



Protective effect of *Indigofera tinctoria* on tissue antioxidant defence system against D-galactosamine and endotoxin-induced hepatopathy in rats

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Abstract:

Objective: The effects of pre-treatment with alcoholic extract of *Indigofera tinctoria* Linn (Leguminosae) (500 mg/kg body wt/day orally 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.B₄) was investigated in male albino rats. **Materials and Methods:** The activities of enzymic antioxidant such as superoxide dismutase, catalase, glutathione peroxidase and glutathione-s-transferase were assayed and the levels of total reduced glutathione was estimated in the liver of normal and experimental groups of rats. The activities of non-enzymic antioxidant (ceruloplasmin) and iron, ferritin was also estimated. 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 was used as an index of oxidative stress. **Results:** 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. *Indigofera tinctoria* (IT) 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. **Conclusion:** Our results indicate that pretreatment with IT extract in rats is very effective in ameliorating D-GalN/endotoxin-induced oxidative stress and thereby suggest an antioxidant effect.

Key words: *Indigofera tinctoria*, D-Galactosamine/endotoxin oxidative stress, antioxidant, lipid peroxidation.

1. Introduction

Oxidative damage to crucial biomolecules 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

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during various pathophysiological states can lead to alteration 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 has attracted a great deal of attention is the possible use of antioxidant supplement 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] and Indirubin, a component isolated from the plant has been proved to be very effective anticancer agent in both pharmacological and clinical trails [8, 9].

Earlier studies with the alcoholic extract of the plant has confirmed its hepatoprotective effects against carbon tetrachloride-induced liver injury in rats, rabbits and mice [10, 11]. Previous studies in our laboratory has also confirmed the hepatoprotective effects of *Indigofera tinctoria* extract against D-Galactosamine/endotoxin-induced hepatitis in rats [12]. D-galactosamine 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].

Previous report by Sakaguchi *et al.* [16] showed that endotoxin injection has been observed to result 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 hrs 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 our present investigation. The effect of pre-administration with *Indigofera tinctoria* extract during D-Galactosamine/endotoxin-induced liver injury, with respect to antioxidant enzymes and non enzymic antioxidant has not been studied earlier.

Hence, we have now attempted to assess the hepatoprotective effects of methanolic extract of *Indigofera tinctoria* on tissue defence system in GalN/LPS-induced hepatitis in rats.

2. Materials and methods

2.1 Plant material

The plant was collected in Chennai from the garden of the Central Research Institute for Siddha, Arumbakkam, Chennai. 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.

2.2 Preparation of the extract

Shade dried and coarsely powdered plant material (whole plant) was extracted with methanol in cold (48 h). The extract was filtered, concentrated on a water bath and then dried in vaccum (yield 10%).

2.3 Chemicals

D-Galactosamine and endotoxin (bacterial lipopolysacchride from *E.coli* serotype 0111. B₄ extracted by phenol water method) was obtained from sigma chemicals, St. Louis, MO, USA. All other chemicals used were of analytical grade.

2.4 Animals

Adult male albino rats of Wistar strain weighing about 140–180g obtained from the Fredric Institute for plant protection and Toxicology, Padappai, Chennai were used for our study. 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*.

2.5 Studies on oxidative injury

The experimental animals were divided into four groups of six animals each. Group 1 served as the control. Group II were normal animals orally treated with *Indigofera tinctoria* (500 mg/kg body wt/day) for 21 days. Group III animals were intraperitoneally (i.p.) injected with GalN/LPS (300mg/kg body wt/day) and LPS (30mg/kg body wt/day) for the induction of hepatitis [19]. Group IV animals were orally pretreated with *Indigofera tinctoria* and then i.p. injected with GalN/LPS.

At the end of the experiment, the animals were killed by decapitation. Blood was collected without any anticoagulant and serum was separated for the assay of Ceruloplasmin [20].

The liver was excised immediately and homogenized in ice-cold 0.1M Tris-HCl buffer using a Potter-Elvehjem homogenizer. The homogenate was used for the estimation of iron [21] ferritin [22], lipid peroxides (LPO) [23], SOD [24], CAT [25], GSH [26], GST [27] and GPX [28].

2.6 Statistical analysis

Results are expressed as mean $\pm$ S.D. and student's *t* - test was used to assess statistical significance.

3. Results

Intraperitoneal administration of GalN/LPS caused a significant increase in lipid peroxidation

in liver, the level of hepatic iron was increased whereas the levels of liver ferritin was significantly decreased as compared to normal control rats (Table 1) thereby showing the severity of oxidative stress. The activities of the antioxidant enzymes (SOD, CAT, GPX, and GST) in liver and the level of ceruloplasmin in serum were significantly decreased in GalN/LPS- intoxicated rats as compared with their levels in normal control rats.

