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Hepatoprotective activity of chitosan against isoniazid and rifampicin-induced toxicity in experimental rats

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Abstract

Tuberculosis is a dangerous disease and its death toll is increasing year by year. Intake of isoniazid and rifampicin, the most common antitubercular drugs, lead to fatal hepatotoxic condition. We have studied the protective effect of chitosan supplementation against the hepatotoxicity induced by antitubercular drugs with respect to the changes in the levels of protein, albumin–globulin ratio, urea and bilirubin in the serum and diagnostic marker enzymes (alanine amino transferase, aspartate amino transferase, acid phosphatase and alkaline phosphatase), protein, glycoprotein conjugates (hexose, hexosamine and sialic acid), lipid peroxidation and reduced glutathione in the liver tissue of normal and experimental groups of rats. Co-administration of chitosan was found to significantly prevent the antitubercular drugs-induced alterations in the levels of diagnostic marker enzymes, bilirubin and albumin/globulin ratio in experimental groups of rats. Isoniazid and rifampicin-induced lipid peroxidation was also found to be prevented by the administration of chitosan. Further, chitosan administration increased the levels of urea and protein (in serum and liver) in experimental groups compared to hepatotoxicity-induced group of rats. Levels of glycoconjugates were also maintained to near normal level by chitosan co-administration. From the results obtained, it can be concluded that chitosan is beneficial against antitubercualr drugs-induced hepatoxicity.

Keywords: Chitosan; Glucosamine; Isoniazid; Rifampicin; Diagnostic marker enzyme; Urea; Lipid peroxidation

1. Introduction

Tuberculosis is one of the fatal communicative diseases and is spread easily amongst people. Over one-third of the world's population is estimated to be infected with *Mycobacterium tuberculosis* and over 2 million people a year will die of the disease (Shishoo et al., 2001). Multi-drug resistant (MDR) strains of *M. tuberculosis* have emerged and a co-infection with AIDS was found out. This turned out that the WHO declared tuberculosis as 'Global health emergency' (Anon, 1997). The administration of isoniazid and rifampicin, the most common medication prescribed against tuberculosis, produces many metabolic and morphological aberrations in liver due to the fact that liver is the main detoxifying site for these antitubercular drugs. These antitubercular drugs induce hepatitis by a multiple step mechanism. It is characterized by a fall in serum albumin concentration and a rise in serum globulin concentration, which is related to the severity and duration of the disease. Peroxidation of endogenous lipids has been shown to be a major factor in the cytotoxic action of isoniazid and rifampicin. Antitubercular drugs mediated oxidative damage is generally attributed to the formation of the highly reactive oxygen species, which act as stimulator of lipid peroxidation and source for destruction and damage to the cell membrane (Georgieva et al., 2004). Alterations of various cellular defense mechanisms consisting of enzymatic and non-enzymatic components [reduced glutathione (GSH)] have been reported in isoniazid and rifampicin-induced hepatoxicity (Tasduq et al., 2005).

Marine polysaccharides were proved to have a lot of medicinal applications. Chitosan is a marine polysaccharide obtained as a by-product of the shrimp processing industry (Sini et al., 2005). Structure of chitosan is similar to that of cellulose.

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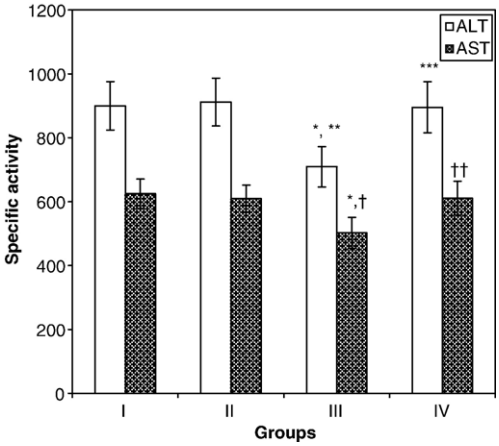

Fig. 1. Levels of ALT and AST (μmoles of pyruvate liberated/mg protein) in the liver of normal and experimental groups of rats.
Group I: Normal control.
Group II: Chitosan administered, 100 mg/kg body weight/day (intragastric intubation) for 30 days.
Group III: Administered 200 mg of isoniazid and rifampicin each/kg body weight/day (intragastric intubation) for 30 days.
Group IV: Chitosan co-administered (as in Group II) with antitubercular drugs (as in Group III) for 30 days.
Values are shown as the mean±S.D. for six animals.
* $P<0.001$ – compared with Group-I animals.
** $P<0.001$ – compared with Group-II animals.
*** $P<0.001$ – compared with Group-III animals.
† $P<0.01$ – compared with Group-II animals.
†† $P<0.01$ – compared with Group-III animals.

