA and plasma membrane proteins[25,26]. Oxidative tissue damage triggered by D-galactosamine is believed to be due to the formation of highly reactive hydroxyl radicals which are the initiators of lipid peroxidation chain reaction which subsequently provokes inflammatory reaction, and hence destruction and damage to cell membrane[27-29]. Significant decrease in the activities of antioxidant enzymes superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase and increase in the levels of lipid peroxides with a concomitant decrease in the levels of total reduced glutathione in the Group 2 rats indicate the severity of oxidative stress induced as a result of administration of D-galactosamine/-endotoxin. (Table 1) Considerable increase in the activities of antioxidant enzymes, decrease in the levels of lipid peroxides and improvement in hepatic GSH status in the *I. tinctoria* pre-treated rats clearly indicate the protection offered by pre-treatment with the plant extract and thereby suggest an antioxidant effect.

The hepatoprotection was associated with a significant enhancement in hepatic GSH status, as indicated by substantial increase in tissue GSH levels in *I. tinctoria* pretreated rats. GSH antioxidant system consists of an array of non-enzymatic and enzymatic reaction pathways involved in the neutralization of reactive free radical species. While D-galN/endotoxin pretreatment produced drastic decreases in hepatic GSH status, IT pretreatment could effect an increase in the activity of the enzyme in D-galN/endotoxin-intoxicated animals. Reactive oxygen species generated from D-galN/endotoxin can increase the formation of lipid hydroperoxides. The increased activity of GST in IT pretreated rats can hasten the decomposition of lipid hydroperoxides, and thereby account for the protective effect. Eventhough there was a drop in the activity of hepatic GPx after D-galN/endotoxin challenge, IT pretreatment increased the activity of this enzyme.

Thus the results of the present study indicate that pre-treatment with IT extract improves hepatic

Table 1—Effect of *I. tinctoria* extract on liver antioxidant defense system and the levels of lipid peroxides (LPO), total reduced glutathione (GSH) in the liver during D-galactosamine/endotoxin - induced hepatitis in rats

[Values are mean ± SD for 6 animals in each group)

| Parameters | Group 1 | Group 2 | Group 3 | Group 4 |
|---|---|---|---|---|
| SOD | 7.12 ± 0.64 | 4.03 ± 0.25[a] | 7.06 ± 0.69[NS] | 6.27 ± 0.58[a] |
| CAT | 60.12 ± 0.53 | 41.03 ± 0.39[a] | 60.58 ± 0.62[NS] | 53.17 ± 0.49[a] |
| GPx | 115.21 ± 10.50 | 70.93 ± 6.88[a] | 115.99 ± 11.33[NS] | 97.09 ± 8.23[a] |
| GST | 1552 ± 150 | 1198 ± 117[a] | 1549 ± 147[NS] | 1421 ± 137[b] |
| LPO | 1.28 ± 0.09 | 2.39 ± 0.15[a] | 1.26 ± 0.08[NS] | 1.92 ± 0.10[a] |
| GSH | 8.42 ± 0.84 | 4.33 ± 0.41[a] | 8.39 ± 0.78[NS] | 6.20 ± 0.59[a] |

Units: SOD: Units / mg protein; CAT: n moles of $H_2O_2$ decomposed/min/mg protein; GPx: n moles of GSH oxidized /min/mg protein; GST: n moles of CDNB conjugated /min/mg protein; LPO: n moles of malondialdehyde / mg protein; GSH: n moles / g of wet tissue.

*P* values :[a]<0.001; [b]<0.001; NS - Non significant.

Student's -*t*-test (Comparisons are made between Group 2 and Group 1; Group 3 and Group 1; Group 4 and Group 2).

enzymic and non-enzymic antioxidant status and decreases the levels of lipid peroxides in rats treated with toxic doses of D-galactosamine and endotoxin.

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## References


1 Cullen W J, Dulchavsky S A, Devasagayam T P A, Venkataraman B V & Dutta S, Effect of Maharishi AK-4 on $H_2O_2$-induced oxidative stress in isolated rat heart, *J Ethno Pharmacol*, 56 (1997) 215.

2 Sies H, In Oxidative stress : Oxidants and antioxidants (Academic Press, New York), 1991, 25.

3 Lin J M, Lin C C, Chen M F, Ujiie T & Takata A, Studies on Taiwan folk medicine, Thang-Kau-Fin (II). Measurement of active oxygen scavenging activity using an ESR technique, *American Journal of Chinese Medicine* 23 (1995) 43.

4 Ira Thabrew M, Gove C D, Hughes R D, Mc Farlane I G & Williams R, Protective effects of *Osbeckia octandra* against galactosamine and tert-butyl hydroperoxide induced hepatocyte damage, *J Ethno Pharmacol*, 49 (1995) 69.