Also, the level of GSH was significantly reduced in GalN/LPS intoxication. Repeated oral administrations of *Indigofera tinctoria* whole plant extract significantly protected against the GalN/LPS-induced oxidative injury and ameliorated the deficit in antioxidant enzyme activities (Table 1).

4. Discussion

D-Galactosamine is a hepatotoxin that induces liver damage similar to human viral hepatitis, via the depletion of uridine nucleotides and subsequently diminishes the synthesis of RNA and plasma membrane proteins [29, 30]. 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. [31, 32]

The significant decrease in the activities of antioxidant enzymes and increase in the levels of lipid peroxides with a concomitant decrease in the levels of total reduced glutathione in the group III rats indicate the severity of oxidative stress induced as a result of administration of D-Galactosamine/endotoxin. The considerable increase in the activities of antioxidant enzymes, decrease in the levels of lipid peroxides and improvement in hepatic GSH status in the *Indigofera tinctoria* pretreated rats clearly indicates the protection

Table 1.

Hepatoprotective effect of the ethanol extract of *Indigofera tinctoria* on tissue defence systems in D-galactosamine Gal-N/LPS-induced hepatitis in rats

Test parameters	Group I Normal control	Group II IT treated	Group III GalN/ LPS intoxicated	Group IV (A+B)
LPO	1.65 ± 0.15 ^a	1.70 ± 0.12 ^a	2.81 ± 0.23 ^b	2.04 ± 0.19 ^{ac}
Iron	151.26 ± 14.30 ^a	153.47 ± 14.23 ^a	314.85 ± 15.21 ^b	182.53 ± 16.24 ^{ac}
Ferritin	32.72 ± 2.9 ^a	33.61 ± 3.30 ^a	21.83 ± 2.3 ^b	29.76 ± 2.7 ^{ac}
SOD	7.12 ± 0.64 ^a	7.06 ± 0.69 ^a	4.03 ± 0.25 ^b	6.27 ± 0.58 ^{ac}
CAT	60.12 ± 6.53 ^a	60.58 ± 5.62 ^a	41.03 ± 3.39 ^b	53.17 ± 4.49 ^{ac}
GSH	8.42 ± 0.84 ^a	8.39 ± 0.78 ^a	4.33 ± 0.41 ^b	6.20 ± 0.59 ^c
GST	1552.00 ± 150 ^a	1549.00 ± 147 ^a	1198.00 ± 117 ^b	1421.00 ± 137 ^{ac}
GPX	115.21 ± 10.50 ^a	115.99 ± 11.33 ^a	70.93 ± 6.88 ^b	97.09 ± 8.23 ^{ac}
Ceruloplasmin	42.13 ± 4.10 ^a	43.94 ± 4.30 ^a	21.06 ± 2.5 ^b	34.25 ± 3.60 ^{ac}

Values are expressed as mean ± SD; n = 6; Student's *t* - test means bearing different superscripts differ significantly (P<0.01) and those that bear atleast one superscript in common are not significantly different.

(A) IT 500 mg/kg per day, P.O. for 21 days ;

(B) GalN/LPS 300 mg/kg per day. 30 mg/kg per day i.p for 2 days

LPO = malondialdehyde/mg protein, Iron = µg/g wet litre, SOD = One unit of SOD activity is the amount of protein required to give 50% inhibition of epinephrine auto-oxidation, CAT = n mol of H₂O₂ decomposed/min/mg protein, GSH = n mol/g wet litre, GST = µ mol of 1-chloro 2, 4-dinitrobenzene conjugate formed per min/mg protein, GPX = µ mol glutathione oxidised/min/mg protein, Serum = ceruloplasmin = Units / mg protein.

offered by pretreatment with the plant extract and thereby suggests an antioxidant effect.

The hepatoprotection offered by pretreatment with *Indigofera tinctoria* was associated with a significant enhancement in hepatic GSH status. The GSH antioxidant system consists of an array of non-enzymic and enzymic reaction pathways involved in the neutralization of reactive free radical species. While D-Galactosamine/endotoxin-pretreatment produced drastic decrease in hepatic GSH status, IT pretreatment could effect a compensatory increase in the level of GSH 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. Even though there was a drop in the activity of hepatic GPX after

D-GalN/endotoxin challenge, IT pretreatment was found to restore the activity of this enzyme.

In our study, oral pre-treatment with IT (500 mg/kg per day) significantly prevented the adverse effects and maintained the level of the evaluated parameters nearly at normal values. The overall antioxidant effect of the extract may be attributed to the ability of IT to increase the activities of the free radical scavenging enzymes.

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