

## Protective Effect of Liv.52 on Alcohol-Induced Fetotoxicity

Gopumadhavan, S., Jagadeesh, S., Chauhan, B.L. and Kulkarni, R.D.,  
R&D Center, The Himalaya Drug Company, Bangalore, India.

### ABSTRACT

*The adverse effects of maternal alcohol consumption on the development of the fetus are well known. The adverse effects of ethanol on the liver are now believed to be due to acetaldehyde formed as an intermediate metabolite of ethanol. Liv.52 has been shown to bring about faster elimination of acetaldehyde from the body and thus prevent alcoholic liver damage. Other toxic effects of alcohol may also be due to acetaldehyde and may be prevented by Liv.52. In this, study, rats were given 20% (v/v) ethanol in drinking water, during the gestation period, and the effect on maternal body weight and fetal outcome was noted. The protective effect of Liv.52 administration during the gestation period was studied. The results show that ethanol ingestion caused a decrease in gestational weight gain, total fetal weight, and number of live fetuses. There were increases in resorptions. Liv.52 administration reduced the deleterious effects of ethanol. The concentration of acetaldehyde in the amniotic fluid of ethanol-consuming animals was 0.727 µg/ml. Liv.52 administration lowered it to 0.244 µg/ml. The protective effect of Liv.52 could be due to the rapid elimination of acetaldehyde.*

**Key Words:** Fetal Alcohol Syndrome, Acetaldehyde, Liv.52.

The adverse effects of maternal alcohol consumption of fetal development are well documented<sup>1-3</sup>. Even moderate drinking is clearly contraindicated during pregnancy. The resulting abnormality on the fetus consists of decelerated growth and a number of major and minor malformations. Stillbirths and spontaneous abortions are much more frequent in alcohol-imbibing pregnant women. Increased accumulation of acetaldehyde, an intermediate metabolite of ethanol, is believed to be an important factor for the adverse effects of ethanol<sup>4,5</sup>. Evaluating the embryotoxicity of two ethanol metabolites, Priscott<sup>6</sup> reported that acetaldehyde in concentrations of 100 and 260 µM in the incubation medium had no deleterious effect on the gross morphology or viability of cultured 10-day old Albino Wistar rat embryos. However, at 800 µM concentration under similar conditions, it was overtly toxic causing rapid death. The other metabolite 2,3-butanediol, at a concentration of 25 µM, had no adverse effect<sup>6</sup>.

Liv.52 is a herbal formulation based on “AYURVEDA” and is known to protect the liver from damage induced by toxic substances, including alcohol, in experimental studies<sup>7</sup>. Liv.52 enhances the absorption of ethanol and rapidly reduces the acetaldehyde levels, which may explain its hepatoprotective effect on ethanol-induced liver damage<sup>8,9</sup>. Keeping these facts in mind, a study was undertaken to elucidate the protective effect, if any, of Liv.52 against the harmful effects of maternal alcohol consumption during the gestation period and also to determine the effect of Liv.52 on the acetaldehyde concentrations in the amniotic fluid of fetuses.

## MATERIALS AND METHODS

Albino rats of the Wistar strain (2.5-3.0 months old and weighing between 200-250 g) were used in the trial. These animals were given a standard diet (Hindustan Lever Pellets, Bangalore), clean tap water *ad libitum*, and kept in an air-conditioned room maintained at  $22 \pm 1^\circ\text{C}$  with 60% relative humidity. All experiments were conducted between August and September, during which day and night periods are equal, and the animals were exposed to this natural day and night cycle. Assigned oral administrations in all the groups were conducted daily, between 9:00 and 11:00 a.m.

The vaginal smear of each rat was examined daily for 12 days for the degree of cornification of epithelium to select animals showing a regular estrous cycle of 5-6 days. In the third cycle, those female rats in proestrus were separated and allowed to mate with males of proven fertility by mass-mating technique. Female and male animals (ratio 1:2) were caged together overnight. The next morning, a vaginal smear from each rat prepared and examine under a microscope for spermatozoa. The positive females were marked and placed in individual cages and the day was termed as Day 1 of pregnancy.

