

Postnatal development of alcohol dehydrogenase in liver & intestine of rats exposed to ethanol *in utero*

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Background & objectives: Ethanol exposure during gestation induces marked aberrations in growth and development of offsprings collectively known as foetal alcohol syndrome (FAS). However, its effects on the postnatal development of alcohol dehydrogenase (ADH) are not adequately investigated. Therefore, ADH activity in liver and intestine of rats exposed to ethanol during gestation was studied in relation to postnatal development.

Methods: Pregnant female rats beginning at day 1 of gestation were fed 1 ml of 30 per cent ethanol daily during the entire gestation period. ADH activity was determined in liver and intestine postnatally at day 4, 8, 14, 20 and 30. DNA and RNA contents and intestinal histology were also examined.

Results: During the first two weeks of postnatal life, there was no difference in ADH levels of rat liver and intestine in control and prenatally ethanol exposed pups but ADH levels were significantly reduced at 3-4 wk in ethanol fed group compared to control. A similar decrease in DNA and RNA contents of intestine and changes in tissue morphology were observed in ethanol exposed pups during postnatal development.

Interpretation & conclusion: The findings of our study suggested that prenatal ethanol exposure modified ADH activity in liver and intestine during postnatal development. This could affect ethanol metabolism under these conditions.

Key words Postnatal development - prenatal ethanol ingestion - rat liver and intestine alcohol dehydrogenase

Chronic ethanol ingestion during gestation is responsible for foetal alcohol syndrome (FAS), which is associated with general growth retardation and multiple birth defects in offsprings^{1,2}. Major consequences of FAS are facial deformities, central nervous system dysfunctions and non specific malfunctions^{3,4}. Earlier studies in experimental animals have demonstrated that prenatal ethanol exposure also retards intestinal growth⁵, modifies the

development of intestinal brush border enzymes⁶ and impairs the absorption of glucose, amino acids and macromolecules^{7,8} during postnatal development in rats.

Traves *et al*⁹ described that ethanol metabolism in developing foetus is negligible, thus intragastric ethanol levels are several times higher than in other foetal compartments. Such high levels of ethanol could

induce structural and functional deformities during the period of intense cell proliferation and differentiation. Ethanol is primarily metabolized to acetaldehyde by the action of alcohol dehydrogenase (ADH). In adults, ADH activity is induced by ethanol ingestion¹⁰. However, the effects of prenatal ethanol exposure on the development of ADH activity are not adequately studied. In the present study, postnatal development of ADH activity was investigated in rat liver and intestine exposed to ethanol during gestation.

Material & Methods

Wistar strain albino rats (100-120 g body weight) were used. Animals were maintained on commercial rat pellet diet (Hindustan Lever, India) *ad libitum* with free access to water. The female animals were kept for mating and the first day of gestation (day 1) was checked by examining the vaginal smears under microscope as described by Baker¹¹. Beginning from day one of pregnancy, 1 ml of 30 per cent ethanol was administered by Ryle's tube daily to pregnant rats, throughout the gestation period. Isocaloric amounts of glucose in 1 ml saline were given to the control animals. Upon delivery, the neonates were kept with their natural mothers and were sacrificed at different days of postnatal age. Usually 4-6 female rats were used in each group. Pups from each of the experimental group were sacrificed between 0900-1000 by decapitation at postnatal day 4, 8, 14, 20 and 30. Intestinal tissue starting from the ligament of Treitz to caecum was removed and washed thoroughly with ice cold normal saline. Intestinal lengths and weights were recorded. Liver was removed and washed with ice cold saline. 10 per cent tissue homogenates (w/v) were made in 50 mM sodium-maleate buffer (pH 6.8), centrifuged at 2000 g for 10 min at 4°C, the supernatant was used for biochemical analyses.

The experimental protocol was approved by the Institute's Ethical Committee. Experiments on animals were conducted in accordance with the Guidelines for use of Laboratory Animals in Medical College, Indian Council of Medical Research, New Delhi¹².

