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Protective effect of Liv.52 on Na^+-K^+-ATPase activity in Paracetamol-induced Hepatotoxicity

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ABSTRACT

Role of Liv.52, a polyherbal formulation, in regulating the sodium pump in hepatic injury induced by paracetamol was investigated. Alterations in sodium pump were induced by chronic administration of paracetamol at the dose of 500 and 1000 mg/kg b. wt. for 28 days. Serum alanine aminotransferase (ALT), Na^+-K^+-ATPase activity estimation and histology of liver were studied. Chronic administration of paracetamol for 4 weeks to rats produced significant increase in ALT and reduction in liver Na^+-K^+-ATPase activity indicating hepatocellular damage. Histological evaluation supported this change with evidence of swelling, hydropic degeneration and necrosis of the hepatocytes. These changes were reversed with simultaneous administration of paracetamol and Liv.52 at 750 mg/kg b. wt. for 28 days. Reversal of Na^+-K^+-ATPase, ALT levels and restricted hepatic damage in Liv.52 treated animals confirms the hepatoprotective effect of Liv.52. Hepatic regeneration and membrane-stabilization by reversal of Na^+-K^+-ATPase towards normal in Liv.52 treated animals could be the probable mode of action. Thus, Na^+-K^+-ATPase may be considered as a marker to evaluate the hepatoprotective effects of various herbs.

INTRODUCTION

The sodium-potassium adenosine triphosphatase (Na^+-K^+-ATPase) is an integral membrane enzyme found in all cells and is responsible for the ATP-dependent transport of sodium and potassium across the cell membrane. This membrane-bound enzyme is related to a number of other ATPase including sarcoplasmic and endoplasmic reticulum calcium ATPase and plasma membrane calcium ATPase. The sodium/potassium ATPase consists of a large, multipass, transmembrane catalytic subunit, termed the alpha subunit, and an associated smaller glycoprotein, termed the beta subunit. The Na^+-K^+ pump is a tetrameric transmembrane protein, with two alpha and two beta subunits.[1]

The different isoforms of the sodium/potassium ATPase exhibit tissue-specific and developmental patterns of expression. The alpha subunit has been found in the kidney, brain and heart, and to a lesser extent the liver and skeletal and smooth muscles.

The pump uses ATP as its energy source and so this was called the sodium-potassium ATPase or the sodium-potassium pump. This pump couples the energy yielding process of ATP hydrolysis to the inward transport of potassium ions and the outward transport of sodium ions.

In addition to maintaining the appropriate intracellular concentrations of both potassium and sodium, it contributes to the charge differential across the membrane (membrane potential) and helps in maintaining the osmotic balance of the cell.

MATERIALS AND METHODS

Animals: Forty inbred male Wistar rats weighing 220-250 g were used for the study. The animals were maintained on a 12-h light and dark cycle, at 22 ± 3°C, fed *ad libitum* with standard pellet diet (Amruth Laboratory, Mumbai) and had free access to water.

Study Protocol: The rats were randomized into five groups comprising 8 animals each. Group I served as normal control, which received 10 ml/kg b. wt. of water as vehicle once a day orally for 28 days. Rats of Groups II and III received paracetamol at a dose of 500 and 1000 mg/kg b.wt. p.o. respectively, for same duration. Group IV rats were administered paracetamol 500 mg/kg b.wt. + Liv.52, 750 mg/kg b.wt. p.o. and Group V rats were administered paracetamol 1000 mg/kg b. wt. + Liv.52 750 mg/kg b. wt. p.o. for the same duration. On day 29, the animals were euthanised by ether anesthesia. Blood was collected for the estimation of serum alanine aminotransferase (ALT).[2] The liver was dissected out immediately, rinsed with cold phosphate buffer and subjected for estimation of Na^+-K^+-ATPase.[3,4] Pieces of liver were trimmed into 10-15 mm thickness and fixed in 10% neutral buffered formalin, processed by the paraffin technique. Sections of 5μ thickness were cut and stained by routine H&E method for histological evaluation.[5]

Statistical Analysis: The significance of difference among the groups was assessed using one way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test. The values expressed as mean ± SEM, $p<0.05$ were considered significant.