The 27 rats selected by the above method were divided into three groups. Group 1 (n=5) served as negative control and received 10 ml/kg water once a day orally for 20 days, from Day 1 of gestation. Group 2 (n=12) served as positive control and received 20% v/v alcohol in water feeding bottles for 20 days from Day 1 of gestation, and 10 ml/kg water orally once daily. Group 3 (n=10) rats received Liv.52 (3g/kg/day) orally for 15 days before and during gestation, in addition to 20% v/v alcohol in drinking water during this gestation period. The quantity of water consumed with Liv.52 was the same as that of Group 1 and Group 2.

General observations of the mother rats were made in regard to any change in daily food and water intake, alcohol consumption, gestational body weight, behavior, and presence of vaginal bleeding.

On Day 20, 3 hours after the assigned drug administration, the animals were anesthetized with ether, the abdomen was opened to expose the uterus, and the number and placement of implantation sites, early and late resorptions, and live and dead fetuses were noted. The ovaries were removed and examined for the number of corpora lutea. Amniotic fluid collected and pooled from fetuses in each dam was subjected to acetaldehyde analysis by the head space gas chromatography method<sup>10</sup>. Due to the limitations of head space gas chromatography and the need to analyze samples soon after collection, estimation of acetaldehyde was conducted from five dams in each group.

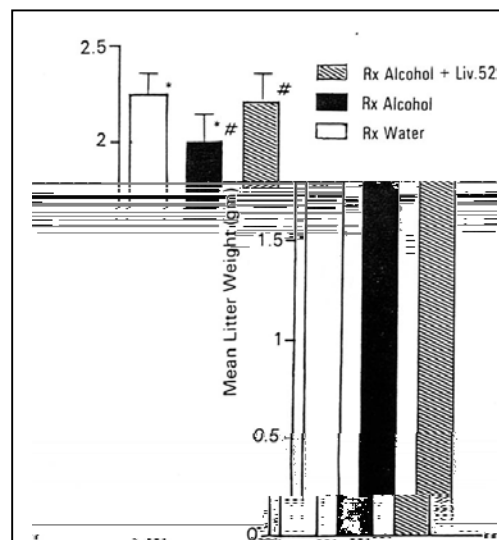
The weights of all fetuses were recorded, and each fetus was examined from gross external variations. Half of the fetuses from each litter were examined for soft tissue malformations by using Wilson's sectioning technique<sup>11</sup>. The remaining fetuses were examined for skeletal malformations by using the Alizarin Red stain technique<sup>12</sup>. Fertility and gestation indices were calculated by standard method<sup>13</sup>. All parameters were statistically determined by one-way analysis of variance with appropriate post-hoc analysis using SPSS/PC<sup>+</sup> on an IBM-compatible AT 286.

## RESULTS

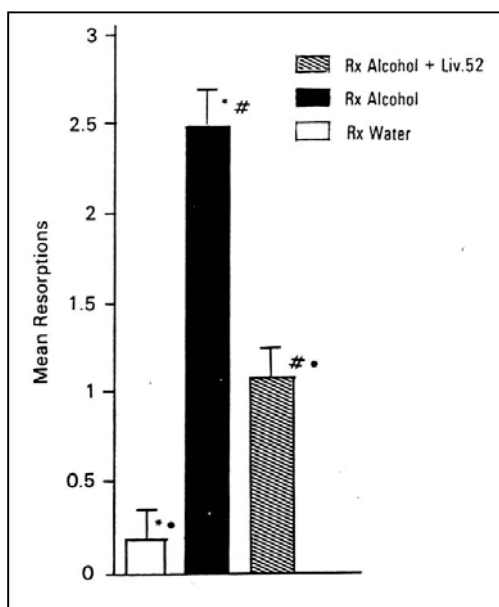
The mean alcohol consumed per rat was 12.53 g/kg body weight/day in Group 2 (treatment alcohol + Liv.52). Two animals in Group 2 (treated with only alcohol) and one animal in Group 3 (alcohol + Liv.52-treated) did not show signs of pregnancy and were excluded on Day 4, thus leaving 10 and 9 animals, respectively, for evaluation. Table 1 shows the mean alcohol consumption, maternal body weight gain, gestational food intake, and total number of live fetuses in each of the treatment groups. Twenty percent v/v alcohol ingestion in drinking water during the gestation period (Group 2) produced a significant reduction in the gestational weight gain and number of live fetuses as compared with the control group. Liv.52 treatment group showed no significant difference from the control group in these respects. The food intake was similar in all groups, and mean alcohol intake was not different between the alcohol alone and alcohol + Liv.52 groups. Figure 1 shows the mean litter weight of dams in the three groups. The mean litter weight was significantly lower in Group 2 treated with only alcohol as compared with the control group treated with tap water. The mean litter weight in the alcohol + Liv.52 group was not different from the control group, but was significantly higher than the alcohol alone group.