Biochemical determinations: Intestinal DNA and RNA contents were determined following the standard methods^{13,14}. ADH activity was assayed by

the method of Bonnichsen and Brink¹⁵. Protein was determined by the method of Lowry *et al*¹⁶ using bovine serum albumin as the standard.

Statistical analysis of the data was done by unpaired student t-test.

Histology of the liver and intestine was studied under light microscopy. Tissues were fixed in molten paraffin wax. 5 µm thick sections were cut with a fine razor attached to a speneer microtome (UK). The sections were stained with haematoxylin and eosin (H & E) and observed under light microscope¹⁷.

Results

There was no difference in food intake and body weight of pregnant female rats in control and ethanol exposed groups. The gestation period was lengthened by a day, from 21.5 ± 0.7 in controls to 22.5 ± 0.9 days in ethanol-exposed group. The litter size was reduced from 11.8 ± 0.9 in control to 9.6 ± 0.5 pups in ethanol-exposed group. At day 4 after birth, pups exhibited low body weight in ethanol group compared to controls (5.8 ± 0.22 g vs 8.42 ± 0.37 g). The observed decline in body weight of ethanol-exposed pups persisted after 4 wk of birth. At day 30, the body

Table. Effects of prenatal ethanol exposure on certain parameters of developing rat intestine

| Parameter | Control group | Ethanol exposed group |
|------------------------|---------------|-----------------------|
| Intestinal wt (g) | | |
| Day 8 | 0.62 ± 0.01 | 0.58 ± 0.01* |
| Day 30 | 3.40 ± 0.31 | 3.02 ± 0.17* |
| Intestinal length (cm) | | |
| Day 8 | 33.1 ± 0.87 | 28.56 ± 0.97* |
| Day 30 | 80.7 ± 0.68 | 78.98 ± 0.42* |
| DNA (µg/mg protein) | | |
| Day 8 | 29.4 ± 1.9 | 20.7 ± 2.5** |
| Day 30 | 38.0 ± 1.6 | 35.3 ± 1.3* |
| RNA (µg/mg protein) | | |
| Day 8 | 74.8 ± 2.7 | 67.9 ± 1.9* |
| Day 30 | 82.1 ± 1.5 | 75.7 ± 2.9* |

Values are mean ± SD, *P<0.05, **<0.01 compared to control (n=6-8), from 4-6 litters

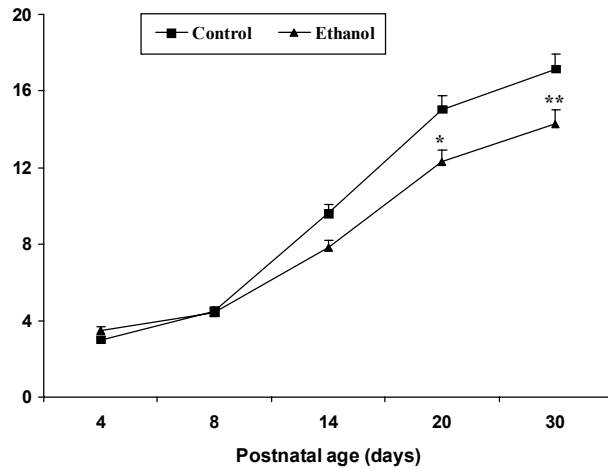


Fig.1. Alcohol dehydrogenase (ADH) activity in rat liver during postnatal development. Values are mean \pm SD of 6 observations from 4 litters. ** $P < 0.01$, * $P < 0.05$ compared to control.

weight of control animals was 68.0 ± 1.9 g compared to 63.1 ± 0.1 g in prenatally ethanol-exposed pups. The intestinal weight and intestinal length were also low in ethanol group compared to controls (Table). The weight to length ratio of intestine and intestinal weight to body weight ratios were not affected in pups during postnatal development in control and experimental animals (data not shown).

