PRENATAL EXPOSURE OF AN ALCOHOLIC BEVERAGE (ARRACK) ON FETAL LIPID METABOLISM IN RATS

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Abstract: The objective of this study was to determine the effects of a country liquor (Arrack) and the equivalent quantity of ethanol on liver function and lipid metabolism in utero. Female rats of average weight 125 g were exposed to Arrack (12 ml/kg body weight/day) and ethanol (3.2 ml/kg body weight/day) for 15 days before conception and throughout gestation. On 13th day and 19th day of gestation, altered liver function and hyperlipidemia was seen in the fetus of both the treated groups. Altered liver function was evidenced by the increased activity of alcohol dehydrogenase and glutamic pyruvic transaminase or alanine amino transferase (GPT). Hyperlipidemia was caused by increased biosynthesis since the incorporation of ^14C acetate to lipids and activities of HMG CoA reductase and lipogenic enzymes were elevated. Arrack seemed to potentiate the toxicity induced by alcohol indicating the role of non ethanolic portion. Hepatic functions of the 13th day fetuses were effected to a lesser degree than the 19th day hepatic liver.

Key words: liver function ethanol lipid metabolism FAS pregnancy arrack

INTRODUCTION

It had been established that consumption of alcohol and behavioural effects on the fetus and offspring termed as Fetal Alcohol Syndrome (FAS) (1, 2). Alcoholism among women has increased during the last decades. The excess intake of alcohol for a long time causes fatty liver (3) and accumulation of fat in brain (4) heart and kidneys (5). Almost all research work done in alcohol metabolism was carried out with pure ethyl alcohol. But in reality it is alcoholic beverages that are being consumed. Alcoholic beverages contain many pharmacologically active substances other than ethanol. There are reports that progenies of pregnant rats fed 10% country liquor have higher mortality rates (6). Vinson and Hontz (1995) reported that red wines had significantly higher antioxidant index than white wines due to its higher phenolic content (7).
A popular country liquor consumed by the low social-economic group in South India is Arrack. Arrack is a distilled alcoholic beverages. But no data is available in India about the consumption of the Arrack during pregnancy because of the taboo prevailing in the society about liquor consumption. Recently Alcohol and Drugs Information Centre (ADIC-INDIA) came out with a report that there is an emerging group of female social drinkers in Kerala. In the American society the prevalence of FAS is more common in the low socio economic group than in the higher economic group. Hence we have designed this study to generate data about the ill effects of consumption of Arrack during pregnancy. Our previous studies have shown that maternal consumption of alcohol beverages reduced the body weight of both dams and fetus, altered carbohydrate metabolism (9-11) and postnatal lipid metabolism of the offspring (12). So the aim of the present study was to examine the effects of in utero exposure of Arrack and its equivalent quantity of ethanol on liver function and lipid metabolism in the 13th day and 19th day fetus of rats.

METHODS

Female albino rats (Sprague Dawley strain) of average weight of 125 g were used. They were maintained in laboratory conditions with light and dark cycles of 12 h duration. Rats were fed with pelleted diet (Supplied by Lipton India Ltd.). Animals were grouped into 3 of 12 rats each.

Arrack was purchased from government licensed shop and its ethanol content was estimated by the method given in AOAC (13). The ethanolic content of our sample was 42%. The consumption of absolute ethanol in Arrack group and ethanol group was same. We have arrived at this dosage taking into consideration that a habitual drinker on an average consumes 650 ml of Arrack. Arrack/ethanol was administered by gastric intubation. Arrack was diluted in the ratio 1:1.25 and ethanol 1:7. Fluid intake was adjusted to be same in all the case. Rats were treated as shown above for 15 days, after which they were allowed to mate with normal male (Sprague Dawley strain) rats. Pregnancy was detected by microscopical examination of vaginal smear, and the day of detection of the spermatozoa was considered as the first day of gestation. On 13th day of gestation, half of the rats were starved overnight and sacrificed. The fetuses were cut opened and liver was collected. The rest of the animals were sacrificed on 19th day of gestation. Weights of the fetuses were also recorded.

Tissues were stored in precooled containers (4°C) for biochemical analysis. The tissues were homogenized and extracted with chloroform : methanol (2:1). The residue washed with
chloroform: methanol at least 3 times. The filtrates were combined. To the filtrate, 0.7% KCl (20% of the total volume of the extract) was added and mixed. The aqueous upper phase was removed with a pasteur pipette and the lower layer was washed 3 times with 5 ml of chloroform: methanol: KCl (2:48:47) solution. The washed lower layer of chloroform was evaporated to dryness and the residue was redissolved in a known volume of chloroform. Aliquots of the extracts were used for the estimation of various lipids. Cholesterol, Triglycerides, Phospholipids and free fatty acids of liver was estimated by the methods previously reported by Menon and Kurup (14). Activity of glutamic pyruvic transaminase or alanine amino transferase (GPT) (E.C.2.6.1.2) was estimated by the method of Bergmeyer and Bernt (15). Activity of alcohol dehydrogenase (E.C.1.1.1.1) was estimated by the method of Zorzano and Herrera (16). Activity of HMG CoA reductase (EC1.1.1.34) was estimated by the method of Rao and Ramakrishnan (17).