Figure 2 shows the mean of resorptions of dams in each group. The mean of resorptions in the female rats treated with alcohol alone was significantly higher as compared with the control group. In the Liv.52 + alcohol group the mean of resorptions was less than in the alcohol alone group, and the difference was statistically significant in spite of high variation in the alcohol alone group. The mean of resorptions in the Liv.52 + alcohol group was also significantly higher than in the control group.

The amniotic fluid of all fetuses from each dam was pooled for the estimation of acetaldehyde concentration. The mean results of five rats in each group treated with only alcohol and alcohol + Liv.52, respectively, are shown in Figure 3. The mean acetaldehyde concentrations in rats treated with only alcohol were significantly higher ( $0.727 \pm 0.046$ ) as compared with the alcohol + Liv.52-treated rats ( $0.244 \pm 0.027$ ).



**Figure 1:** Mean litter weight. Dams of treatment (Rx) alcohol group (n=10) had significantly lower mean litter weights as compared with Rx water group (n=5). \* $p < 0.050$  and Rx Alcohol + Liv.52 group (n=9). # $p < 0.015$ . No significant difference between Rx water and Rx alcohol + Liv.52 groups.



**Figure 2:** Mean resorptions. Dams of treatment (Rx) alcohol group (n=10) and Rx alcohol + Liv.52 group (n=9) had significantly higher resorptions as compared with Rx water group (n=5). \* $p < 0.006$  and • $p < 0.003$ , respectively. Mean resorptions in Rx alcohol + Liv.52 group were significantly lower as compared with the Rx alcohol group (# $p < 0.050$ ).

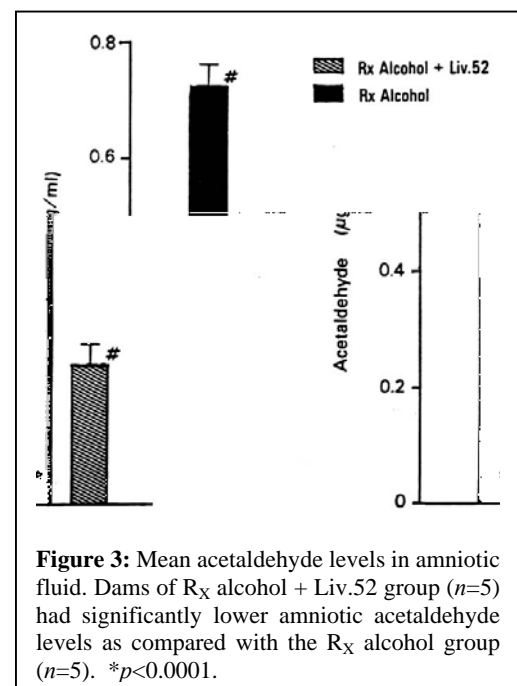
<b>Table 1: Maternal and fetal parameters following exposure to 20% v/v alcohol in water-feeding bottles during gestation period</b>				
Group dose	R <sub>x</sub> water (n=5) 10 ml/kg	R <sub>x</sub> alcohol (n=10) 20% v/v alcohol <i>ad libitum</i> ± 10 ml/kg water	R <sub>x</sub> alcohol + Liv.52 (n=9) 20% v/v alcohol <i>ad libitum</i> + Liv.52 3 g/kg with 10 ml/kg water as vehicle	ANOVA
Alcohol consumed (g/kg/day)	—	12.53 ± 0.42	11.82 ± 0.46	NS
Maternal body weight gain (g)	68.80 ± 8.69	47.30 ± 4.46	62.55 ± 4.06	<i>p</i> <0.038*
Gestational food intake (g/rat/day)	16.80 ± 0.81	14.70 ± 0.55	15.66 ± 0.38	NS
No. of live fetuses	10.40 ± 0.22	08.70 ± 0.82	10.44 ± 0.42	NS
Mean litter weight (g)	02.25 ± 0.08	02.02 ± 0.05	02.23 ± 0.05	<i>p</i> <0.030 <sup>•</sup>
Mean resorptions	00.20 ± 0.18	02.50 ± 0.70	01.11 ± 0.11	<i>p</i> <0.030 <sup>○</sup>
Acetaldehyde level in amniotic fluid (µg/ml)	—	0.727 ± 0.046	0.244 ± 0.027	<i>p</i> <0.0001 <sup>■</sup>

