ORIGINAL ARTICLE

Hepatoprotective Properties of Sugarcane Juice and Vitamin C were compared in a Mouse Model of Liver Injury Induced by INH (Isoniazid)

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ABSTRACT

Aim: To compare the hepatoprotective properties of sugarcane (*Saccharum officinarum.* L) juice and vitamin C in mice on hepatotoxicity induced by INH (Isoniazid).

Methods: Ten mice were randomly placed in four groups A, B, C and D. **Group A** served as control, **Group B** received an oral dose of INH 100 mg/kg/day for 30 days and **Group C** was given sugarcane juice 15ml/kg/day along with INH 100 mg/kg/day orally for 30 days. **Group D** was given vitamin C 20mg/kg/day along with INH 100 mg/kg/day orally for 30 days. At the end of experimental period the animals were anesthetized and blood was obtained for the estimation of biochemical markers. The animals were then dissected and livers were taken and processed for microscopic examination.

Results: Sugarcane juice showed histological regression of central vein congestion, periportal inflammation and vacuolar degeneration in hepatocytes. Simultaneously, sugarcane juice significantly lowered the raise in aminotransferases (ALT, AST) and alkaline phosphatase (ALP) levels, which show the liver functional status. Effects of vitamin C were less pronounced in terms of histological as well as biochemical evaluation.

Conclusion: Sugarcane juice was found to be more effective as a hepatoprotective agent than vitamin C in INH induced oxidative liver injury.

Key words: Isoniazid (INH), Sugarcane (*Saccharum officinarum*. L), Hepatoprotective.

INTRODUCTION

Liver being the primary organ for transformation of various chemicals and therapeutic agents is susceptible to the possible damaging effects of drugs or their metabolites. Overdose of various drugs and sometimes their therapeutic dose may become a cause of hepatic insult[1]. Generation of free oxygen radicals is associated with lipid peroxidation in the liver. This has stirred an incessant evaluation of antioxidants in order to prevent hepatotoxicity[2].

INH is a known effective agent for preventing and treating tuberculous infections and is also known for its neurotoxic and hepatotoxic potential[3]. The mechanism of INH induced liver injury is attributed to the production of free oxygen radicals by lipid peroxidation,[4] initiated by cytochrome P450 (CYP) mediated metabolism of INH to its oxidative reactive metabolites i.e. acetylhydrazine and hydrazine[5].

Sugarcane juice can be considered as a rich antioxidant source. It is rich in apigenin which is known to be potent inhibitor of free radical-induced lipid peroxidation[6,7].

Vitamin C is also considered to be an important free radical scavenger in extracellular fluids. It protects the biomembranes from peroxide damage by trapping radicals especially singlet oxygen, superoxide, hydroxyl and water soluble peroxyl radical[8]. This water soluble vitamin is reported to act by rapid electron transfer to reactive oxygen species thus inhibiting lipid peroxidation process[9,10]. Vitamin C is reported to act synergistically either directly or indirectly with other antioxidants such as vitamin E[11].

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MATERIALS AND METHODS

Animals: A randomized control Trial (RCT) was done on forty adult albino mice. They were taken from Veterinary Research Institute (VRI) Lahore and were randomly alloted into four groups, containing ten mice in each. All mice were kept for one week for acclimatization, at a temperature 25±2°C, relative humidity 50±5% and 12 hour light and dark cycle. The mice were fed on standard mouse diet and tap water *ad libitum*. The mice were weighed at the start and then regularly after every week till the end of experimental period.

Experimental procedure: Random placement of 10 animals each in 4 groups A, B, C and D was done. **Group A** served as control, **Group B** was given an oral dose of INH 100 mg/kg/day for 30 days and **Group C** was given sugarcane juice 15ml/kg/day along with INH 100 mg/kg/day orally for 30 days. **Group D** was given Vitamin C 20mg/kg/day along with INH 100 mg/kg/day orally for 30 days. At the end of the experimental period the animals were anesthetized and blood was drawn through cardiac puncture for the estimation of biochemical markers. The animals were dissected and liver exposed. Liver from each mice was taken and processed for histological examination.

Statistical analysis: SPSS 18.0 was used for analyzing our data. One way ANOVA (Analysis of Variance) was applied to see the differences among groups. . Post Hoc Tukey test was applied to observe mean differences among the groups. For observation of association of qualitative variables between groups Chi-square and Fisher exact test were applied. A p-value of ≤ 0.05 was considered as statistically significant.

