Estimation of Tissue Lipid Peroxidation Level and Organ Weight in Litters of Wistar Rats Exposed to Prenatal Alcohol Ingestion

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Abstract

Alcohol (ethanol) is among some of the potentially harmful factors that are transmitted directly to the embryo. The present study was aimed to find out the changes in the tissue lipid peroxidation level and organ weight in the litters exposed to prenatal alcohol consumption. Adult female wistar rats (n = 6/group) were divided into control group (saline treated) and experimental group (ethanol treated 2 g/kg BW). Changes in the body weight, number of litters delivered, birth weight of the litters, organ weight and lipid peroxidation level was evaluated in both the groups. Ethanol intoxication produced a significant increase (P<0.0001) in the body weight. The birth weight of the litters (P<0.0001) and the number of litters (P<0.0001) were significantly (P<0.0001) reduced when compared to the saline treated control group. There was a significant increase in the weight of the liver, brain and the testis in the treated group litters. Also in these litters, a significant elevation in the lipid peroxidation level was observed in the liver, brain, kidneys and testis. The present data suggest that prenatal ethanol exposure might activate the free radical process leading the increase in the lipid peroxidation level and consequent decrease in the organ weight in these litters.

Key Words: *ethanol, lipid peroxidation, organ weight, litters, Wistar rats*

Alcohol-related problems now rank among the world's major public health concerns, not only in most industrialized countries but also in developing countries. There is increasing evidence that the prenatal environment can influence the risk of developing some chronic diseases in the offspring's later in life.[1-3] This is thought to occur through fetal programming, which refers to the ability of changes in environmental factors (e.g. nutrition, stress, exposure to toxicants) at the critical periods during development which permanently alter the structure, physiology or metabolism of the body, resulting in a lifelong effect on the organism.[3] Alcohol (ethanol) is among some of the potentially harmful factors that are transmitted directly to the embryo.[4] Exposure to ethanol in utero is known to have serious long-term implications for the offspring and can lead to a diverse array of health problems, described collectively as Fetal Alcohol Spectrum Disorder.[5] A wide variety of variable pregnancy outcome have been reported after controlled human studies on this aspect; which includes brain malformation, cerebral palsy, intrauterine growth retardation, decreased birth weight etc.[6-8] Reports also show that alcohol consumed by mothers during pregnancy particularly during the first trimester causes many congenital defects in offspring of both human and experimental animal models.[9-12]

Ethanol manifests its harmful effects either through direct generation of reactive metabolites, including free radical species that react with most of the cell components, changing their structures and functions, or by contributing to other mechanisms that finally promote enhanced oxidative damage.[13-15] Studies show that chronic alcohol consumption leads to several metabolic disorders including hepatic and extra hepatic diseases[16] which are initiated by reactive oxygen species (ROS) generation and lipid peroxidation as found in liver and heart[16] or brain[17] leading to cellular damage. Recent studies suggest that oxidative stress is an important factor contributing to obesity, insulin resistance, and type 2 diabetes, and rats with intra uterine growth restrictions has been shown to have increased systemic oxidative stress with damage to liver and skeletal muscle.[18-20] Because heavy ethanol exposure causes oxidative stress,[21] and alcohol is transmitted to embryo we hypothesized that rats exposed to ethanol in utero might also have oxidative damage in various tissues of the offspring also. In the present study we evaluated the oxidative stress induced damage by prenatal ethanol intoxication by measuring thiobarbituric reactive substances (TBARS) levels in the litters.

Methods

Healthy adult female albino rats of Wistar strain weighing approximately 150-200 g were selected for the expe-

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riment. The animals were housed four per cage under 50% of humidity and 24°C. Animals had free access to water and standard pallet diet. The animals were divided into control group (saline treated, n=6) and experimental group (ethanol treated, n=6). In the first set of the experiment the experimental group was injected with ethanol (2 g/kg body weight) and the control group of rats was injected with saline intraperitonially. After 15 days the changes in the body weight was observed. The animals were allowed to male rat by placing a male rat overnight and the vagina was examined next morning for plugs. Day 1 of pregnancy was regarded as the day vaginal plugs were found or sperms observed in the vaginal washing next morning. Once the pregnancy is confirmed the pregnant animals were placed one in a cage until delivery. During the entire gestational period also ethanol injection was given. At the time of the birth, the litter weight, number of litter were observed. After one month all the litters were anaesthetized with sodium pentobarbital (40 mg/kg body weight) and then liver, kidney, brain, testis were quickly removed and weighed. The wet weights of the organs were determined and organ weight was expressed as grams per 100 g of body weight.