RESULTS

The findings of ALT and Na^+-K^+-ATPase have been summarized in Table 1. It is seen that paracetamol at the dose of 1000 mg/kg b.wt. showed significant elevation in serum ALT and decrease in Na^+-K^+-ATPase activity compared to Group I (control). No significant difference was observed in biochemical parameters with 500 mg/kg b.wt. dose of paracetamol. Simultaneous treatment with Liv.52 at 750 mg/kg b.wt. significantly prevented the changes of elevated serum ALT and reduced hepatic Na^+-K^+-ATPase activity, induced by paracetamol intoxication as compared to Group III.

Table 1: Effect of Liv.52 on serum ALT and Na^+-K^+-ATPase in Paracetamol-induced Hepatotoxicity

Group	N	Alanine aminotransferase (IU/L)	Na^+-K^+-ATPase (U/mg protein)
Control	8	33.38 ± 2.11	7.394 ± 0.264
Paracetamol (500 mg/kg)	8	38.17 ± 1.82	6.823 ± 0.196
Paracetamol (1000 mg/kg)	8	78.00 ± 10.35$^{\#}$	6.529 ± 0.397$^{\#}$
Paracetamol (500 mg/kg) + Liv.52 (750 mg/kg)	8	30.88 ± 1.74	7.083 ± 0.278
Paracetamol (1000 mg/kg) + Liv.52 (750 mg/kg)	8	37.25 ± 5.89*	7.165 ± 0.323*

Values are Mean ± SEM.
$^{\#}p<0.05$ in comparison with control
*$p<0.05$ in comparison with Paracetamol (1000 mg/kg)

The histopathological observations of the liver showed normal structural and architectural intactness without any apparent damages or disruptions in Group I (Figure 1). The animals in Group III showed damage to the hepatic architecture characterized by congestion, cloudy swelling, vacuolar degeneration, karyolysis, necrosis, hemorrhage and inflammatory cell collections (Figure 2). The hepatic damage in Group V was minimal with distinct preservation of structural and architectural frame (Figure 3).

DISCUSSION

Liv.52 has proved the hepatoprotective activity in chemical and drug-induced hepatic damage.[6-8] It is also found to possess free radical scavenging activity in CCl_4-induced hepatotoxicity in rats.[9-10]

The active transport of sodium-potassium across the cell membrane is controlled by sodium-potassium-adenosine triphosphatase (Na^+-K^+-ATPase) enzyme, which is an integral plasma membrane protein responsible for a large part of the energy consumption constituting the cellular metabolic rate. Na^+-K^+-ATPase controls cell volume, nerve and muscle signals and drives the transport of amino acids and sugars. Emphasis on the early pathogenesis of paracetamol hepatotoxicity was established with respect to lowered Na^+-K^+-ATPase activity. In the present study, it was aimed to review the evidence for a role of Liv.52, a polyherbal formulation in regulating the sodium pump in hepatic injury produced by paracetamol. Paracetamol, a commonly used analgesic-antipyretic agent, gets metabolized in the liver to an active metabolite, N-acetyl-p-benzoquinone imine, by the cytochrome-P-450 microsomal enzyme system, which results in oxidative stress-producing liver glutathione and glycogen depletion and hepatic necrosis.[11,12] In the present study, paracetamol intoxication resulted in hepatic necrosis, which is in concurrence with published reports.[12-16]

Figure 1: Photomicrograph of liver in control group showing normal structural and architectural intactness without any apparent damages or disruptions (H&E, 1000x)

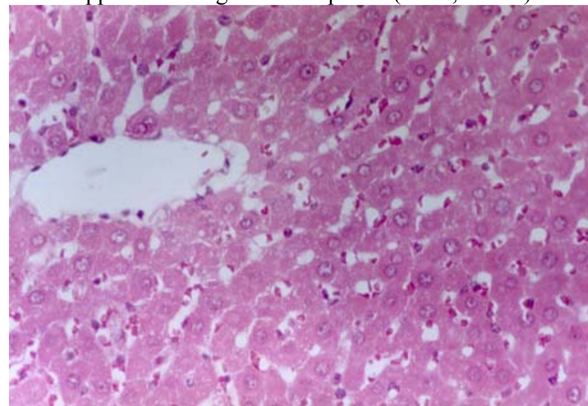


Figure 2: Photomicrograph of liver in rats treated with Paracetamol (1000 mg/kg b.wt.) showing severe degree of cell swelling, vacuolations, necrosis, hemorrhage and inflammatory cell collections (H&E, 1000x)