Analysis of DNA and RNA contents showed a decrease ($P < 0.05$) at day 8 and 30 of postnatal development in ethanol-exposed pups compared to controls (Table). As shown in Fig. 1, ADH activity in liver increased progressively with age. The enzyme levels were enhanced 4 folds in 4 wk old animals compared to that at day 4 after birth. There was essentially no difference in liver ADH activity in control and ethanol-exposed 4-14 day old pups. However, at day 20 and 30, ADH activity was significantly reduced in pups born to mothers fed ethanol during gestation compared to controls.

Analysis of ADH activity in intestine during postnatal development revealed a progressive increase in enzyme activity with age in control animals (Fig. 2). However, the enzyme levels were essentially unchanged during postnatal development in ethanol-exposed animals *in utero*. There was no difference in

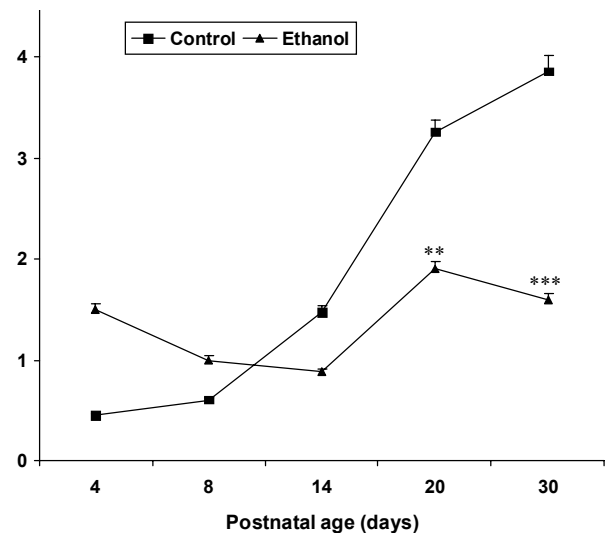


Fig.2. Alcohol dehydrogenase (ADH) activity in rat intestine during postnatal development. Values are mean \pm SD of 6 observations from 4 litters. *** $P < 0.001$, ** $P < 0.01$ compared to control.

ADH activity in first two weeks of postnatal age in control and ethanol groups but it was significantly low at day 20 ($P < 0.01$) and at day 30 ($P < 0.001$) of postnatal development in rats exposed to ethanol prenatally, compared to controls.

The light microscopy of intestinal tissue in 30 day old pups showed that goblet cells were not well formed, luminal part of the villi was damaged with a portion shredded off in the lumen with necrosis. Villi were denuded and showed atrophy (Fig. 3). Similar changes in liver morphology revealed hypertrophy of the cells with few cytoplasmic granules. Hepatocytes showed vacuolation along with few pycnotic nuclei and confluence of cell was observed in ethanol exposed group (Fig. 4).

Discussion

The model of chronic ethanol consumption employed in this study was comparable to the long-term ethanol ingestion among alcoholics. Female rats were exposed to ethanol in the dose of 2 g/kg body weight, which is apparently equivalent to 200 ml of whisky consumed by a 70 kg person¹⁸. Control animals received isocaloric amounts of glucose to minimize the calorie imbalance, which may arise due to high energy content of ethanol. There was no