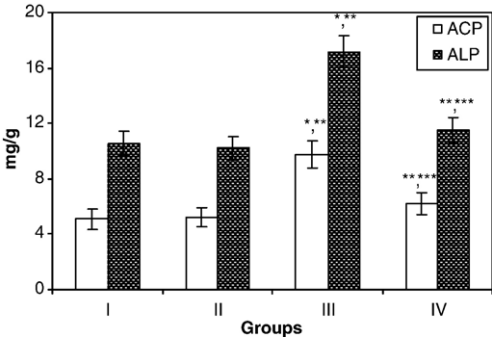

Fig. 2. Levels of acid phosphatase (ACP) and alkaline phosphatase (ALP) in the liver of normal and experimental groups of rats.
Group I: Normal control.
Group II: Chitosan administered, 100 mg/kg body weight/day (intragastric intubation) for 30 days.
Group III: Administered 200 mg of isoniazid and rifampicin each/kg body weight/day (intragastric intubation) for 30 days.
Group IV: Chitosan co-administered (as in Group II) with antitubercular drugs (as in Group III) for 30 days.
Values are shown as the mean±S.D. for six animals.
* $P<0.001$ – compared with Group-I animals.
** $P<0.001$ – compared with Group-II animals.
*** $P<0.001$ – compared with Group-III animals.

Glucosamine is the basic monomeric unit of chitosan (Santhosh et al., 2005). Chitosan has profound applications in the fields of pharmaceuticals and biomedicines since it is having antibacterial, haemostatic, fungistatic, antitumoral and anticholesteremic properties (Krajewska, 2005). The main advantage of chitosan is that it is non-toxic (Arai et al., 1968). Dissolution properties and bioavailability of poorly soluble drugs can be improved by grinding them with chitosan. Shigemasa and Minami (1996) studied the application of chitosan in wound healing. Han et al. (1999) has reported that supplementation of chitosan reduced high fat diet-induced lipidemia by its hypolipidemic property. The free radical quenching property of this marine polysaccharide has also been studied in detail (Yan et al., 2006). Membrane stabilizing property of chitosan was mentioned by Filipovic-Grcic et al. (2001). Earlier in our laboratory Anandan et al. (2004) observed that chitosan can be beneficially used against HCl–ethanol-induced gastric injury in rats.

2. Materials and methods

2.1. Chemicals and drugs

Isoniazid and rifampicin were supplied by M/s. Lupin Pharmaceuticals Ltd., Mumbai; bovine serum albumin and tetraethoxy propane were from M/s Sigma Chemical Company, St. Louis, MO, USA. Chitosan (M_W 750000 Da; viscosity 8 cps; deacetylation rate 85–87%; purity 98.6%) used in this study was prepared from chitin in our laboratory according to the method of Madhavan (1992). All other chemicals used were of analytical grade.

2.2. Animals

Wistar strain male albino rats, having weight range of 120–150 g, were used for the experiment. The animals were housed in polypropylene cages under hygienic condition and maintained at 28±2 °C temperature. The animals were allowed to have food and water *ad libitum*. The experiment was conducted according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India and approved by the Institutional Animal Ethics Committee (IAEC).

Table 1
Levels of protein (g/dl), albumin/globulin ratio (g/dl) urea (mg/dl) and bilirubin (mg/dl) levels in the serum of normal and experimental groups of rats

	Group I control	Group II chitosan (A)	Group III hepatotoxicity (B)	Group IV (A+B)
Protein	6.65±0.44	6.52±0.42	4.05±0.23[a,b]	5.75±0.37[c]
Albumin/globulin ratio	1.25±0.08	1.19±0.09	0.53±0.03[a,b]	1.04±0.08[c]
Urea	22.1±1.90	20.8±2.20	11.4±1.30[a,b]	18.6±1.55[c]
Bilirubin	0.42±0.04	0.41±0.03	0.92±0.08[a,b]	0.65±0.05[c]

A: Chitosan, 100 mg/kg body weight/day (intragastric intubation) for 30 days.
B: 200 mg of isoniazid and rifampicin each/kg body weight/day (intragastric intubation) for 30 days.
A+B: Chitosan co-administered (as in A) with antitubercular drugs (as in B) for 30 days.
Values are expressed as the mean±S.D. for six animals.
[a] $P<0.001$ — compared with Group-I control animals.
[b] $P<0.001$ — compared with Group-II chitosan control animals.
[c] $P<0.001$ — compared with Group-III antitubercular drugs administered animals.