Activity of malic enzyme (EC 1.1.1.40) was estimated by the method of Ochoa (18), and glucose-6-Phosphate dehydrogenase (EC 1.1.1.49) activity by the method of Kornberg and Horecker (19). Protein in the enzyme extract was determined after the TCA precipitation by the method of Lowry et al (20). 14C acetate incorporation was carried out as described by Molly Thomas et al (21). Statistical analysis was performed using one-way analysis of variance (ANOVA). Differences between treatment means were determined by the methods of Snedecor and Cochran (22).

RESULTS

Alcohol dehydrogenase activity was not detected on 13th day fetus. On 19th day the Arrack/ethanol caused increased activity, but Arrack group showed higher activity than ethanol group (Table 1).

TABLE I: Effect of ingestion of ethanol/Arrack by dams on hepatic alcohol dehydrogenase and glutamic pyruvic transaminase (GPT) activities of day 19 old fetus.

<table>
<thead>
<tr>
<th></th>
<th>Alcohol dehydrogenase (NAD+) formed/ min/g protein</th>
<th>GPT (μ moles of pyruvate lib./ min./mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12.38±0.39</td>
<td>40.33±2.94</td>
</tr>
<tr>
<td>Ethanol</td>
<td>15.09±0.86a</td>
<td>55.66±2.56a</td>
</tr>
<tr>
<td>Arrack</td>
<td>18.8±0.74ab</td>
<td>61.68±3.35ab</td>
</tr>
<tr>
<td>CD*</td>
<td>0.85</td>
<td>3.65</td>
</tr>
</tbody>
</table>

Average of values of 6 rats in each group ± SD.

* Critical difference [5%] to compare treatment means.

a-indicates that the corresponding result is significantly different from the control.

b-indicates that the effects of ethanol and Arrack are significantly different.

NS-not significant.

Administration of Arrack/ethanol enhanced the levels of hepatic GPT in 19th day fetus. Toxicity induced by Arrack was significantly higher than ethanol (Table I).
TABLE II: Lipid profile of the fetal liver of animals prenatally exposed to ethanol/Arrack (mg/100 g tissue).

<table>
<thead>
<tr>
<th></th>
<th>Cholesterol</th>
<th>Triglycerides</th>
<th>Phospho lipids</th>
<th>Free fatty acids</th>
<th>Cholesterol</th>
<th>Triglycerides</th>
<th>Phospho lipids</th>
<th>Free fatty acids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>13 day old fetus</td>
<td></td>
<td></td>
<td></td>
<td>19 day old fetus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>324.5 ± 14.7</td>
<td>259.0 ± 12.0</td>
<td>1648.5 ± 75.4</td>
<td>585.5 ± 26.7</td>
<td>342.8 ± 25.3</td>
<td>164.0 ± 17.0</td>
<td>2179.1 ± 110.69</td>
<td>675.8 ± 30.2</td>
</tr>
<tr>
<td>Ethanol</td>
<td>327.6 ± 15.0</td>
<td>262.5 ± 12.1</td>
<td>1801.6 ± 78.3a</td>
<td>589.6 ± 26.3</td>
<td>647.0 ± 33.2a</td>
<td>255.0 ± 12.6a</td>
<td>2468.3 ± 93.9a</td>
<td>1650.1 ± 133.9a</td>
</tr>
<tr>
<td>Arrack</td>
<td>330.6 ± 15.4</td>
<td>280.0 ± 13.1a</td>
<td>1900.7 ± 101.1a</td>
<td>596.1 ± 27.0</td>
<td>771.5 ± 36.5a</td>
<td>275.8 ± 11.7a</td>
<td>2539.1 ± 92.8a</td>
<td>1766.2 ± 103.0a</td>
</tr>
<tr>
<td>CD*</td>
<td>NS</td>
<td>14.8</td>
<td>105.4</td>
<td>NS</td>
<td>39.4</td>
<td>12.1</td>
<td>122.3</td>
<td>121.8</td>
</tr>
</tbody>
</table>

Average of values of 6 rats in each group ± SD.
*Critical difference [5%] to compare treatment means.
a-indicates that the corresponding result is significantly different from the control.
b-indicates that the effects of ethanol and Arrack are significantly different.
NS-not significant.