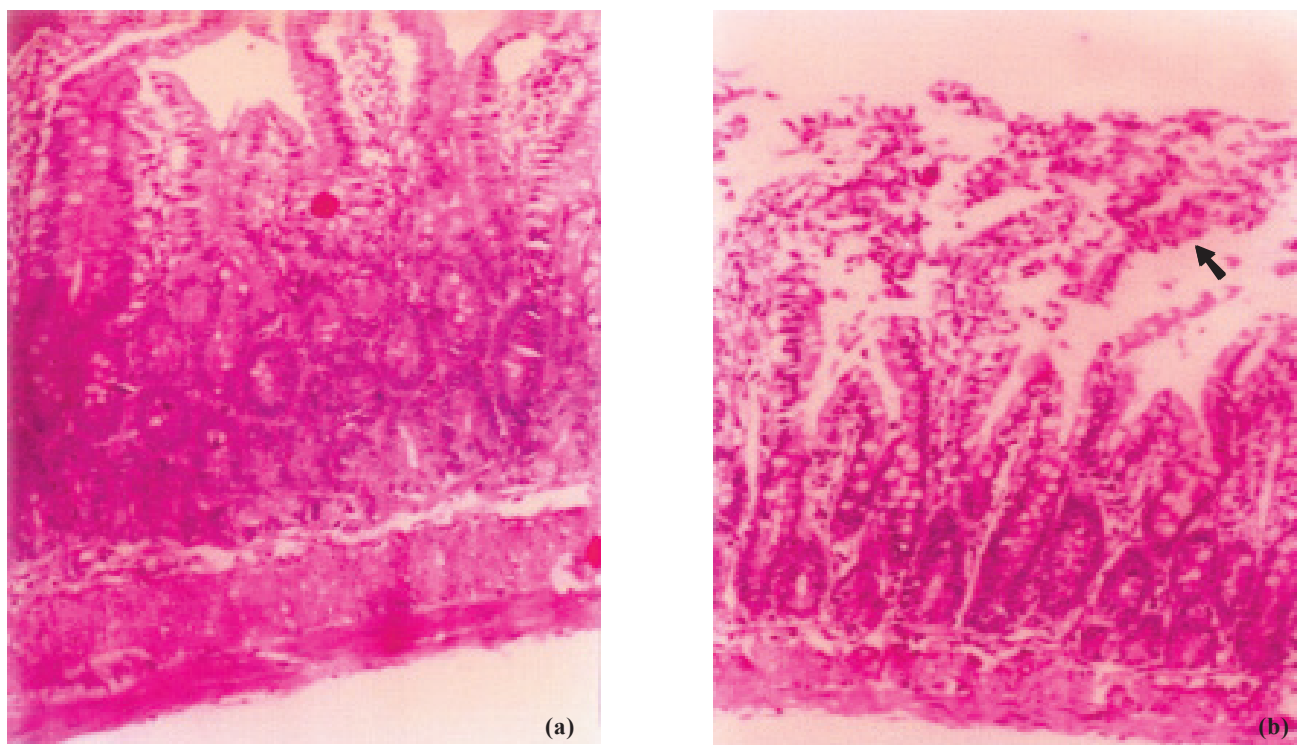


Fig. 3. Histology of intestinal tissue (x150) from 30 days old (a) Control and (b) prenatally ethanol exposed rats. Arrows in test group (b), indicate the damaged villi and necrosis in the epithelial layer. Villi tips are denuded with atrophy.

difference in gestation periods of control and ethanol-exposed mothers. These findings were similar to those reported by Abel¹⁹ who observed no delay in the gestation period when pregnant female rats were administered ethanol in the dose of 1-2 g/kg body weight. Feeding ethanol during gestation induced a marked decrease in body weight of pups during postnatal development compared to controls. These observations were in agreement to earlier reports⁴. Recently Klug *et al*²⁰ have reported low body mass of children born to mothers consuming alcohol during pregnancy.

The present findings indicated that ADH levels were low in newborn pups both in liver and intestine, which might explain high concentration of ethanol circulating in the foetal tissue and its poor metabolic degradation. Enzyme activity expressed in terms of per mg protein showed several fold more activity in liver than in intestine during postnatal development. This also suggests that liver is the primary site of ethanol metabolism. The contribution of intestine to this process is quite meager. ADH levels were not

different in control and ethanol fed animals both in liver and intestine during the first two weeks of life. However, the enzyme levels were markedly stimulated in the adult liver and intestine but remained quite low in ethanol fed group compared to controls. A decrease in DNA content of mucosa in ethanol exposed pups during postnatal development indicates reduced number of enterocytes in intestine. These findings were similar to those of Camps *et al*⁶ who described reduced number of epithelial cells in pups exposed to ethanol during gestation. The observed decrease in intestinal cell mass may explain low ADH activity observed in intestine under these conditions.

