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Anti-fibrotic effect of Noni (*Morinda citrifolia*. L) on carbon tetrachloride induced liver fibrosis

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Abstract : A study was carried out to investigate the effect of Noni against carbon tetrachloride (CCl_4), induced liver fibrosis. Liver fibrosis was induced by twice/week administration of CCl_4 at a dose of 1 ml/kg weight mixed with an equal volume of corn oil. The extent of liver fibrosis was assessed by the content of hydroxyproline in liver, serum level of asparate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and bilirubin. Treatment with Noni reduced the hydroxyproline content of liver, serum enzyme levels and total bilirubin. These observations confirm the antifibrotic effect of extract.

Introduction

Fibrosis is seen as a scar formation in liver (Borchers *et. al.,* 2000). Hepatic fibrosis is the result of chronic viral, toxic auto immune or cholestatic liver injury (Wasmuth, *et. al.,* 2003). Viral infection seems to be a crucial factor in liver fibrosis. Numerous chemicals and drugs can harm the liver (Chojkier and Brenner, 2003). In many experimental fibrotic models, CCl_4 was used to induce hepatic injury (Madro, *et. al.*, 2002). Some workers have used N-nitro dimethyl amine (NMDA) to injure rat liver and have reported that hyaluronic acid plays a role in the pathogenesis of liver fibrosis (George, *et. al.*, 2004). Likewise, bile duct ligation technique was used by many workers to induce fibrosis (Kountouras, J., *et. al.*, 1984; Turkacaper, N., *et. al.,* 2003).

In this study CCl_4 induced liver fibrosis was used as a model to evaluate the antifibrotic effect of Noni.

Materials and Methods

Animals

Male albino Wistar rats (150 –200 g) were purchased from Chellamuthu Trust, Madurai. They were housed in groups of 3 to 4/cage, maintained at 25 $\pm$ 2^0C under 12 hour light -dark cycle. They were fed with standard pellet diet and water ad libitum.

Induction of liver fibrosis by Carbon Tetrachloride

CCl_4 was given to rats orally twice a week for 28 days at the dose of 1 ml/kg body weight mixed with an equal volume of corn oil (Bickel, *et. al.,* 1991). Three days after the last dose, rats were sacrificed under light anesthesia and blood and liver samples were collected for biochemical studies.

Treatment with Noni

The diluted Noni extract was given orally by gavage for 28 days at a dose of 5 ml. The control group received equal amount of distilled water, given orally for 28 days. For comparison a group of normal rats was used throughout. The body weight of the animals was recorded every day for this study.

Estimation of serum biochemical parameters

After 28 days, the rats were sacrificed under light anesthesia and blood was collected by cardiac puncture. A part of it was used for biochemical estimation and centrifuged at 3000 rpm to obtain serum. The levels of asparate transaminase (AST), alananine transaminase (ALT), alkaline phosphatase (ALP) and total billirubin were estimated by standard procedures.

Determination of Hydroxyproline content in liver

The hydroxyproline content of liver was determined by the method suggested by Jamall, *et. al.,* (1981). The specimens of liver were weighed and hydrolysed completely in 6 M HCl. A fraction of the sample was derivatised using Chloramine T solution and Erhlich's reagent. The density was measured at 558 nm.

Statistical analysis

The values are expressed as mean $\pm$SEM. The data were analysed using one way ANOVA followed by Newman Keul's multiple range tests. Differences below $P < 0.05$ implied significance.

Results

The results are presented in Tables 1 and 2.

Table 1: Effect of Noni on the biochemical parameters of rats treated with CCl_4

Treatments	AST IU/L	ALT/IU/L	ALP IU/L	Total Bilirubin mg/dl
Normal rats	159 $\pm$6	47$\pm$ 2.2	215 $\pm$ 6.8	0.48 $\pm$ 0.01
CCL4 treated rats	289 $\pm$1.1*	102 $\pm$5*	396$\pm$8*	1.6 $\pm$ 0.08*
CCL4 + Noni treated rats	175$\pm$ 8*[a]	61$\pm$4*[a]	235 $\pm$13.2*[a]	0.7 $\pm$0.03*[a]

Data are mean + SEM. n=6, Newman Keul's multiple test was used (P<0.05).

* Significantly different from normal rats.

*a significantly different from CCl4 treated rats.

Table 2: Hydroxyproline content of liver and liver weight following various treatments

Treatment	Hydroxyproline Content (mg/g liver)	Liver weight on day 28
Normal rats	53 ± 3	3.5 ± 0.02
CCL4 treated rats	124 ±7	4.5 ± 0.06
CCL4 + Noni treated rats	77 ± 3 *a	3.8 ± 0.07 *a

Data are mean + SEM. n = 6, Newman Keul's multiple test was used (P<0.05)

* significantly different from normal rats.

*a significantly different from CCl_4 treated rats.

Serum parameters

Treatment with CCl_4 altered various tissue and serum parameters and also the architecture of liver. In CCl4 treated rats, the serum parameters AST, ALT, and ALP were significantly elevated. The bilirubin level was also high. However, in CCl_4 + Noni treated rats, the serum levels of these enzymes and bilirubin were significantly low when compared to CCl_4 alone treated rats.

Tissue parameters

The main tissue parameters assessed were hydroxyproline content and weight of liver. Hydroxyproline was elevated following CCl_4 treatment. Treatment with the extract reduced this parameter. Liver weight was increased in CCl_4 treated rats which was reduced by Noni.

Discussion

Large literature states that collagen content in the extracellular matrix is high in fibrosis, the extent of which could be assessed by the hydroxyproline content (Muriel and Escobar, 2003). Many earlier studies have reported high hydroxyproline content in association with liver fibrosis. Present study showed a high hydroxyproline content in CCl_4 treated rats, indicating the development of fibrosis, which was brought down by co-treatment with Noni.

In the present study, CCl_4 the toxicant that injures liver is converted to trichloromethyl radical by the enzyme cytochrome P450 which initiates lipid

peroxidation and liver damage. Hence, liver damage was reflected by high levels of serum AST, ALT, ALP and bilirubin in CCl_4 treated rats. The rats treated with CCl_4 + Noni showed low levels of AST, ALT, ALP and bilirubin. These observations suggest that Noni is effective against CCl_4 induced liver fibrosis in rats.

Conclusion

This study has indicated the protective effect of Noni against CCl_4 induced chronic liver injury. This conclusion was based on the correction of serum as well as tissue parameters by Noni.

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