Table 2
Levels of protein (mg/g tissue), hexose (mg/g tissue), hexosamine (mg/g tissue), sialic acid (mg/g tissue), lipid peroxides (nmole MDA/mg protein) and reduced glutathione (nmoles/g wet tissue) levels in the liver of normal and experimental groups of rats

	Group I control	Group II chitosan (A)	Group III hepatotoxicity (B)	Group IV (A+B)
Protein	192±12.3	189±14.7	153±8.46[a,b]	185±11.9[c]
Hexose	24.7±1.55	28.9±1.68	15.6±1.21[a,b]	24.3±1.45[c]
Hexosamine	8.18±0.49	10.7±0.65	5.11±0.44[a,b]	7.87±0.56[c]
Sialic acid	0.32±0.02	0.36±0.03	0.21±0.01[a,b]	0.28±0.02[c]
Lipid peroxides	0.96±0.07	0.87±0.04	1.91±1.65[a,b]	0.98±0.05[c]
Reduced glutathione	4.84±0.32	5.18±0.34	3.38±0.28[a,b]	4.95±0.33[c]

A: Chitosan, 100 mg/kg body weight/day (intragastric intubation) for 30 days.
B: 200 mg of isoniazid and rifampicin each /kg body weight/day (intragastric intubation) for 30 days.
A+B: Chitosan co-administered (as in A) with antitubercular drugs (as in B) for 30 days.
Values are expressed as the mean±S.D. for six animals.
[a] $P<0.001$ — compared with Group-I control animals.
[b] $P<0.001$ — compared with Group-II chitosan control animals.
[c] $P<0.001$ — compared with Group-III antitubercular drugs administered animals.

2.3. Experimental design

Animals were grouped into four. Each group was provided with six rats. Group I (normal control) rats were fed with standard diet. In Group II, normal rats were treated with chitosan (100 mg/kg body weight/animal/day, intragastric intubation for 30 days). Hepatotoxicity was induced in Group III, by the oral administration of isoniazid and rifampicin (200 mg each/kg body weight/animal/day, intragastric intubation for 30 days). Group IV animals, chitosan was co-administered (100 mg/kg body weight/animal/day, intragastric intubation for 30 days) with antitubercular drugs described for Group III.

At the end of the experiment, rats were sacrificed and blood was collected without any anticoagulant for the separation of serum. Protein (Lowry et al., 1951), urea (Natelson et al., 1951), albumin/globulin ratio (Varley et al., 1980) and bilirubin (Mally and Evelyn, 1937) were estimated in the serum. The liver tissue was excised immediately and washed with ice-cold saline. Accurately weighed liver tissue was taken, homogenized with 0.1 M Tris HCl buffer, pH 7.4 and the homogenate was used for the determination of protein (Lowry et al., 1951), lipid peroxidation (Ohkawa et al., 1979), hexose (Niebes, 1972), hexosamine (Wagner, 1979), sialic acid (Warren, 1959), reduced glutathione [GSH] (Ellman, 1959), alanine aminotransferase [ALT] (Mohur and Cook, 1957), aspartate aminotransferase [AST] (Mohur and Cook, 1957), acid phosphatase [ACP] (King, 1965) and alkaline phosphatase [ALP] (King, 1965).

2.4. Statistical analysis

Results were expressed as mean±S.D. One-way analysis of variance (ANOVA) was carried out and the statistical comparisons among the groups were performed with Tukey's test using a statistical package program (SPSS 10.0 for Windows).

3. Results

Fig. 1 shows the levels of diagnostic marker enzymes (ALT and AST) in the liver tissue of normal and experimental groups of rats. Levels of diagnostic marker enzymes were significantly ($P<0.001$) declined in antitubercular drugs-induced animals of Group III comparative to that of the normal animals in Group I. The cytoprotective effect of chitosan significantly elevated the fall in ALT ($P<0.001$) and AST ($P<0.01$) levels, characterized by antitubercular drugs, to the normal behaviour in Group IV chitosan co-administered animals.

