EFFECT OF ETHANOL/ARRACK ON THE LIPID METABOLISM OF MAMMARY GLAND DURING PREGNANCY AND LACTATION IN RATS

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(Received on February 12, 1999)

Abstract: Female rats were exposed to arrack (12.0 ml/kg body weight/day) and ethanol (4.0 g/kg body weight/day) before conception and throughout gestation and lactation. On 19th day of gestation and 21st day of lactation there was increase in the cholesterol phospholipids, triglycerides and free fatty acids in the mammary gland of rats administered arrack/ethanol in comparison with the controls. The lipoprotein lipase activity showed significant increase in the treated groups, in which the activity decreased on 21st day in comparison with 19th day. The absolute and relative weight of mammary gland also showed a significant decrease in ethanol/arrack treated group. The biochemical alterations produced in the mammary gland by arrack and its equivalent alcohol were different showing that non-alcoholic portion of arrack interferes with the toxicity induced by alcohol. Arrack was found to be a potent hyperlipidemic agent than ethanol.

Key words: ethanol lactation arrack pregnancy mammary gland

INTRODUCTION

Alcohol consumption during pregnancy is known to cause fetal malformations collectively called fetal alcohol syndrome (1). Vilaro et al (2) reported that chronic ethanol consumption profoundly altered normal mammary gland function, impaired milk production, altered mammary gland composition etc. They have also observed that the alcohol exposed rats produced milk with increased lipid content. Ethanol intake during lactation decreased the protein content of mammary gland but increased the rate of lipogenesis in this gland. Female rats receiving 20% of ethanol in drinking water during lactation when compared to pairfed controls had higher triglycerides on 17th day of lactation (3).

Alcoholic beverages contain in addition to alcohol certain non ethanolic components which are also pharmacologically active. There are reports that red wines due to the presence of resveratrol in it had a significantly higher antioxidant index and

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it reduces human LDL oxidation (4) and platelet aggregation (5). It has also been observed that progenies of pregnant rats fed 10% country liquor had higher mortality rates (6). Arrack is a distilled alcoholic beverage consumed by the low socio-economic strata of the society in India. It is made in distillaries by distilling molasses supplied by sugar factories. Scientific data of its impact on various metabolic processes is lacking. Its alcoholic content is of the range 40.0 – 42.0%.

A substantial portion of mammary gland is composed of fat tissue interspaced among the mammary gland epithelium. So it was decided to study the alterations in the cholesterol, phospholipid, triglycerides and free fatty acids of mammary gland during pregnancy and lactation in rats administered arrack and its equivalent quantity of alcohol.

**METHODS**

Femal albino rats (Sprague Dawley strain) of average weight of 125 gm were used. They were maintained in laboratory condition in a light and dark cycle of 12 h duration. Rats were fed with rat feed supplied by Lipton India Ltd. Banglore*. Animals in the experimental group were fed ad libitum. Animals were divided into three groups of 12 rats each.

- **Group 1. Pair fed control**
- **Group 2. Arrack (12.00 ml/kg body weight/day)**
- **Group 3. Ethanol (4.0 gm/kg body weight/day)**

Arrack was purchased from government licenced shop and its alcohol content was estimated by the method given in AOAC (7). The alcoholic content of our sample was 42%. The consumption of absolute alcohol 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. 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 a vaginal smear, and the day of detection of the spermatozoa was considered as the first day of gestation. Throughtout the gestation and lactation periods rats were administered arrack/ethanol. Weight of rats were recorded periodically. On 19th day of pregnancy (19thP) half of the rats were randomly selected from all groups, they were deprived of food overnight and sacrificed by decapitation. The total mammary gland was dissected and immediately washed throughly with saline until no milk exuded from tissue (in order to remove excess of milk) blotted with tissue paper and collected in pre-cooled containers for the estimation of lipids (8) and activity of lipoprotein lipase (LPL) (EC.3.1.1.34) (9) and protein was estimated by Lowry et al. (10) metod. Rest of the animals were allowed to deliver. The pups were nursed by their own mothers. The day of delivery was considered as the 1st day of lactation. On 21st day of lactation (21stL), dams were sacrificed and mammary glands were removed for the above mentioned estimations. Statistical analysis was carried out by Student’s ‘t’ test.
RESULTS

There was significant difference in the absolute and relative weight of the mammary gland (Fig. 1).

![Graph showing absolute and relative weight of mammary gland](image)

*P<0.05 between control and other groups

Fig. 1: Absolute and relative weight of mammary gland

The lipid profile of the mammary gland is shown in the Tables 1 and 2. There was a significant increase in the concentration of cholesterol, phospholipids, triglycerides and free fatty acids in the arrack/ethanol groups on both the days studied (19thP and 21stL) in comparison with their controls. Most of the lipid parameters studied were decreased on 21stL day from that on the 19thP day. The activity of LPL (Table 3) significantly increased in the arrack/ethanol treated groups. In both the cases lactating rats showed lesser LPL activity than non-lactating rats. Cholesterol: phospholipid ratio was enhanced. Maximum increase was noted in arrack than its equivalent quantity of ethanol.

### TABLE I: Lipid profile of mammary gland on 19th day of pregnancy values expressed as mean ± SE (Mean of six rats) concentration expressed as mg/100 gm wet tissue.

