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Role of Liv.52 – A Herbal Formulation on ^{14}C-Ethanol Metabolism and ^{14}C-Acetaldehyde Accumulation in Rat Liver

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ABSTRACT

Metabolism of ^{14}C-ethanol was studied in rat liver following Liv.52 treatment (0.5 ml/100 g body weight) for 10 days. Liv.52 significantly reduced the in-vivo accumulation of ethanol metabolite to hepatic organelles. Similarly, it also inhibited the in-vivo incorporation of ^{14}C-accetaldehyde in liver. It is thus concluded that Liv.52 can reduce the hepatic damage caused by alcohol consumption possibly by rapid ethanol-acetaldehyde conversion and by significant inhibition of acetaldehyde accumulation in the hepatic organelles.

INTRODUCTION

Ethanol consumption is a major cause of liver disease. Ethanol and its metabolites have been shown to be directly injurious to the liver[1]. Alcohol is known to be readily and completely absorbed from the proximal portions of the gastrointestinal tract[2]. The fraction of ethanol that does not appear in the blood is likely to be oxidized before entering the systemic circulation[3,4]. Alcohol is mainly metabolized in the liver, which is also the major target organ of ethanol induced toxicity[5]. Many of the hepatic structural and functional changes produced by ethanol consumption have been attributed to acetaldehyde formation, which is the first by-product of ethanol oxidation[6,7]. Toxicity of acetaldehyde to hepatic cell constituents such as mitochondria, endoplasmic reticulum and microtubules is well-established[8,9]. However, acetaldehyde induced hepatotoxicity has been reported to be influenced by genetic, nutritional and environmental factors[10].

Liv.52, a herbal formulation based on Ayurvedic principles, contains a number of hepatoprotective ingredients and is widely prescribed in Indian and European countries for various liver disorders[11]. It has earlier been reported to protect liver from damage produced by toxic substances including alcohol[12,13]. However, the action of this drug on *in-vivo* alcohol hepatic metabolism together with *in-vitro* hepatic acetaldehyde incorporation has not been explored so far. Therefore, it was of our interest to investigate the role of Liv.52 on ^{14}C-ethanol metabolism and ^{14}C-acetaldehyde accumulation in liver by using rat as an experimental model.

MATERIALS AND METHODS

Forty five male Sprague Dawley rats in the weight range of 120-130 g used in this study were procured from the central animal house of Punjab University, Chandigarh. These were acclimatized in the departmental animal house for one week prior to subjecting them to Liv.52 treatment. These animals were divided in two main groups viz., control group-I (G-I) and Liv.52 treated Group-II

(G-II). Each of the two groups was further sub-divided into three subgroups viz., G-Ia, G-Ib and G-Ic, (6 animals in each subgroup) and G-IIa, G-IIb, G-IIC (9 animals in each subgroup). All animals in group-II were daily-administered Liv.52 syrup (supplied by The Himalaya Drug Company, Bangalore, India) in single dose by intubation at a dose level of 0.5 mL/100 g body weight (b. wt.) for a period of 10 days.

The animals of G-Ia and G-IIa were sacrificed under light ether anesthesia on the last day 910th day), 2 hour after administration of the last dose of Liv.52. Livers of these animals were incised and perfused with ice-cold normal saline. The perfused liver was cut into thin slices. Each slice was incubated by tissue accumulation method[14] in a medium containing radiolabelled acetaldehyde (5.0 mL of normal saline and 0.037 MBq activity of ^{14}C-acetaldehyde supplied by NEN, USA) at 37^{o}C for 1 hour with a metabolic shaker. The standard method of cutting tissue into uniform slices by a sharp surgical blade and confirming equal weight of the cut slices by digital balance was performed. On day 10, 2 hours after the last dose of Liv.52, animals of G-IIb and G-IIc and the respective controls (G-Ib and G-Ic) were orally administered 5.0 mL of 5% ethanol containing 0.06 MBq ^{14}C-ethanol (procured from NEN, USA) as the radiotracer. Animals of G-Ib and G-IIb, and G-Ic and G-IIc, were sacrificed under light ether anesthesia at 2 hour and 4 hour respectively following ^{14}C-ethanol administration. Liver was extracted and perfused with chilled normal saline. Blood was drawn from the retro-orbital plexus for subsequent testing before sacrificing the animals. Uniform weight of liver tissue (500 mg) from all the animals was digested in 30% potassium hydroxide overnight and then transferred to scintillation vials containing fixed volume (5.0 mL) of Bray's fluid.

Radioactivity present in the liver and blood was counted using beta-counter (LKB, Germany). Radioactivity in the liver was expressed as percent incorporation per gram of wet tissue whereas in the blood it was expressed as percent activity per ml of blood.

RESULTS

Results of *in-vitro* incorporation ^{14}C-acetaldehyde in liver of control and Liv.52 treated rats are presented in Table 1. Liv.52 treated significantly reduced 17% at $p<0.005$) the accumulation of acetaldehyde in liver when compared with the respective controls. Similarly, Liv.52 treatment also reduced the hepatic accumulation of ethanol metabolite i.e. acetaldehyde produced as a result of *in-vivo* ethanol metabolism and this reduction was 21% ($p<0.05$) and 46% ($p<0.01$) at 2 hours and 4 hours respectively (Table 2). The circulating levels of radioactivity measured at 2 hours after ethanol administration in blood was also significantly reduced (35%, $p<0.05$) with Liv.52 treatment. Interestingly, no activity of ethanol metabolite (acetaldehyde) was noticed in blood of control and Liv.52 supplemented rats after 4 hours of *in-vivo* ethanol metabolism (Table 2).

Table 1: *In-vitro* incorporation of ^{14}C-acetaldehyde in liver of control and Liv.52 treated rats

Group	% Incorporation of acetaldehyde / g liver (Values are Mean ± SD)
G-Ia (Control)	0.502 ± 0.034 (6)
G-IIa (Liv.52 treated)	0.417 ± 0.083 (9)

The values in parentheses represent the number of animals $p<0.05$.

DISCUSSION

Ethanol is primarily metabolized in the liver where it gets converted into acetaldehyde as result of its oxidation and immediately binds to hepatic organelles such as mitochondria, endoplasmic reticulum and microtubules[9,15,16]. The activity present in the liver and blood 2 hours after oral administration of ^{14}C-ethanol would reflect the co-existence of ethanol and acetaldehyde due to the incomplete metabolism and clearance of ethanol within 2 hours[12]. In the present study, Liv.52 treatment significantly reduced the hepatic accumulation of ^{14}C-ethanol-acetaldehyde activity and at the same time facilitated its clearance as evidenced by low blood activity when compared with the control. Similarly, a significant reduction in the liver accumulation of acetaldehyde 4 hours after *in-vivo* ethanol metabolism was seen in Liv.52 treated rats. But no blood activity was observed at this time, which could be due to complete hepatic metabolism of ethanol. Near complete metabolism of ethanol to acetaldehyde in liver after 4 hours of its oral administration has already been reported by earlier workers[3,12]. Reduction in the residual activity of ethanol metabolites at 4 hours by Liv.52 treatment has been observed. Liv.52 induced inhibition of hepatic acetaldehyde accumulation during *in-vivo* ethanol metabolism in the present study was confirmed by direct *in-vitro* inhibition of ^{14}C-acetaldehyde incorporation in the liver slices. Previously, we have reported Liv.52 to protect liver from carbon tetrachloride toxicity[17-19].

Table 2: Metabolism of ^{14}C-ethanol in blood and liver of control and Liv.52 treated rats as a function of ^{14}C-acetaldehyde activity

Group	Percent activity/g liver	Percent activity/dL blood
G-Ib (6)	0.331 ± 0.056	0.014 ± 0.001
G-IIb (9)	0.260 ± 0.063	0.0094 ± 0.002*
G-Ic (6)	0.240 ± 0.044	Nil
G-IIc (9)	0.130 ± 0.0462	Nil

In groups G-1b, G-IIb animals were sacrificed at 2 h after ^{14}C-ethanol administration.
In groups G-1b, G-IIb animals were sacrificed at 4 h after ^{14}C-ethanol administration.
The value in parentheses represents the number of animals.
*p<0.05; p<0.001.

Thus, we conclude that Liv.52 protects liver from ethanol toxicity by reducing the accumulation of ethanol metabolite and by enhancing its clearance from the blood pool. Liv.52 had earlier been reported[12,13] to raise the blood acetaldehyde levels in the 1st hour after alcohol administration and thereafter stimulating its elimination. This quick clearance of acetaldehyde was attributed to stimulation of ADH (alcohol dehydrogenase) activity. Hasumura *et al*[20] demonstrated a reduction in the capacity of liver mitochondria to oxidize acetaldehyde following alcohol consumption in experimental rats. They assigned this reduction to altered redox state (NADH/NAD) ratio in the cytosol and mitochondria compartments which was further linked to disturbed hepatic structure and function. Thus, the hepatoprotective action of Liv.52 can be assigned to its capacity to enable the mitochondria to restore its redox state, reduce acetaldehyde accumulation in hepatic organelles and to stimulate the ADH activity at sub-cellular level.

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