Levels of acid phosphatase (ACP) and alkaline phosphatase (ALP) in the liver tissue were depicted in Fig. 2. Significant increase ($P<0.001$) in the levels of ACP and ALP were found in hepatotoxicity induced rats (Group III) as compared to normal control rats. But when chitosan was co-administered in Group IV animals, levels of these enzymes were brought back to near normal status at a significant level ($P<0.001$).

Table 1 shows the levels of protein, albumin/globulin ratio, urea and bilirubin in the serum of normal and experimental groups of rats. The levels of protein, albumin/globulin ratio and urea were decreased significantly ($P<0.001$) and bilirubin was found to be significantly increased ($P<0.001$) in Group III, antitubercular drugs-induced animals as compared to that of Group I normal rats. In Group IV animals, chitosan was co-administered with antitubercular drugs. In this Group, protein, albumin/globulin ratio, urea and bilirubin were maintained to near normalcy at a significant level ($P<0.001$).

Table 2 shows the protein, hexose, hexosamine, sialic acid, lipid peroxidation and reduced glutathione levels in the liver tissue of normal and experimental groups of rats. Administration of isoniazid and rifampicin in Group III animals significantly ($P<0.001$) elevated the level of lipid peroxidation as compared to Group I normal animals. But, the levels of protein, hexose, hexosamine, sialic acid, and reduced glutathione were lowered ($P<0.001$) in Group III, hepatotoxicity induced rats. Rats in Group IV, chitosan co-administered with antitubercular drugs, significantly ($P<0.001$) maintained these alterations to the normal status.

The rats administered with chitosan alone, in Group II, were not seemed to show any adverse effect supporting the non-toxic behaviour of chitosan.

4. Discussion

Amino transferases are important class of enzymes linking carbohydrate and amino acid metabolism, thereby clearly establishing the relationship between the intermediates of the citric acid and amino acids. Alanine amino transferase and aspartate amino transferase are well known diagnostic indicators of liver disease. In cases of liver damage with hepatocellular lesions and parenchymal cell necrosis, these marker enzymes are released from the damaged tissues into the blood stream. In the present study, the activities of these enzymes were significantly lower in the liver tissue of Group III, antitubercular drugs-administered rats as compared to that of control rats. Observations of significant decrease in the activities of these enzymes

have already been reported in the liver tissue of isoniazid and rifampicin intoxicated rats (Saraswathy et al., 1998).

The fall in alanine amino transferase activity is almost always due to hepatocellular damage and is usually accompanied by a lowering in the activity of asparate amino transferase. Increased protein catabolism and urea formation that are seen in antitubercular drugs-induced hepatocellular damage may also be responsible for the decline of these amino tranferases activities in liver. In the present study, the activities of alanine amino transferase and aspartate amino transferase were maintained at near normal level in the orally chitosan co-administered rats as compared to that of Group III antitubercular drugs-induced hepatotoxic rats, showing the normalizing effect of chitosan on protein metabolism.

ACP and ALP were found to increase in the hepatotoxic animals (Deepa and Varalakshmi, 2003) Alakaline phosphatase activity on endothelial cell surfaces is responsible for the conversion of adenosine nucleotides to adenosine, a potent vasodilator and anti-inflammatory mediator that results from injury. So, following injury, accumulation of interleukin-6 can lead to production of adenosine by alkaline phosphatase and subsequent protection from ischemic injury. This may be the reason for the increment in ALP in intoxicated rats, which have cell necrosis. Co-administration of chitosan deactivates the reactive metabolites, which damages the liver cells, caused by antitubercular regimens by virtue of chitosan's membrane stabilizing action (Filipovic-Grcic et al., 2001). As a result, the chitosan treatment could brought back the levels of ACP and ALP to near normalcy showing its antihepatotoxic potential.

