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POSTNATAL CHANGES IN THE BRAIN LIPIDS, GLYCOLIPIDS AND GANGLIOSIDES OF RATS EXPOSED TO ARRACK/ETHANOL DURING GESTATION AND LACTATION

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Abstract : Effects of exposure of an alcoholic beverage (arrack and its equivalent quantity of alcohol throughout pregnancy and lactation on brain lipids were investigated. Female rats were exposed to arrack (12.00 ml/kg body weigh/day) and ethanol (4.00 g/kg body weight day) before conception and throughout gestation and lactation. For 21 days pups were nursed by their own mothers, afterwards they were fed normal laboratory feed. We found that the level of cholesterol, phospholipids, triaceyl gycerols, free fatty acids, cerebrosides, ceramide dihexosides, ceramide polyhexosides, sulfatids,, mono and diglycosyl diglycerides and gangliosides were increased in the brain of 21st and 45th day pups. The alterations in the glycolipid profile of the brain persisted even when pups were not directly exposed to alcohol. These alterations in the glycolipid and ganglioside metabolism may be associated with the developmental abnormalities of the brain seen in FAS. The elevation produced in the glycolipid profile of arrack administered pups were more than that caused by its equivalent quantity of ethanol. This suggested an interaction of congeners in the arrack with the alcohol.

Key words : FAS lipids glycolipids ethanol beverage lactation

INTRODUCTION

Maternal consumption of alcohol may lead to fetal malformations, collectively called Fetal alcohol syndrome (FAS) (1). It is characterized by abnormalities of the central nervous system. Animal research has revealed that prenatal exposure at critical periods of development leads to altered cytoarchitecture and metabolic activity of the numerous brain regions (5). It has been shown that *in utero* exposure of alcohol adversely affects the normal brain development in the offspring (6). Maternal alcohol consumption increased total gangliosides in the brain and spinal cord and also resulted in a decrease in the ganglioside catabolising enzymes in rats (7).

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Lalitha *et al* (8) reported that lipid composition of the brain was altered in rat pups exposed to alcohol during prenatal and postnatal period. Studies in this laboratory showed that maternal beverage/ethanol exposure could induce hyperlipidemia among the offspring of rats (9, 10, 11).

However, studies on the effect of *in utero* exposure of alcoholic beverages on brain gangliosides of offspring have been scarce. Most of the studies conducted have been with pure ethanol. Alcoholic beverages contain many substances other than ethanol, which modify the effects of ethanol. Various alcoholic beverages are consumed by men according to their socio-economic status. Arrack is a popular distilled alcoholic beverage in India. It is mainly consumed by the people belonging to low socio-economic strata. Hence studies were carried out to determine the effects of maternal consumption of arrack and its equivalent quantity of ethanol on the brain glycolipids of pups at various stages of development.

METHODS

Female albino rats (Sprague Dawley strain) of average weight of 125 g were randomly selected and divided into three groups of 12 rats each. They were maintained in normal laboratory conditions in light and dark cycle of 12 h duration Rats were fed with pelleted diet supplied by Lipton India Ltd. and tap water *ad libitum*.

Group 1. Control + glucose solution

Group 2. Arrack (12.00 ml/kg body weight/day)

Group 3. Ethanol (4.00 g/kg body weight/day)

Arrack was purchased from Govt. Licensed shop and its alcohol content was estimated (40-42%) as described in AOAC (12). The ethanol group was fed with pure ethanol, which was equivalent to the alcohol content of that of arrack. Control rats were fed with an equicaloric amount of glucose solution. Rats were treated as shown above for 15 days. Arrack/ethanol was administered by gastric intubation after diluting in the ratio 1:1.25 and 1:7 respectively. After 15 days they were allowed to mate with normal male rats. Pregnancy was detected by microscopical examination of vaginal smear, and the day of detection of spermatozoa was considered as the first day gestation. Throughout gestation and lactation, rats were administered arrack/ ethanol. Weights of rats were recorded periodically. Pups were nursed by their own mothers for 21 days. On 21st day (21 P), half of the pups were deprived of food overnight and sacrificed by cervical dislocation. The whole brain was dissected immediately washed with physiological saline and stored in pre-cooled containers for various biochemical estimations. Rests of the offspring were fed normal pelleted laboratory diet and they were allowed to grow upto 45 days. On the 45th day (45 P), they were also sacrificed after 12 hrs fasting and the brain was removed as above. Cholesterol, phospholipids, triacylglycerols and free fatty acids were estimated as reported earlier (13) For estimating glycolipid level, lipid extraction was done by the method of Folch *et al (14)*. The lipid extract was shaken with 0.1 mol/L KCl. Upper and lower layers were washed three times and combined upper phases were dialyzed against distilled water and lyophilized. The lyophilized material was

extracted with chloroform: methanol: water (10:5:1). Sialic acid content was estimated to determine the gangliosides content (15). Glycolipid fractions namely cerebrosides, ceramide dihexoside+ceramide polyhexoside, monoglycosyl diglyceride and diglycosyl diglyceride and sulfatides were separated by diethyl aminoethyl (DEAE) cellulose ion-exchange column chromatography (16). Total hexose (17) and sulfate (18) levels were determined quantitatively to estimate the levels of various glycolipid fractions. Statistical analysis was carried out by one way ANOVA. Differences between treatment means were determined by the method of Snedecor and Cochran (19).