RESULTS

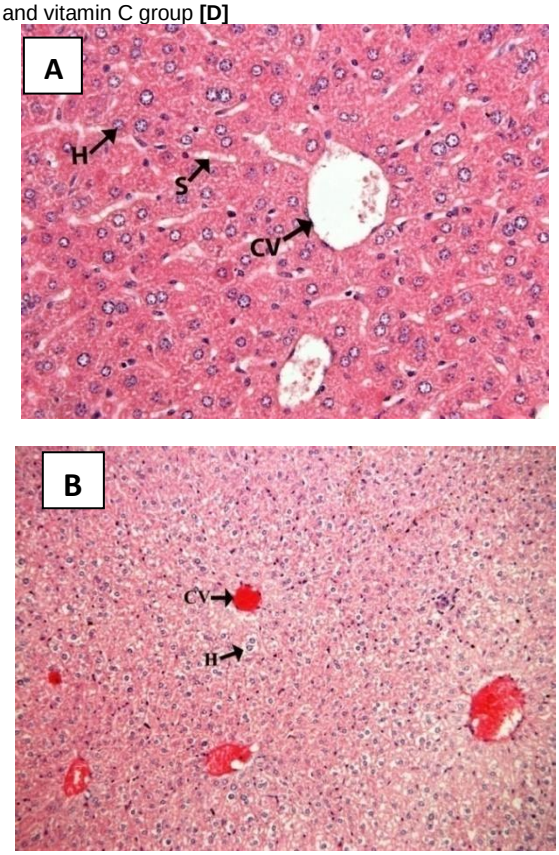
Microscopic examination of the liver tissue of all the mice was done. The control group showed normal morphology in all animals. In INH treated group, 8/10 (80%) animals showed central vein congestion, moderate to heavy

periportal inflammation, moderate to severe vacuolar degeneration of hepatocytes and focal areas of necrosis (**Fig 1B, 2A, 2C; Table 1**), while 2/10 (20%) animals showed only mild periportal inflammation and vacuolar degeneration (**Table 1**).

The histopathalogical examination of group C (INH + sugarcane juice) revealed that only 2/10 animals showed focal areas of necrosis and central vein congestion while 8/10 animals showed mild or no periportal inflammation and vacuolar degeneration; showing the hepatoprotective effects of sugarcane juice (**Fig. 1C, 2B; Table 1**). In liver sections of group D (INH+ Vitamin C) central vein congestion and moderate periportal inflammation was seen in 7/10 (70%) animals. Moderate to severe vacuolar degeneration was still obvious in 6/10 (60%) animals and 5/10 (50%) animals showed focal areas of necrosis (**Fig 1D, 2D; Table 1**); thus this group of animals showed mild to none hepatoprotection.

The histopathalogical findings correlated with markedly increased plasma ALT, AST and ALP activities, (used for assessing liver function) in mice treated with INH only and with significantly lower activities of these enzymes in animals additionally treated with sugarcane juice and vitamin C (**Graph 1**). The results of Group C which was given sugarcane juice and INH showed remarkable improvement when compared with Group D receiving vitamin C and INH.

Fig. 1: Central vein in control **[A]**, INH group **[B]**, sugarcane group **[C]** and vitamin C group **[D]**