TABLE III: 14C acetate incorporation into lipids in fetal liver prenatally exposed to ethanol/Arrack (Values are expressed as counts/min/g tissue).

<table>
<thead>
<tr>
<th></th>
<th>Cholesterol</th>
<th>Cholesterol ester</th>
<th>Triglycerides</th>
<th>Phospho lipids</th>
<th>Cholesterol</th>
<th>Cholesterol ester</th>
<th>Triglycerides</th>
<th>Phospholipids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>13 day old fetus</td>
<td></td>
<td></td>
<td></td>
<td>19 day old fetus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>179.4 ± 8.2</td>
<td>316.8 ± 13.43</td>
<td>547.3 ± 24.4</td>
<td>388.0 ± 17.4</td>
<td>130.5 ± 27.42</td>
<td>359.3 ± 20.06</td>
<td>1306.5 ± 59.0</td>
<td>134.3 ± 24.4</td>
</tr>
<tr>
<td>Ethanol</td>
<td>184.8 ± 8.54</td>
<td>316.7 ± 14.0</td>
<td>552.8 ± 24.3a</td>
<td>430.1 ± 19.2</td>
<td>743.1 ± 45.7a</td>
<td>352.2 ± 13.3</td>
<td>2642.9 ± 106.0a</td>
<td>609.6 ± 55.9</td>
</tr>
<tr>
<td>Arrack</td>
<td>191.8 ± 8.61</td>
<td>344.0 ± 15.9a,b</td>
<td>588.3 ± 26.1a,</td>
<td>350 ± 15.6a,b</td>
<td>787.6 ± 22.3a,b</td>
<td>339.2 ± 12.1</td>
<td>2717.8 ± 68.8a</td>
<td>624.02 ± 35.5a</td>
</tr>
<tr>
<td>CD*</td>
<td>NS</td>
<td>17.6</td>
<td>30.7</td>
<td>21.5</td>
<td>41.0</td>
<td>NS</td>
<td>99.2</td>
<td>50.2</td>
</tr>
</tbody>
</table>

Average of values of 6 rats in each group ± SD.
*Critical difference [5%] to compare treatment means.
a-indicates that the corresponding result is significantly different from the control.
b-indicates that the effects of ethanol and Arrack are significantly different.
NS-not significant.
Consumption of Arrack/ethanol during pregnancy produced significant alterations in the lipid metabolism. 13th day and 19th day fetuses were affected. Arrack groups were severely affected in comparison with ethanol.

Cholesterol and free fatty acid levels were unaltered on 13th day of gestation after in utero exposure to ethanol/Arrack. But Arrack caused elevation of triglycerides and phospholipids. On 19th day of gestation all the parameters i.e., cholesterol, triglycerides, phospholipids and free fatty acids were enhanced after the administration of ethanol/Arrack. But lipemia produced by Arrack was significantly higher than that of ethanol (Table II).

Incorporation of 14C acetate to cholesterol ester, triglycerides and phospholipids increased on 13th day fetus of Arrack group. But in both the treated groups, there was an increased incorporation of 14C acetate to cholesterol, TG and phospholipids in the 19th day fetus. (Table III).

Activity of HMG CoA reductase in the liver of both 13th and 19th day fetuses of experimental animals showed significant increase in comparison with controls. But 13th day fetus of Arrack treated rats showed significant increase in activity than ethanol (Table IV).

Administration of ethanol/Arrack enhanced the activities of hepatic glucose-6-phosphate dehydrogenase and malic enzyme in both the stages of the fetus (Table IV).

### Table IV: Activity of HMG CoA reductase, glucose-6-phosphate dehydrogenase and malic enzymes in fetal livers prenatally exposed to ethanol/Arrack.

<table>
<thead>
<tr>
<th></th>
<th>13 day old fetus</th>
<th>19 day old fetus</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HMG CoA reductase</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3.0±0.14</td>
<td>2.66±0.15</td>
</tr>
<tr>
<td>Ethanol</td>
<td>2.0±0.14</td>
<td>1.26±0.070**</td>
</tr>
<tr>
<td>Arrack</td>
<td>1.26±0.070**</td>
<td>1.05</td>
</tr>
<tr>
<td><strong>CD</strong>*</td>
<td>1.06</td>
<td>1.05</td>
</tr>
</tbody>
</table>

**CD***: Critical difference (5%) to compare treatment means.

*Significant difference from control.

- **HMG CoA reductase**
- **Glucose-6-phosphate dehydrogenase**
- **Malic enzyme**
As a whole drastic alterations were seen in Arrack exposed rats then in ethanol exposed ones.