5 Chopra R N, Nayar S L & Chopra I C in *Glossary of Indian medicinal plants*, (CSIR, New Delhi), 1956, 141.

6 Liang H & Jin Z, Development of medicinal chemistry in China, in Comprehensive medicinal chemistry, Vol.1, edited by P D Kennewell, (Pergamon Press, Oxford) 1997, 99.

7 Nadkarni K M, *Indian Materia Medica* (Popular Prakasham Private Limited Bombay) 1976, 680.

8 Rui H, Recent progress in the study of anticancer drugs originating from plants and traditional medicine in China, *Chinese Med J*, 108 (1995) 729.

9 Xiujuan J, Furong Z, Liang J & Yuting X, Studies on the antineoplastic action and toxicity of synthetic Indirubin. *Acta Pharm Sin*, 16 (1981) 146.

10 Anand K K, Chand D & Ray Ghatak B J, Protective effect of the alcoholic extract of *Indigofera tinctoria* Linn. in experimental liver injury, *Indian J Exp Biol*, 17 (1979) 685.

11 Anand K K, Chand D, Ray Ghatak & Arya B K, Histological evidence of protection of *Indigofera tinctoria* Linn. against carbon tetra chloride induced hepatotoxicity, *Indian J Exp Biol*, 19 (1981) 298.

12 Sreepriya M, Devaki T, Balakrishna K & Apparanantham T, Biochemical studies evaluating the efficacy of *Indigofera tinctoria* Linn. in the alleviation of D-galactosamine/lipopolysaccharide-induced liver dysfunction in rats. *Anc Sci Life* (1999) (in press).

13 Galanos C, Freudenberg M A & Reutter W, Galactosamine-induced sensitization to the lethal effects of endotoxin. *Proc Natl Acad Sci USA*, 76 (1979) 5939.

14 Decker K & Keppler D, Galactosamine hepatitis: Key role of the nucleotide deficiency period in the pathogenesis of cell injury and cell death, *Rev Physiol Biochem Pharmacol*, 71 (1974) 77.

15 Tiegs G, Wolter M & Wendel A, Tumour necrosis factor is a terminal mediator in galactosamine/endotoxin-induced hepatitis in mice, *Biochem Pharmacol*, 38 (1989) 628.

16 Sakaguchi S & Yokota K, Role of $Ca^{2+}$ on endotoxin-sensitivity by galactosamine challenge: Lipid peroxide formation and hepatotoxicity in Zymosan-primed mice, *Pharmacol Toxicol*, 77 (1995) 81.

17 Hase K, Li J, Basnet P, Xiong Q, Takamura S, Namba T &, Kadota S, Hepatoprotective principles of *Swertia japonica* Makino on D-galactosamine/lipopolysaccharide-induced liver injury in mice, *Chem Pharm Bull*, 45 (1997) 1823.

18 Kondo Y, Takano F, Yoshida K & Hojo H, Protection by sinomenine against endotoxin-induced fulminant hepatitis in galactosamine-sensitized mice, *Biochem Pharmacol*, 48 (1994) 1050.

19 Misra H P & Fridovich I, The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase, *J Biol Chem*, 247 (1972) 3170.

20 Bergmeyer H V, Gowehn K & Grassel M, in methods of enzymatic analysis (Academic Press, Inc., Newyork) 1974, 438.

21 Rotruck J T, Pop A L, Ganther H F, Swanson A B, Hafeman B G & Hoeksira W G, Selenium: Biochemical role as a component of glutathione peroxidase, *Science*, 179 (1973) 588.

22 Habig W H, Pabst M J & Jakoby W B, Glutathione-S-transferases. The first enzymatic step in mercapturic acid formation, *J Biol Chem*, 249 (1974) 7130.

23 Moron M S, Depierre J W & Mannervik B, Levels of glutathione, glutathione reductase and glutathione-S-transferase activities in rat lung and liver, *Biochim Biophys Acta*, 582 (1979) 62.

24 Ohkawa H, Ohishi N & Yagi K, Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction, *Anal Biochem*, 95 (1979) 351.

25 Keppler D, Lesch R, Reutter W & Decker K, Experimental hepatitis induced by D-galactosamine, *Exp Mol Pathol* 9 (1968) 279.

26 Decker K & Keppler D, Galactosamine induced liver injury, in Progress in liver diseases, edited by H.Popper and F.Schaffner (Grune & Stratton, New York) 1972, 183.

27 Lin W S, Chan W C & Hew C S, Superoxide and traditional chinese medicines, *J Ethno Pharmacol* 48 (1995) 165.

28 Genc S, Gurdol F, Iyidogan Y & Onaran I, The effect of melatonin administration on ethanol-induced lipid peroxidation in rats, *Pharmacological Research*, 37 (1998) 37.

29 Anandan R & Devaki T, Hepatoprotective effect of *Picrorrhiza kurroa* on tissue defence system in D-galactosamine-induced hepatitis in rats, *Fitoterapia*, 70 (1999) 54.