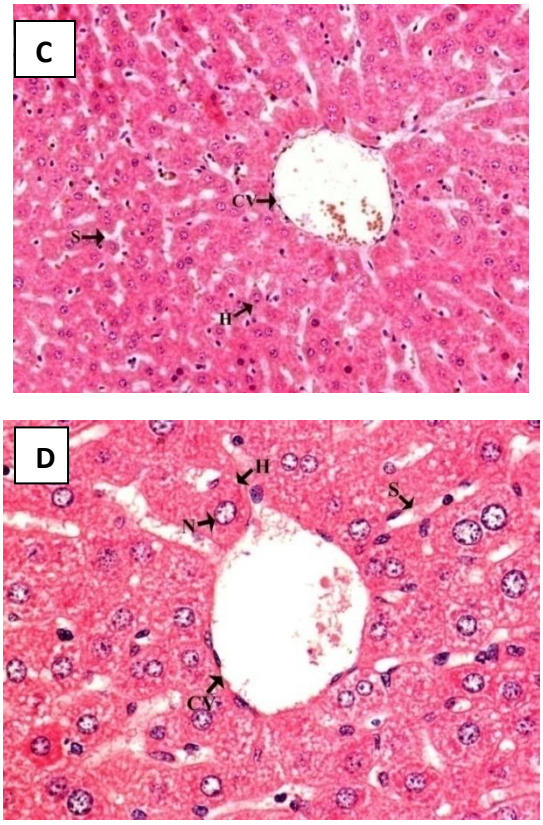
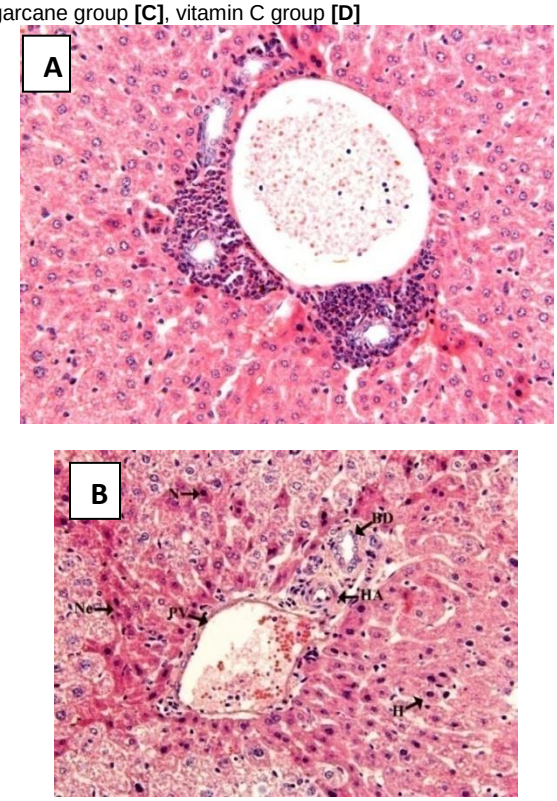
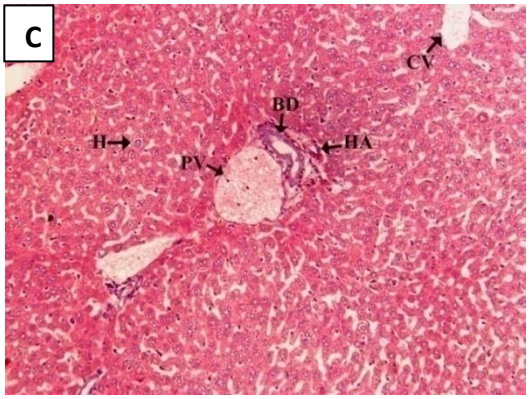
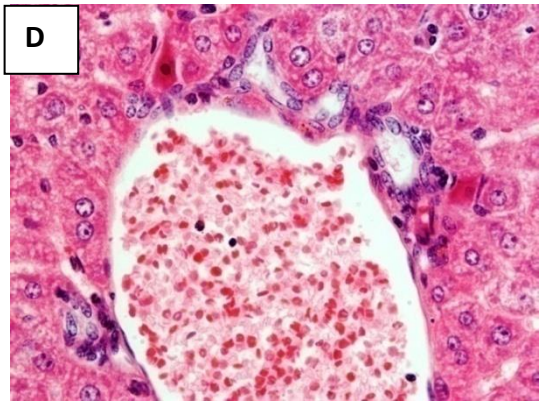


Fig.2: Portal triad inflammation and necrosis in INH group **[A]**, **[B]**, sugarcane group **[C]**, vitamin C group **[D]**







Graph 1: Difference in plasma aminotransferases (ALT & AST) and alkaline phosphatase (ALP) levels in groups A, B, C and D