Values are mean ± SE. R<sub>x</sub>, treatment; ANOVA, one-way analysis of variance; NS, not significant.  
\* R<sub>x</sub> water vs. R<sub>x</sub> alcohol (*p*<0.050); R<sub>x</sub> water vs. R<sub>x</sub> alcohol + Liv.52 (NS); R<sub>x</sub> alcohol vs. R<sub>x</sub> alcohol + Liv.52 (*p*<0.015).  
<sup>•</sup> R<sub>x</sub> water vs. R<sub>x</sub> alcohol (*p*<0.022); R<sub>x</sub> water vs. R<sub>x</sub> alcohol + Liv.52 (NS); R<sub>x</sub> alcohol vs. R<sub>x</sub> alcohol + Liv.52 (*p*<0.013).  
<sup>○</sup> R<sub>x</sub> water vs. R<sub>x</sub> alcohol (*p*<0.006); R<sub>x</sub> water vs. R<sub>x</sub> alcohol + Liv.52 (*p*<0.003); R<sub>x</sub> alcohol vs. R<sub>x</sub> alcohol + Liv.52 (*p*<0.050).  
<sup>■</sup> R<sub>x</sub> water vs. R<sub>x</sub> alcohol + Liv.52 (*p*<0.0001).

## DISCUSSION

It is known from previous studies that embryos exposed to acetaldehyde sustain fetal damage<sup>5</sup>. The mechanism of this deleterious effect of acetaldehyde is not certain, but acetaldehyde has been shown in other cell systems to form adducts with amino acids<sup>14</sup> and to impair mitochondrial and other cellular transport functions<sup>15</sup>. More specifically, Priscott and Ford<sup>16</sup> have studied the effects of acetaldehyde *in vitro* by incubating fetuses with and without placenta in a medium containing acetaldehyde. They demonstrated that the placenta can remove significant amounts of acetaldehyde from the medium and propose that placenta may be acting as an effective barrier to protect the fetus from the deleterious effects of acetaldehyde in maternal blood<sup>16</sup>. But as mentioned previously, Priscott has demonstrated direct toxic effects

of acetaldehyde on fetuses in the *in vitro* system when acetaldehyde concentration in the amniotic fluid was 0.727 µg/ml (16.77 µM), which is far below the toxic concentration demonstrated by Priscott. The discrepancy may be due to the fact that Priscott incubated the embryos in the medium containing acetaldehyde for only 48 hr from Day 10 to Day 12, whereas in our experiments the dams were fed ethanol from Day 1 of pregnancy through Day 20 and the period of greatest sensitivity to acetaldehyde may occur at a time in development outside the range of Priscott's exposure period. Acetaldehyde reaching the fetus through the blood supply may be more toxic and the placental barrier function in early pregnancy may not be adequate; also one does not know the acetaldehyde concentration inside the fetus in Priscott's experiments. Further, blood acetaldehyde concentration in the mother may be higher than the amniotic fluid concentration. Unfortunately we did not measure maternal blood acetaldehyde concentrations.



**Figure 3:** Mean acetaldehyde levels in amniotic fluid. Dams of R<sub>x</sub> alcohol + Liv.52 group (n=5) had significantly lower amniotic acetaldehyde levels as compared with the R<sub>x</sub> alcohol group (n=5). \**p*<0.0001.