RESULT

The lipid profile of brain showed and increased concentration of cholesterol, phospholipids, triacylglycerol and free fatty acids in the arrack/ethanol treated groups. This increase was more prominent

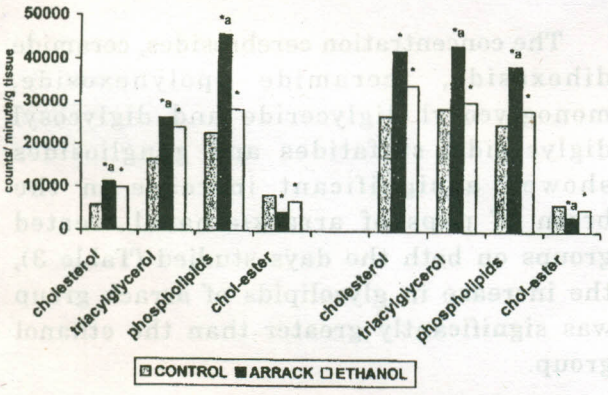


Fig. 1: 14 C acetate incorporation to lipids in the brain of pups.
Values are Mean ± SD from six rats
*- Indicates that the corresponding resultsis significant in comparison with dontrol
a- Indicates that the effects of arrack and ethanol are significantly different

TABLE I : Concentration of cholesterol, phospholipids, triacylglycerols and free fatty acids of the brain of pups on 21st and 45th day pups. (mg/100 gm wet tissue)

Group	Colesterol		Triacylglycerol		Phospholipids		Free fatty acids	
	21st	45th	21st	45th	21th	45th	21st	45th
Control	685.20±18.76	875.96^{c}±21.64	614.27±18.02	825.57^{c}±14.57	1.59±0.08	2.07±0.09	467.73±31.63	528.67^{abc}±23.48
Arrack	917.28^{ab}±23.98	1101.07^{abc}±82.30	960.38^{ab}±97.11	1093.95^{ab}±71.58	2.00^{a}±0.08	2.86^{ab}±0.12	654.72^{ab}±23.38	748.45^{abc}±33.54
Ethanol	866.6^{c}±19.25	1045.89^{ac}±13.39	883.83^{a}±49.38	937.38^{ac}±38.29	1.86^{a}±0.53	2.27±0.11	557.23^{a}±27.97	643.90^{ac}±20.21

Values are Mean ± SD from six rats (a, b, c- $P<0.05$)
a- Indicates that the corresponding results is significant in comparison with control
b- Indicates that the effect of arrack and ethanol are significant different
c- Indicates that the change between 21st day pups ad 45th day pups are significantly different

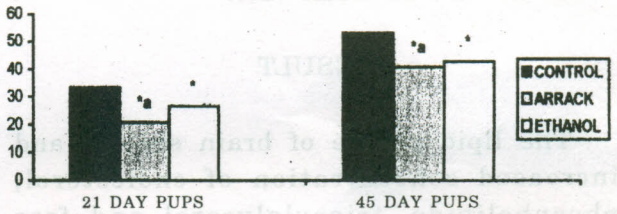
TABLE II: Activities of HMG Co-A reductase, glucose 6- phosphate dehydrogenease and malic enzyme in the liver of 45th day pups prenatally and postnatally exposed to Arrack/ethanol.

Group	*HMG CoA reductase (HMG Co–A / Mevalonate)*	*Glucose -6- phosphate dehydogenase**	*Malic enzyme***
Control	0.20±0.15	44.95±1.60	87.82±5.10
Arrack	2.12±0.12^{ab}	83.46±1.47^{ab}	146.59±4.80^{ab}
Ethanol	3.22±0.15^{a}	62.28±0.94^{a}	130.31±2.79^{a}

Values are Mean ± SD from six rats (a, b P<0.05)
a- Indicates that the corresponding results is significant in comparison with control
b- Indicates that the effect of arrack and ethanol are significant different
*- The amount of enzyme which causes an increase in OD of 0.01/min/g protein
**-The amount of enzyme which causes an increase in OD of 1.0/min/g protein

in the arrack treated group. The 45th day pups showed significant increase in these lipids compared to that of 21st day pups- ^{11}C acetate incorporation to lipids also showed that there was a marked and significant increase in the concentration of various fractions of lipids in the brain of offspring exposed to beverages compared to the control and ethanol exposed groups (Fig. 1). Here also we observed a marked difference between 21st and 45th day pups.

The activity of HMG Co-A reductase, malic enzyme and glucose -6- phosphate dehydrogenase showed a significant increase in the arrack/ ethanol exposed pups (Table 2). The bile acid content (Fig. 2) of the liver of pups exposed to arrack/ethanol on both the days showed a significant reduction compared to control.