Alterations in protein metabolism have been considered for decades to be one of the conditions associated with hepatic dysfunction. Our results showed decreased levels of protein in the serum and the liver of isoniazid and rifampicin-administered rats as compared to the Group I controls. Also a significant decline in the serum albumin/globulin ratio and urea were observed. The disaggregation of polyribosomal profiles induced by antitubercular drugs is also associated with the inhibition of protein synthesis, which may be partially responsible for the fatty liver, probably not necrosis although it contributes to disabling of the cell. Consumption of isoniazid and rifampicin increased the bilirubin level in the serum of Group III rats, which is in parallel with the report by Tasduq et al. (2005). Hepatotoxicity is characterized by cirrhotic liver condition which in turn increased the bilirubin release (Man-Fung et al., 2003). Co-administration of chitosan restored the level of bilirubin to near normal status by its cytoprotectivity and may be also due to the inhibitory effect on cytochrome P_{450}.

Drug induced hepatotoxicity is associated with failure to maintain serum albumin level whereas serum globulins, particularly γ-globulin, which is formed in the reticuloendothelial system are increased. Formation of urea is the mode of disposal of nitrogen. Liver is the most important organ in the maintenance of blood ammonia levels through the urea cycle (Stryer, 1995). In hepatotoxic condition, due to the failure of the liver to convert amino acids and ammonia to urea, a significant decrease in urea was observed (Reicher and Paumgartner, 1980). There is an increased catabolism of proteins coupled with the diminished ability of kidneys to excrete the nitrogenous waste. These could be possibly the reasons for the lowered level of urea. This shoulders with a previous report (Eule et al., 1986). Co-administration of chitosan in our study significantly prevented antitubercular drugs-induced alterations in the levels of protein, albumin/globulin ratio and urea in Group IV rats as compared to that of Group III rats. It probably did so by reducing the accumulation of toxic antitubercular drugs derived metabolites, which may contribute to the changes in the rough endoplasmic reticulum and the disturbance of protein metabolism in liver.

Group III, isoniazid and rifampicin administered rats showed a decrease in the levels of hexose, hexosamine and sialic acid. This is in line with an earlier reported study (Leinweber and Mahrt, 1976). The inhibition of protein synthesis in isoniazid and rifampicin-administered rats disturbed the glycoprotein synthesis. Thus, the glycoprotein conjugates (hexose, hexosamine and sialic acid) were found decreased in the Group III rats. Since, chitosan is a mucopolysaccharide, co-administration of chitosan might have accelerated the synthesis of hexose, amino sugars and sialic acid. As a result, tissue glycoprotein conjugate levels were brought back to normal level in Group IV animals.

Isoniazid and rifampicin induced hepatitis is due to their biotransformation to reactive metabolites that are capable of binding to cellular macromolecules (Georgieva et al., 2004). As an alternative to inducing cellular damage by covalent binding, there is evidence that these antitubercular drugs cause cellular damage through the induction of oxidative stress, a consequence of dysfunction of hepatic antioxidant defense system. The role of oxidative stress in the mechanism of isoniazid and rifampicin-induced hepatitis has been reported by Attri et al. (2000). Our findings confirm the same pattern and show significant increase in the level of lipid peroxidation in the serum and liver tissue of Group III antitubercular drugs administered rats as compared to that of Group I control rats. This was paralleled by significant decrease in the level of non-enzymatic (reduced glutathione) free radical scavengers.

Increase in the level of lipid peroxides in liver reflected the hepatocellular damage. The depletion of antioxidant defenses and/or raise in free radical production deteriorates the prooxidant-antioxidant balance, leading to oxidative stress-induced cell death (Sodhi et al., 1997). Depletion of reduced glutathione (GSH) is known to result in enhanced lipid peroxidation and excessive lipid peroxidation can cause increased glutathione consumption (Onyema et al., 2006), as observed in the present study. Co-administration of chitosan resulted in reduction in the level of lipid peroxidation (Shevtsova, 2000), an important cause of destruction and damage to hepatocellular membranes, and elevation in the level of GSH (Benassi et al., 2006) in liver. Report by Jeon et al. (2003) indicated the antioxidant role of chitosan against experimentally induced hepatic injury. Xing et al. (2005) have also reported the antioxidant and free radical scavenging properties of several marine polysaccharides including chitosan.

In conclusion, the antitubercular drugs (isoniazid and rifampicin)-induced alterations on protein metabolism and hepatic antioxidant defense system were normalized by chitosan co-administration, indicating a possible cytoprotective role of

chitosan against drug induced hepatitis. Thus chitosan can be classified as an antihepatotoxic agent.

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