In this study we observed significant deleterious effects on the fetus due to alcohol ingestion by the mother during gestation. Treatment with Liv.52 simultaneously prevented these deleterious effects on maternal gestational weight gain, the total number of live fetuses and mean litter weight (Fig.1). The mean resorptions in the Liv.52-treated group, although less than in the alcohol alone group, were nevertheless significantly more than in the control group (Fig. 2). It is also seen that the mean amniotic acetaldehyde level in the Liv.52-treated group was significantly lower than in the alcohol alone group (Fig. 3). It is, therefore, likely that the rapid acetaldehyde elimination action of Liv.52 may have caused reduction in maternal blood acetaldehyde levels<sup>8,17</sup>. This could have resulted in lesser exposure of fetuses to acetaldehyde and been responsible for the beneficial effects of Liv.52 administration. Only one dose of Liv.52 was used in this study. The possibility of Liv.52 preventing the binding of acetaldehyde to fetal tissue cannot be excluded. More definitive studies will have to be undertaken regarding acetaldehyde binding to fetal tissue and dose response of Liv.52 in preventing deleterious effects of acetaldehyde, as also simultaneous study of the effect of Liv.52 on maternal ethanol and acetaldehyde metabolism.

## REFERENCES

1. Tze WJ, Lee M: Adverse effects of maternal alcohol consumption on pregnancy and fetal growth in rats. *Nature* 257: 479-480, 1975.
2. Daniels M, Evans MA: Effect of maternal alcohol consumption on fetal and newborn development. *Fed Proc* 39: 846, 1980 (abstr).
3. George I, Henderson, et al: The effects of chronic and acute alcohol administration on fetal development in the rat. *Alcohol Clin Exp Res* 3: 99-106, 1979.
4. Sreenathan RN, Padmanaban R, Singh S: Teratogenic effects of acetaldehyde in rats. *Drug Alcohol Depend* 9:339-350, 1982.
5. Campbell MA, Fantel AG: Teratogenicity of acetaldehyde *in vitro*: relevance of fetal alcohol syndrome. *Life Sci* 32: 2641-2647, 1983.
6. Priscott PK: The effects of acetaldehyde and 2,3-butanediol on rat embryos developing *in vitro*. *Biochem Pharmacol* 34: 529-532, 1985.
7. Karandikar SM, Joglekar GV, Chitale GK, et al: Protection by indigenous drugs against hepatotoxic effects on carbon tetrachloride – A long-term study. *Acta Pharmacol Toxicol* 20:274, 1963 (abstr)
8. Chauhan BL, Kulkarni RD: Effect of Liv.52, a herbal preparation, on absorption and metabolism of ethanol in humans. *Eur J Clin Pharmacol* 40: 189-191, 1991.
9. Chauhan BL, Kulkarni RD: Alcohol hangover and Liv.52. *Eur J Clin Pharmacol* 40:187-188, 1991.
10. Mendenhall CL, McGee J, Green ES: Simple, rapid and sensitive method for the simultaneous quantitation of ethanol and acetaldehyde in biological materials using head space gas chromatography. *J Chromatogr* 190: 197, 1980 (abstr).

11. Wilson JG, et al: Method of examining rat fetuses for malformations, in Wilson JG, and Wartany J (eds): *Teratology: Principles and Techniques*. Chicago, University of Chicago Press, 1965.
12. Chaube S, et al: Procedure for staining rat skeleton with Alizarin red, in Wilson JG and Wartany J (eds): *Teratology: Principles and Techniques*. Chicago, University of Chicago Press, 1965.
13. Manson JM, Zenick H, Costlow RD: Teratology test methods for laboratory animals, in Wallace HA (ed): *Principles and Methods of Toxicology*. New York, Raven Press 1982, pp 141-184.
14. Israel Y, Hurwitz E, Niemela O, et al: Monoclonal and polyclonal antibodies against acetaldehyde-containing epitopes in acetaldehyde-protein adducts. *Proc Natl Acad Sci USA* 83:7923-7927, 1986.
15. Lieber CS, et al: Acetaldehyde and acetate, in Smith LH (ed): *Medical Disorders of Alcoholism: Pathogenesis and Treatment. Major Problems in Internal Medicine, Vol. 22*. Philadelphia, WB Saunders, 1982, pp 43-64.
16. Priscott PK, Ford JR: An *in vitro* model of acetaldehyde metabolism by rodent conceptuses. *In Vitro Cell Develop Biol* 21:88-92, 1985.
17. Chauhan BL, Kulkarni RD: Dose response of ethanol after chronic administration of alcohol and effect of Liv.52 on blood, liver ethanol and acetaldehyde levels in rats. Presented at the XIth International Congress of Pharmacology, Amsterdam. The Netherlands. *Eur J Pharmacol* 236: 1864, 1990.