<table>
<thead>
<tr>
<th>Group</th>
<th>Cholesterol</th>
<th>Phospholipids</th>
<th>Triglycerides</th>
<th>Free fatty acids</th>
<th>Cholesterol/Phospholipids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>172.73±24.43</td>
<td>2030.00±60.00</td>
<td>243.09±12.06</td>
<td>869.63±37.84</td>
<td>0.088±0.003</td>
</tr>
<tr>
<td>Arrack</td>
<td>305.92±17.01*</td>
<td>3150.00±119.0*</td>
<td>289.30±15.00*</td>
<td>1300.4±95.20*</td>
<td>0.097±0.001</td>
</tr>
<tr>
<td>Et-OH</td>
<td>285.23±17.25*</td>
<td>2800.00±89.80*</td>
<td>286.37±10.67*</td>
<td>1252.70±83.40*</td>
<td>0.103±0.001</td>
</tr>
</tbody>
</table>

*P<0.05 between control and other groups

### TABLE II: Lipid profile of mammary gland on 21st day of lactation values expressed as mean ± SE (Mean of six rats) concentration expressed as mg/100 gm wet tissue.

<table>
<thead>
<tr>
<th>Group</th>
<th>Cholesterol</th>
<th>Phospholipids</th>
<th>Triglycerides</th>
<th>Free fatty acids</th>
<th>Cholesterol/Phospholipids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>107.64±12.81</td>
<td>2250.00±67.00</td>
<td>222.87±10.06</td>
<td>902.36±22.82</td>
<td>0.049±0.001</td>
</tr>
<tr>
<td>Arrack</td>
<td>244.03±15.78*</td>
<td>2950.00±89.00*</td>
<td>299.78±16.15*</td>
<td>1132.95±62.34*</td>
<td>0.084±0.001</td>
</tr>
<tr>
<td>Et-OH</td>
<td>178.89±18.61**</td>
<td>2450.00±66.00**</td>
<td>273.99±12.80*</td>
<td>1045.21±61.20**</td>
<td>0.075±0.002</td>
</tr>
</tbody>
</table>

*P<0.05 between control and other groups

a-P<0.05 between arrack and ethanol groups
TABLE III: Activity of lipoprotein lipase of mammary gland on 19th day of pregnancy and 21st day of lactation (activity/mg protein/30 min)  
Values expressed as Mean ± SE (Mean of six rats)

<table>
<thead>
<tr>
<th>Group</th>
<th>19th day</th>
<th>21st day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>180.33 ± 7.85</td>
<td>154.87 ± 6.43</td>
</tr>
<tr>
<td>Arrack</td>
<td>257.95 ± 5.23*</td>
<td>228.96 ± 6.88*</td>
</tr>
<tr>
<td>Et-OH</td>
<td>250.10 ± 2.78*</td>
<td>230.67 ± 3.54*</td>
</tr>
</tbody>
</table>

*P<0.05 between control and other groups  
**P<0.05 between arrack and ethanol groups

DISCUSSION

Our analysis of the alcohol content of the arrack showed that it contains 42% alcohol. Present results showed that hyperlipidemia induced by arrack was more than that of ethanol. This study was designed in such a way that the alcohol content of ethanol group was equivalent to that in the arrack group. Alcohol/Arrack produced reproductive toxicity as indicated by the decrease in the weight of pups, number of pups and decrease in the weight gain in the last trimester of pregnancy. The observed decrease in the absolute and relative weight of mammary gland in the arrack treated group showed impairment in the development and dysfunction of mammary gland. It has been observed by Vilaro et al. (11) that alcohol exposed rats produced milk with increased lipid concentration. The high lipid content seen in the mammary gland can be correlated with this and also with the reported enhanced lipogenesis (12). Biological membranes are composed of phospholipids and proteins. Addition of cholesterol causes a transition from gel to crystalline phase resulting in alteration in the nature and extent of lipid interaction and decrease in amplitude of motion of the chain axes and reduced fluidity of the system (13). The altered cholesterol:phospholipid ratio will affect the structure and stability of cell membrane resulting in membrane dysfunction. This may account for the observed variation in amino acid (14) and glucose uptake (2) by mammary gland. Phospholipids are vital components of biomolecules and their composition and metabolism greatly affect the properties and function of the membranes in the control and segregation of various cellular activities including signal transduction for a major group of hormones, neurotransmitters and growth factors. It has been observed that lack of appropriate response to the suckling stimulus contribute to the impairment in the development and dysfunction of mammary gland. The observed alteration in the phospholipid composition may alter the signal transduction capacity of the mammary gland and it may account for the hormonal changes reported in the mammary gland of alcohol administered experimental animals (2).

Decrease in the lipid parameters in the lactating dams in comparison with the non lactating dams indicate utilisation of lipids for milk production. LPL is involved in the uptake of triglyceride rich lipoproteins by extrahepatic tissues. Our studies indicate the greater uptake of triglycerides by mammary gland. This result is in agreement with the studies of Ramirez et al. (15). This study points out that consumption of alcohol/arrack alters the composition of mammary tissues during pregnancy and lactation. The hyperlipidemia observed may cause the production of milk with higher fat content. This will directly affect the quality and quantity of the milk.
produced and indirectly the growth of progeny.

So it can be concluded that arrack/ethanol intake can affect mammary gland function. Our study also reveals that alcohol induced toxicity is potentiated by the nonethanolic portions of arrack. Since the ethanol consumed by group 2 and 3 were same, this observed hyperlipidemia might have been induced by other nonethanolic components in arrack. Various studies (6, 16–21) have been conducted to analyse the effect of congeners. It has been found that higher alcohols in the alcoholic beverages competitively inhibit alcohol dehydrogenase and thus potentiate the toxicity induced by alcohol (16). So this study points out that more experiments should be conducted to elucidate the actual role of congeners.

REFERENCES