The effect of maternal ethanol consumption during gestation on class 1 ADH activity in developing rats suggests that the primary effect of ethanol is on an important regulatory mechanism of the enzyme. ADH class 1 is under hormonal control, performed to a significant degree by the hypothalamus-hypophysis axis^{21,22}. The impaired function of this endocrine system is known after prenatal ethanol exposure^{23,24}. The observed decrease in enzyme activity may result

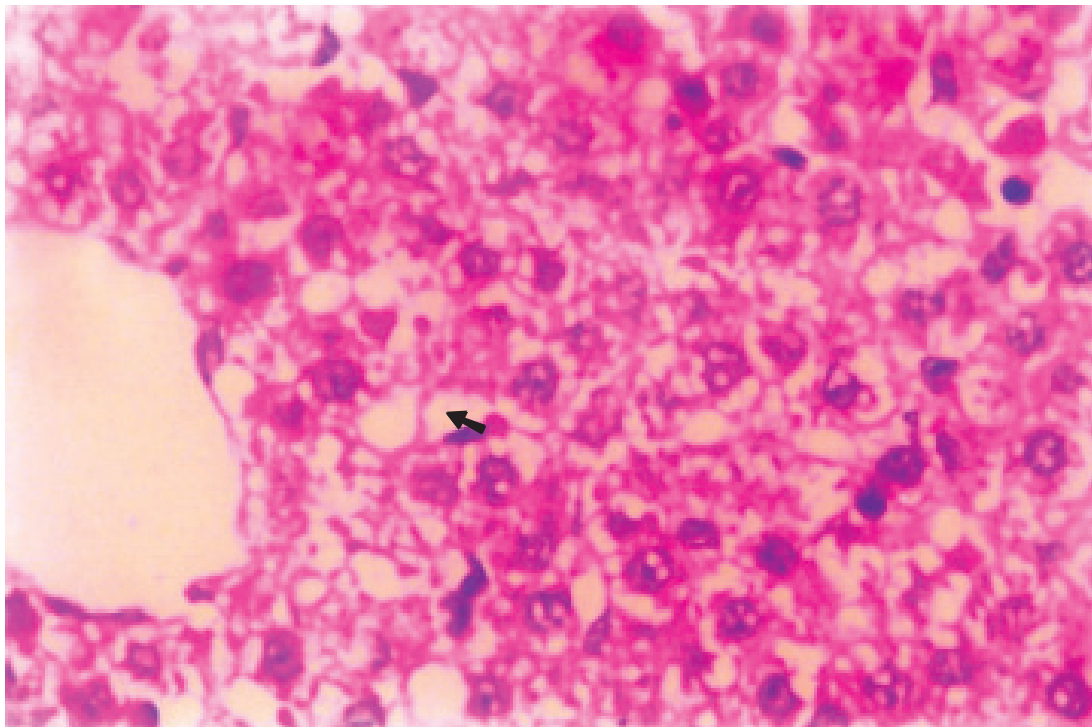
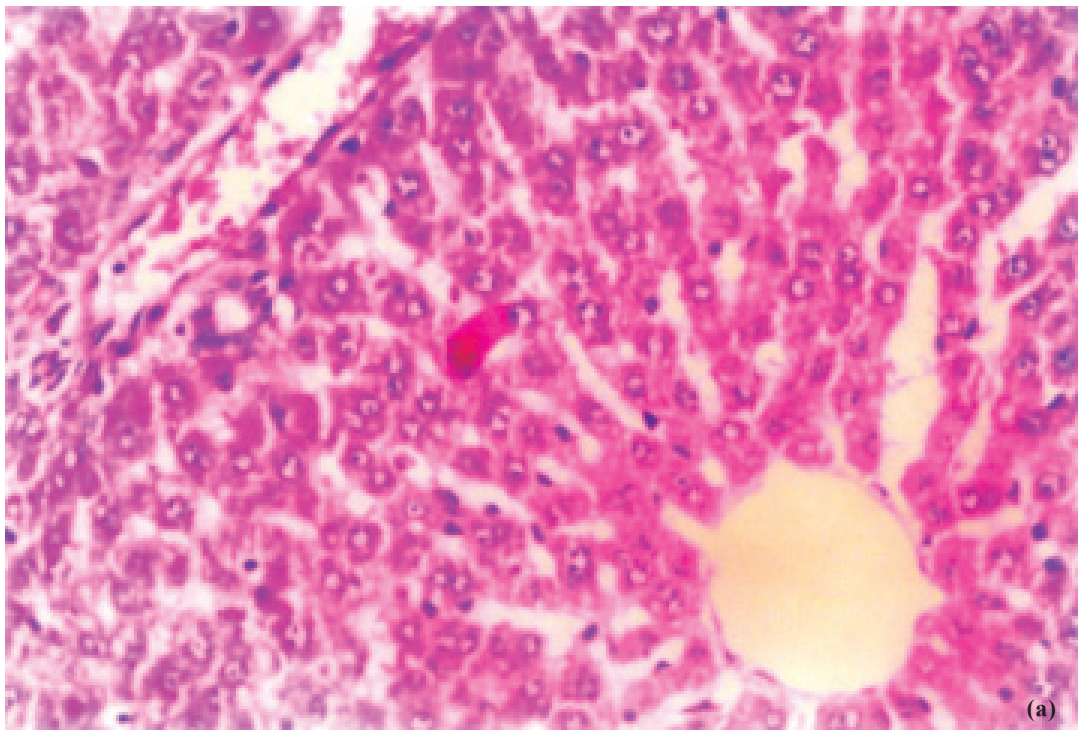