**DISCUSSION**

Our previous studies have shown that in utero exposure of Arrack causes reduction in weight of fetuses, altered carbohydrate metabolism (9), postnatal growth retardation and postnatal hyperlipidemia than its equivalent quantity of ethanol (12). Since carbohydrate metabolism and lipid metabolism are interrelated, in the present study we have analysed in detail the liver function and lipid metabolism of the fetus. The biochemical observations of the present study revealed that the hepatotoxicity induced by Arrack was greater than its equivalent quantity of ethanol.

Hyperlipodemia was observed on in utero exposure to Arrack/ethanol. Lipid accumulation is caused by enhanced synthesis. Observations of increased activities of HMG CoA reductase and lipogenic enzymes, labelled acetate incorporation to lipids support this. Operation of pentose phosphate pathway was enhanced as seen by the elevated activity of Glucose-6-phosphate dehydrogenase. Our previous studies (9) on carbohydrate metabolism have also shown that administration of Arrack/ethanol causes hypoglycemia and reduces the operations of citric acid cycle causing decreased oxidation of pyruvate. Pyruvate is used for the production of lipids via acetyl CoA. So it can be concluded that glucose is shunted for the production of lipids. This is in agreement with the observations of Ramakrishnan (4). Increase in the production of lipids content after alcohol administration has been previously reported by Druse (23) and Lalitha et al. (24). Moreover, it has been observed that in the 19th day fetal liver of Arrack group there was almost 2.5 times increase in the concentration of free fatty acids. It has been reported that administration of pregnant rats with ethanol during gestation days leads to accumulation of fatty acid ethyl esters (FAEE) (25) of long chain fatty acids both in the maternal and fetal organs. Hence the accumulated free fatty acids may serve as a substrate for fatty acid ethyl ester synthase for the enhanced synthesis of FAEE. Drastic increase in the concentration of phospholipids was seen both in 13th day and 19th day fetuses. Phospholipids are vital components of biomembranes (1). Their composition greatly affects the properties and functions of the membranes including signal transduction and hence the alterations in the membrane composition may be a reason for the teratogenic effects caused by maternal alcohol consumption.

Henry (26) in studying the effects of alcoholic beverages containing large and small amounts of congeners on BP and EEG had suggested that the congeners in alcoholic beverages have significant pharmacological effects. Studies on wines revealed that red wines had higher antioxidant index than white wines (7). The non-ethanolic components can be
hepatotoxic or hepatoprotective. In the present study significant alterations were seen in Arrack group than that of ethanol. Since absolute intake of ethanol was similar in both the experimental groups, the observed effects were probably due to non-ethanolic components present in Arrack other than ethanol. So the synergistic action of these non-ethanolic components may have produced the accumulation of lipids.

Alcohol freely passes through the placental barriers to the amniotic fluid and fetus (27). So the offspring is exposed to alcohol from the prenatal periods itself and increased lipid content seen in the fetus in comparison with control may be due to the alcohol passed on to the fetus through the placenta during pregnancy. Hyperlipidemia was observed both on 13th day fetuses and 19th day fetuses. But on 19th day fetus was more lipidemic them 13th day. Alcohol dehydrogenase is not detected on 13th day fetal liver. This reflects the immaturity of the fetal liver at this stage of organogenesis and this is in agreement with the observation of Raiha et al (28), Sanchis and Grerri (29) and Rovinki et al (30). Alcohol is cycled back to maternal body for detoxification and so fetus at this stage is partially safe.

In 13 day old fetuses, activity of HMG Co-A reductase was enhanced, but increased incorporation of labelled acetate was observed only in the cholesterol ester and not in the cholesterol. The cholesterol ester generated may be transported from the tissues and plasma to the liver by HDL, where the latter may be metabolized and excreted. On 13th day of gestation alcohol is metabolized in the maternal system (28). Hence excess of the substrate i.e., acetate is not available in the fetal system for the synthesis of cholesterol (29). Hence it may be concluded that catabolism of cholesterol might be greater than synthesis and it is reflected in the cholesterol levels on 13th day fetus.

In short, this study indirectly shows that the young ones born to alcoholic mothers have an inborn hyperlipidemia from the stage of organogenesis. hyperlipidemia is a positive risk factor for cardiovascular diseases. Lipid accumulation seen in the liver may alter the fluidity of membranes. Hence to may affect the intrauterine growth, and neurotransmission. This can explain to an extent the intrauterine growth retardation and neurological problems associated with FAS. So it can be concluded that congeners in Arrack potentiate the hepatotoxicity induced by ethanol and also that in utero exposure to alcohol/Arrack leads to lipid accumulation in fetal liver during the last trimester.

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