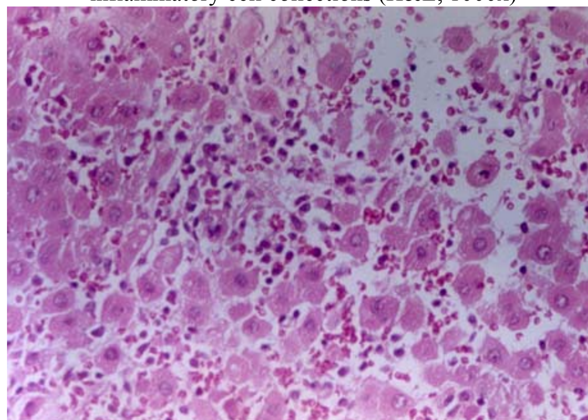
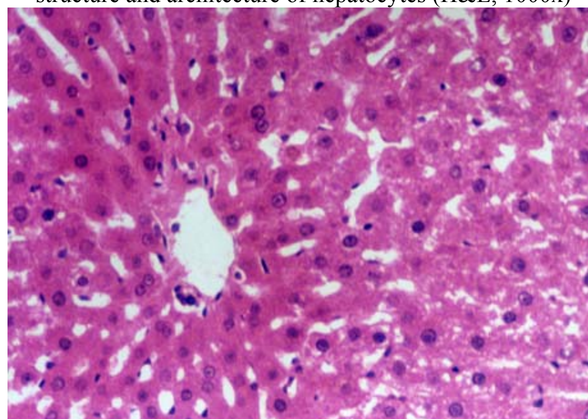


Figure 3: Photomicrograph of liver in rats treated with Paracetamol (1000 mg/kg b.wt.) + Liv.52 (750 mg/kg b.wt.) showing minimal damage with distinct preservation of structure and architecture of hepatocytes (H&E, 1000x)



An obvious sign of hepatic injury is the leakage of cellular enzymes into the plasma.[17] It is established that serum enzymes like ALT are elevated in paracetamol-induced hepatotoxicity.[12] The present observation is in concurrence with published reports. The elevated serum enzyme levels were significantly reduced in animals, which received Liv.52 indicating hepatocellular protection. The alterations in the serum enzyme levels were correspondingly reflected in the histological findings.

The Na^+-K^+ pump is found in the membranes of many types of cells. This pump illustrates "active transport" since it moves Na^+ and K^+ against their concentration gradients. Since the pump requires

an ATP every time it works, ATP must be constantly supplied to the cell. ATP is created during the processes called "cellular respiration", which occur inside the cell. Part of cellular respiration happens in the cytoplasm, and part in the mitochondrion. Pathological processes that interfere with the production of ATP may interfere with sodium pump activity, which in turn results in decreased hepatocellular function. In the present experiment, decrease in liver Na^{+}-K^{+}-ATPase activity, cloudy swelling and cell necrosis were noticed in paracetamol-treated rats. It has been hypothesized that oxidative damage of membrane ATPase is crucial for cell necrosis.[18,19] So under oxidative stress, the hepatocyte membrane appears to be the critical locus of damage and oxidative alterations are responsible for membrane damage in paracetamol-induced hepatotoxicity in rats. Hence the postulation can be made that covalent binding of paracetamol and its metabolites to cellular proteins may induce a series of events, which produce hepatocellular necrosis. This observation is well correlated with histological changes in hepatic parenchyma described as cloudy swelling, which is an early indicator of degenerative changes and latter necrosis. The hepatic regeneration and prevention of necrosis was observed in Liv.52-treated animals indicating its usefulness in early pathogenesis of paracetamol hepatotoxicity.

Thus, hepatic regeneration and membrane-stabilization by reversal of Na^{+}-K^{+}-ATPase towards normal in Liv.52-treated animals could be the probable mode of action. Hence, clinically Liv.52 may be recommended for early prophylaxis of patients with paracetamol-induced hepatic damage. In addition, Na^{+}-K^{+}-ATPase may be considered as a marker for assessing hepatocellular damage induced by hepatotoxic agents.

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