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

Fig. 2: Bile acid content of the liver of pups.

The concentration cerebrosides, ceramide dihexoside, ceramide polyhexoside, monoglycosyl diglyceride and diglycosyl diglyceride, sulfatides and gangliosides showed a significant increase in the brain of pups of arrack/ethanol treated groups on both the days studied (Table 3), the increase in glycolipids of arrack group was significantly greater than the ethanol group.

TABLE III : Concentration of Glycolipids in the brain of Pups on 21st and 45th day of development.

Group	Cerebrosides		Sulfatides		CDH+CPH		MGDG		DGDG		Gangliosides	
	21st	*45th*	*21st*	*45th*	*21th*	*45th*	*21st*	*45th*	*21th*	*45th*	*21st*	*45th*
Control	1862.02± 49.52	2035.94[c]± 144.94	369.86± 18.18	446.72[c]± 13.59	51.38± 1.22	58.37± 1.51	29.37± 0.89	34.34[c]± 1.47	10.69± 0.45	24.18± 1.42	11.93± 0.43	18.00[c]± 0.7
Arrack	3570.44± 44.09[ab]	3841.06± 142.10[ab]	658.56± 23.26[ab]	745.21± 14.07[abc]	86.54± 1.50[ab]	89.42± 1.45[ab]	64.76± 2.75[abc]	79.54± 1.15[abc]	28.46± 0.81[ab]	36.83± 1.27[abc]	35.19± 0.68[ab]	42.2[abc]± 1.1
Ethanol	3072.28± 45.72[a]	35.31.40± 158.23[ac]	536.73± 13.16[a]	662.36± 21.33[ac]	61.37± 2.24[a]	64.39± 1.54[a]	50.72± 1.70[a]	66.43± 2.12[ac]	24.28± 0.89[a]	31.97± 0.74[ac]	27.48± 0.93	35.1[ac]± 0.9

Values are Mean ± SD from six rats (a, b, c- $P<0.05$)
CDH- Ceramide dihexoside, CPH- Ceramide polyhexoside MGDG- Monoglycosyl diglyceride, DGDG- Diglycosyl diglyceride
a- Indicates that the corresponding results is significant in comparison with control
b- Indicates that the effect of arrack and ethanol are significant different
c- Indicates that the change between 21st day pups ad 45th day pups are significantly different

DISCUSSION

Hyperlipidemia was observed in the brain of pups even on the 45th day, ie when they were no longer exposed to arrack/ ethanol. It may be due to the higher level of alcohol exposed prenatally and postnatally. In the present investigation. It was observed that ingestion of arrack/ ethanol altered the concentration of lipids in various tissues of the pups on 21st day of lactation. These observations are in agreement with those of Lalitha *et al.* 8) and Druse (20). Alcohol freely passes through the placental barriers to the amniotic fluid and then to the fetus (21). Milk from alcoholic rats contains alcohol and its derivative (22). Our earlier studies have shown that administration of ethanol and arrack during pregnancy and lactation cause hyperlipidemia in the mammary glands (23). Hence the milk produced will be of higher fat content. This will indirectly affect the growth of the progeny. Vilaro *et al.* (24). also observed that alcohol exposed rats produced milk with high lipid concentration. The increased concentration of various lipids may be due to the increased biosynthesis and decreased catabolism of lipids. The peak period of lipid accumulation in rat brain occurs in the first four weeks of postnatal life (25). This study reveals that exposure to alcohol during gestation and lactation causes hyperlipidemia in brain, and abstaining from alcohol cannot reverse it. The altered lipid pattern may affect the fluidity of membranes, and neurotransmission and it may lead to various abnormalities associated with FAS. We have observed a persistent lag in the growth of pups exposed to arrack/ ethanol.

Glycolipids are involved in a number of processes such as cell membrane functions, immunological reactions and virus cell interactions (26), and sulfatids are involved in the binding of thrombospondin (27) and laminin (28). The increase in the glycolipids of brain of pups exposed to arrack/ethanol may make the membranes more rigid and this may be the underlying factor for alcohol induced changes membrane's physical properties. Reduced membrane fluidity has been reported by some investigators (7). The ganglioside concentration of offspring of alcoholic rats is in harmony with the findings of Lalitha *et al.* (8). These findings are of great concern with regard to membrane function. It may be an adaptive change leading to the development of tolerance. Alteration in the ganglioside metabolism is associated with metal retardation. Hence alterations in brain glycolipids observed may be factors for the mental retardation seen in FAS. In this study it has been found that alterations in the lipid and glycolipid profile of brain persist even when pups are not directly exposed to alcohol. Hence it can be concluded that membrane's dynamic state may not be normal in these animals.

This study also revealed that non-ethanolic components present in alcoholic beverages modify the toxic effects of alcohol. In this case even though the absolute alcohol consumed by alcohol group and arrack were same, alterations in glycolipid and lipid levels were more affected on exposure to arrack. We have observed in our earlier studies, that congeners in arrack potentiate the toxicity induced by ethanol (10,23). Only further studies will reveal the adaptive changes occurring in the developing brain.

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