The collected tissues were homogenized separately with a motor driven glass homogenizer in ice –cold phosphate buffer for lipid peroxidation level analysis. Lipid peroxidation in these tissues was estimated by thiobarbituric acid reactive substance as previously described by Kartha and Krishnamurthy.[22] All the experimental procedures were in accordance with the ethical standards of animal experimentation and the study was approved by the Institutional Animal Care and Use Committee.

Statistical analysis: The data were expressed as means ± SD from 6 animals per group. The differences between the groups were compared for statistical significance by the Student's *t*-test with the level of significance set at $P<0.05$.

Results

In the mother rats ethanol intoxication produced a significant increase ($P<0.0001$) in the body weight. But the birth weight of the litters ($P<0.0001$) and the number of litters ($P<0.0001$) were significantly ($P<0.0001$) less in these groups when compared to the saline treated control group. The weight of the liver, brain and the testis in the treated group litters were also significantly decreased. Further, in these litters, a significant elevation in the lipid peroxidation level was observed in the tissues of liver, brain, kidney and testis.

Discussion

In the present study, ethanol intoxication also produced a significant increase in the body weight in the mother rats. Further, prenatal exposure of alcohol also resulted in the reduction of various organ weights and at the same time, the lipid peroxidation levels of these tissues were increased. This result of the present study was in controlled conditions of a 12 h light/dark cycle, agreement with the present day hypothesis as "the oxidative stress is the attractive possible mechanism for the alcohol induced tissue damage associated with the fetal alcohol syndrome".

Table 1. Body weight changes in mother rats after ethanol injection for 15 days

Parameters	Day 1	Day 15
Control group	136.66 ± 5.16	168.00 ± 4.00
Treated group	134.62 ± 4.24 NS	196.66 ± 15.05*

Day 1 control group versus treated group and NS
Day 15 control group versus treated group *$P<0.0001$
NS = Not significant

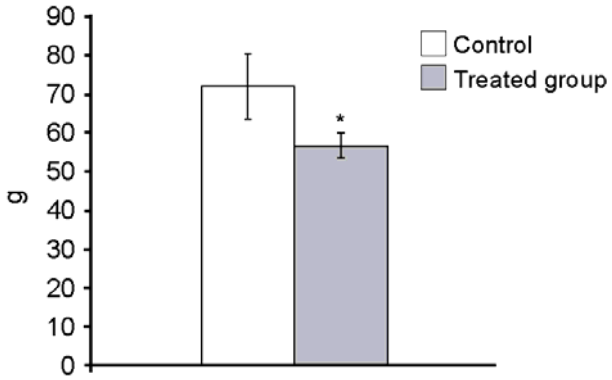

Figure 1. Comparison of birth weight of litters in control group and treated group after gestational ethanol exposure. *$P<0.0001$; control group versus treated group

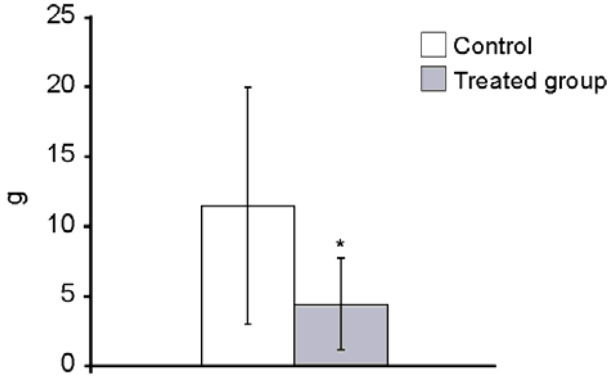

Figure 2. Comparison of number of litters in control group and treated group after gestational ethanol exposure. *$P<0.0001$; control group versus treated group

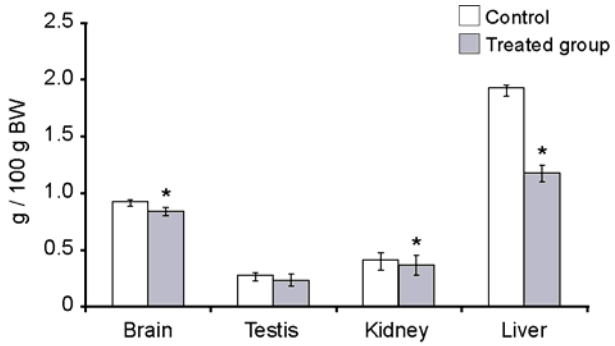

Figure 3. Comparison of different organ weight of the litter in control group and treated group. * *P*<0.0001; control group versus treated group