Fig.4. Histology of the liver tissue (x250) in (a) Control and (b) prenatally ethanol exposed 30 days old rats. Arrows in the test group (b), indicate increased vacuolation of cells, pycnotic nuclei and cell necrosis. Hepatocytes show hypertrophy with few cytoplasmic granules.

in low capacity of the tissues to metabolize ethanol in the offsprings of alcoholic rats with a concomitant rise in their blood alcohol levels. It is known that ethanol metabolism in the foetus is negligible⁹ and ADH isoenzymes that metabolizes ethanol have not been found in the placenta²⁵. Therefore, the establishment of ethanol effects in the foetus depends on the concentration of circulating ethanol in each tissue, which will further rely on the maternal metabolic systems to eliminate ethanol.

Histological data support the contention that prenatal ethanol exposure induces morphological disruptions in intestine. This further corroborates the findings on ADH levels in adult intestine. It also reflects that response of foetal tissue to ethanol is distinct from that in adult animals, as ethanol feeding to adult animals has been shown to induce ADH activity¹⁰. Traves *et al*⁹ did not report any change in liver ADH activity in pups exposed to ethanol *in utero*. The observed differences with the present findings could be attributed to a variations in duration of alcohol exposure, mode of ethanol exposure (in drinking water vs feeding by stomach tube), and different assay methods employed to determine the enzyme activity.

Hepatocytes from 30 day old experimental animals reveal hypertrophy of the cells with intercellular wall damage, confluence of cells and vacuolation along with pycnotic nuclei compared to controls. Thus, it can be deduced that the effects of *in utero* ethanol exposure persists for a longer period after the alcohol was withdrawn at birth. Although the underlying mechanism of these observations is unknown, but these findings are in agreement to those of Guerri and Grisolia²⁶ who observed a marked accumulation of fat in the hepatocytes of prenatally ethanol exposed rats. Nanji *et al*²⁷ reported an increase in tumour necrosis factor α and mRNA in rat liver after ethanol treatment, which might play a role in the pathogenesis of alcohol induced liver injury.

In conclusion, these findings suggested that ethanol exposure *in utero* induced changes in developing embryo, which led to reduced ADH activity in intestine, later in postnatal life. Retarded growth of tissues as a consequence of reduced DNA

content suggested decrease in number of epithelial cells, which might explain the observed decrease in alcohol dehydrogenase levels in 3-4 wk old rat tissues under these conditions.

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