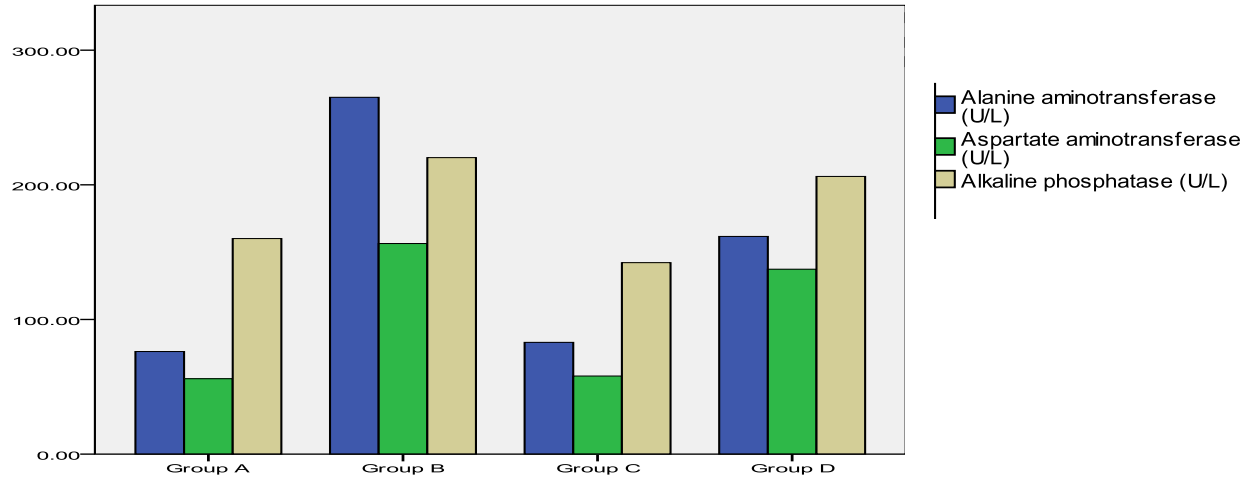


Table 1:

Parameter		Group				*p*-value
		A	B	C	D	
Central vein	Normal	+++	-	+++	++	<0.006
	Congested	-	+++	+	++	
Periportal inflammation	Absent	++++	-	++	-	<0.001
	Mild	-	+	++	+	
	Moderate	-	++	+	++	
	Severe	-	+++	-	+	
Vacuolar degeneration	Absent	+++	-	++	-	<0.0001
	Mild	-	+	++	++	
	Moderate	-	++	+	+	
	Severe	-	+++	-	-	
Necrosis	Absent	+++	+	+++	+	<0.001
	Present	-	+++	+	++	

DISCUSSION

The main finding of this study was that both sugarcane juice and vitamin C administration in mice was associated with a partial reduction of INH induced liver damage; with sugarcane juice being more in efficacy than vitamin C. The primary reason for observed protective effects of the sugarcane juice and vitamin C against INH induced hepatotoxicity may be ascribed to the antioxidant effects of these compounds.

Several researchers have reported the relation of liver damage by INH to the reactive oxidative effects of its metabolites such as acetylhydrazine[12,13,14]. Ergul and Co-researchers found out that drug induced liver injury as a result of oxidative stress was reduced by pretreatment with antioxidant compounds in animal model[4]. Antioxidants like polyphenolic compounds and flavonoids are considered to be valid therapeutical agents for treating many pathologies[15].

Amongst the antioxidants the main constituents of sugarcane juice are potent phenolic compounds anthraquinones and flavones such as naringenin, tricin, apigenin and luteolin derivatives[16,17].

Extracts from sugarcane have been reported to possess antioxidants and exhibit potent effects against hepatotoxicity induced by oxidative stress shown by the improvement in histological picture of liver[18].

Vitamin C has also been evaluated to have a synergistic effect with different agents and amelioration of liver damage evident by normalization of transaminases and inhibition of lipid peroxidation[19].

The present study demonstrated increased levels of ALT, AST and ALP in group B indicating liver damage by INH. Co-administration of sugarcane juice with INH in group C showed normal levels of ALT, AST and ALP as compared to group D animals receiving vitamin C with INH. These findings were well coordinated with a study conducted by Yue et al., in which hepatotoxicity by INH was also found to be correlated with the increased levels of ALT, plasma hydrazine and CYP2E1 indicating the stimulation of CYP2E1 (an enzyme that produces reactive oxygen species) by INH and its metabolites[20].

The histopathological changes observed in the present study were vacuolar degeneration, piecemeal necrosis, central vein congestion, periportal inflammation in INH treated mice amongst which severe to moderate amount of histological damage was observed in most of the animals. Several earlier authors have also reported similar types of histological change due to INH treatment in animals[21,22]. Sugarcane juice was able to show protection against INH induced hepatotoxicity, as evidenced by normalization of the histological structure of liver in most of the animals while mild to moderate histological damage was still existent in mice receiving vitamin C as a protective agent.

Therefore, in our study recovery effects against INH induced hepatotoxicity were suggestive of the role of antioxidants found in sugarcane juice.

CONCLUSION

Sugarcane juice was found to be more effective as a hepatoprotective agent as compared to vitamin C in INH induced oxidative liver injury. Further research studies are required to see if different doses and mode of administration of these antioxidant rich agents have different effects on INH induced liver damage.

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Conflict of interest: There is no conflict of interest.

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