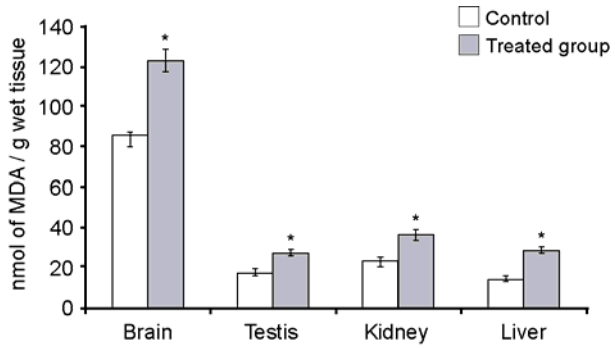

Figure 4. Comparison of tissue lipid peroxidation level in different organs of the litters in control group and treated group. * *P*<0.0001; control group versus treated group

Chronic alcohol consumption leads to several metabolic disorders including hepatic and extrahepatic diseases.[16] It could be possible that due to hepatic metabolic disorders, the plasma protein synthesis may be decreased. The resultant decrease in oncotic pressure leads to generalized edema; might be the cause of increase in the body weight in the treated mother rats. Ethanol exposure in adult animals and humans has shown to elicit significant inhibitory effects on the function of male reproduction but consequences of ethanol exposure on early postnatal testis development are not known. Free radical production and lipid peroxidation are the important mediators in testicular physiology and pathology.[23] Reactive oxygen species (ROS) have been proposed as a major factor affecting male reproductive capacity.[24-26] The testis has been shown to be highly susceptible to ethanol as it crosses the blood testis barrier.[27] In the present study there was a significant reduction in the testicular weight and a significant increase in the lipid peroxidation level in the litters exposed to prenatal ethanol compared to the control group. This observation shows that the increase in the prenatal ethanol exposure might have produced oxidative stress which might lead to functional abnormalities.

The increased lipid peroxidation level observed in the other organs such as brain , liver, kidney in this study indicates that possibly in these organs also the alcohol induced organ damage may be mediated through the oxidative stress. As the brain processes large amounts of O_2 in a relatively small mass, and has a high content of substrates available for oxidation (i.e., polyunsaturated fatty acids and catecholamine) in conjunction with low antioxidant activities, making it extremely susceptible to oxidative damage.

Conclusions

In summary the present study indicate that ethanol intoxication during the gestational period in female rats induced oxidative damage in the litters; which might be related to the oxidative stress or insufficiency of the protective antioxidants in the early prenatal period. The present study confirms the formation of free radicals in the prenatal period due to the gestational exposure of ethanol which might be a contributing factor for fetal alcohol syndrome.

References

1. Vickers MH, Breier BH, D. McCarthy, and Gluckman PD. Sedentary behavior during postnatal life is determined by the prenatal environment and exacerbated by postnatal hypercaloric nutrition. Am J Physiol Regul Integr Comp Physiol. 2003;285:271- 273.
2. Massot M, Clobert J. Influence of maternal food availability on offspring dispersal. Behavioral Ecology and Sociobiology. 1995;37(6).
3. Rebecca M. Reynolds, Keith M. Godfrey, Mary Barker, Clive Osmond, David I. Phillips W.Stress Responsiveness in Adult Life: Influence of Mother's Diet in Late Pregnancy. The Journal of Clinical Endocrinology & Metabolism. 2007;92(6):2208-2210.
4. Noden, D. M.,De Lahunta A. Causes of congenital malformations. In: The Embryology of Domestic Animals. Developmental Mechanisms and Malformations. Williams and Wilkins. Baltimore. 1985.
5. Roebuck TM, Mattson SN, Riley EP. A review of the neuroanatomical findings in children with Fetal Alcohol Syndrome or prenatal exposure to alcohol. Alcohol Clin Exp Res. 1998;22:339-44.
6. Godel JC, Pabst HF, Hodges PE, Johnson KE, Froese GJ, Joffres MR. Exposure to alcohol in utero: Influence on cognitive function and learning in a northern elementary population. Paediatr Child Health. 2000;5:93-100.
7. Coles CD, Smith I, Fernhoff PM, Falek A. Neonatal neurobehavioural characteristics as correlates of maternal alcohol use during gestation. Alcohol Clin Exp Res. 1985;9:454-60.
8. Roebuck TM, Simmons RW, Richardson C, Mattson SN, Riley EP. Neuromuscular responses to disturbances of balance in children with prenatal exposure to alcohol. Alcohol Clin Exp Res. 1998;22:1992-7.
9. Jones KL, Smith DW. Recognition of the fetal alcohol syndrome in early pregnancy. Lancet. 1973;999-1001.
10. Sampson PD, Streisgut AP, Bookstein FL, Ookstein F, Little RE, Clarrens SK et al. