Bioassay for Evaluation of the Hepatoprotective Effect of Liv.52, A Polyherbal Formulation, on Ethanol Metabolism in Chronic Alcohol-Exposed Rats

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SUMMARY
A study was conducted on Wistar male rats to assess the effect of chronic alcohol administration on blood ethanol and acetaldehyde levels and their modification by Liv.52, an Ayurvedic formulation. Following chronic ingestion of 6% alcohol (v/v) through a water feeding bottle for 42 days, the administration of an acute oral dose of 5 ml of 5% alcohol (v/v) resulted in a significant decrease (p<0.05) in blood ethanol and significant increase (p<0.05) in acetaldehyde levels respectively at 30 min. Larger doses (i.e., 5 ml of 10% and 15% respectively) failed to demonstrate an effect of accelerated ethanol metabolism because of saturation kinetics. In the second experiment, temporal observations on blood ethanol and acetaldehyde levels were done at 1, 3 and 4 h. Following chronic ingestion of 6% alcohol for 42 days, stimulation of Phase I and suppression of Phase II metabolism was reproduced at all the time points. As compared with placebo, treatment with Liv.52 in the last two weeks reversed the blood ethanol and acetaldehyde levels (p<0.05) at all the time points and they were comparable to the levels observed on day 1 (basal readings). In the third experiment, chronic exposure to 6% alcohol for 180 days showed further deterioration in ethanol metabolism as compared to 42 days exposure. In a chronic model of 180 days, Liv.52 normalised the blood ethanol and acetaldehyde levels in a dose-related manner. These observations are in agreement with our previous findings in chronic alcohol users. These bioassay findings could be effectively employed to evaluate the comparative efficacy of various polyherbal formulations on ethanol metabolism.

Key Words: Ethanol, acetaldehyde, Liv.52, animal model, bioassay.

Chronic ingestion of alcohol has been known to bring about lowering of blood ethanol levels in humans. Liv.52, a herbal formulation of several plant principles prepared according to Ayurvedic concepts, have shown to reverse this effect in chronic alcohol users and also causes rapid acetaldehyde elimination. Liv.52 is containing active principles of the following herbs: Capparis spinosa, Cichorium intybus, Solanum nigrum, Cassia occidentalis, Terminalia arjuna, Achillea millefolium, Tamarix gallica and Phyllanthus amarus. In order to study the above mentioned phenomenon in detail the following animals study was undertaken. The protective effect of Liv.52 in a chronic alcohol model in animals has been demonstrated.

MATERIAL AND METHODS
Male (Wistar strain) rats bred in our laboratories for over 40 generations, weighing 250-300 gm (3-4 months old) were used. They were housed under ideal laboratory conditions (temperature of 22 ± 2°C) with natural day and night rhythm, maintained on Hindustan Lever Chow and water was allowed ad libitum. The animals were divided into three groups for three different treatment schedules.
In the first experiment, 18 rats were divided into 3 groups consisting of 6 animals each. On day 1 of the experiment, each animal received 5 ml of ethyl alcohol orally after an overnight fast. The concentrations of alcohol for rats of Groups I, II and III were 5%, 10% and 15% (v/v) respectively. Blood was collected by heart puncture after light ether anaesthesia, 30 min after the respective dose of alcohol. The animals then received 6% alcohol (v/v) in place of normal drinking water ad libitum for 42 days. The alcohol intake was recorded daily. On day-42, oral alcohol was administered after overnight fasting (as on day 1) and blood was sampled for ethanol and acetaldehyde estimations.

In the second experiment, 36 animals were divided into 6 groups consisting of 6 animals each. On day 1 of the experiment all animals were given 5 ml of 5% alcohol orally after an overnight fast. Blood was collected at 1, 3 and 4 h from Groups I & IV, II & V and II & VI respectively. All animals were then given 6% alcohol solution in place of drinking water ad libitum for 42 days. From day 28 to day 42, animals in Groups I, II and III received oral dose of Liv.52 (0.5 gm/kg/day), while those in Groups IV, V and VI received placebo (0.5 gm/kg/day) respectively.

In the third experiment, 24 male rats were exposed to 6% alcohol ad libitum in drinking water for 180 days. On day 181 each animal received 5 ml of 5% ethanol orally after an overnight fast and blood was collected after 4 hours for quantification of ethanol and acetaldehyde. The rats were then divided into 3 groups of 8 each and received Liv.52 at doses of 0.5, 1.0 and 1.5 gm/kg respectively, once a day orally for 15 days. Along with Liv.52 treatment they also received 6% alcohol ad libitum. Their daily activity, food and ethanol intake were recorded. As before their blood ethanol and acetaldehyde levels were again assayed at the end of the period, i.e., on the 197th day, after overnight fasting.

Throughout the three experiments, blood samples were subjected immediately for estimation of ethanol and acetaldehyde using the head-space gas chromatography method.4

RESULTS
In all the experiments the average daily ethanol consumption was comparable with that in the control group and no significant difference was observed between the groups.

The data show that the amount of alcohol administered is critical for kinetic studies in animals. 5% alcohol solution at a dose of 5 ml (0.2 gm ethanol per animal) proved optimal for demonstrating the effects of chronic ingestion. Larger quantities failed to clearly demonstrate the effects of accelerated ethanol metabolism because of saturation kinetics.

With 5 ml of 5% ethanol there was a significant decrease in blood ethanol and increase in acetaldehyde concentrations at 30 min after chronic ingestion of ethanol (Figure 1). These effects, viz., stimulation of Phase I and suppression of Phase II metabolism, are known to occur after chronic alcohol consumption in human beings.5

In order to make temporal observations, blood was collected from the different groups at different time points. The data from the second experiment again showed lowered levels of ethanol and raised levels of acetaldehyde after chronic ethanol ingestion (Figure 2). As compared to placebo,
Liv.52 treatment for 15 days reversed the blood ethanol and acetaldehyde levels at all the time points and they were also comparable to the levels observed on day 1 (Figure 3).

![Figure 1](image1)

**Figure 1:** Mean blood ethanol and acetaldehyde levels at 30 min following administration of acute doses of 5 ml of 5%, 10% and 15% ethanol on day 1 and after chronic ingestion of 6% ethanol for six weeks (n=6). *p<0.05 compared to day 1

![Figure 2](image2)

**Figure 2:** Mean blood ethanol and acetaldehyde levels at 1, 3 and 4 h following administration of acute doses of 5 ml of 5% ethanol on day 1 and after chronic ingestion of 6% ethanol for six weeks (n=8). *p<0.05 compared to day 1

In the third experiment, following ingestion of 6% alcohol for 26 weeks, lowered blood ethanol and elevated acetaldehyde levels were highly significant as compared to ingestion of 6% ethanol for 6 weeks (Table 1), suggesting further deterioration in ethanol metabolism. Liv.52 treatment for 15 days (from day 181 to 196) in three different doses demonstrated proportional increases in blood ethanol and decreases in acetaldehyde levels respectively (Figure 4).

![Table 1](image3)

**Table 1:** Duration of the effect of 6% alcohol ingestion on blood ethanol and acetaldehyde levels at 4 h following oral administration of 5 ml of 5% alcohol

<table>
<thead>
<tr>
<th>Study day</th>
<th>Blood ethanol (mg%)</th>
<th>Blood acetaldehyde (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1 basal reading</td>
<td>17.12 ± 3.50</td>
<td>2.43 ± 0.30</td>
</tr>
<tr>
<td>6% alcohol ingestion for 6 weeks</td>
<td>10.37 ± 2.10</td>
<td>12.36 ± 1.32*</td>
</tr>
<tr>
<td>6% alcohol ingestion for 26 weeks</td>
<td>4.13 ± 1.29*</td>
<td>18.83 ± 2.39*</td>
</tr>
</tbody>
</table>

*p<0.05 compared to day 1, *p < 0.05 compared to 6 weeks.

**DISCUSSION**

It has long been believed that ethanol is more rapidly metabolised in chronic alcoholics than in naïve subjects. A number of controlled studies in man and animals has supported this impression, but others have suggested that alcoholism has no effect on ethanol metabolism. A number of mechanisms have been proposed to explain ethanol metabolism in moderate and regular alcohol users. A major problem in such studies, especially in man, is that it is difficult to control important variables such as the nutritional status of the subjects and to determine both the duration...
and quantity of alcohol consumed. Hence it is difficult to compare the results observed in different studies and evaluate the mechanism of ethanol metabolism.

In the present experiment the chronic effect of ethanol ingestion in rats was found to be reproducible in ethanol metabolism. This is an interesting observation and the findings are closer to the real physiologic situation seen in chronic alcohol users.

The probable mechanisms postulated for the effects seen in human being are: (1) inhibition of ethanol-metabolising enzymes in the gastrointestinal tract, and (2) prevention of binding of acetaldehyde to liver cell proteins. Further study of this hypothesis would be possible through proposed animal models as these agree with previous results observed in chronic alcohol users\(^2,3,11\). Thus bioassay could also play an important role as an analytical tool to monitor the comparative hepatoprotective efficacy of different herbal formulations on ethanol metabolism, in a dose-related manner.

REFERENCES


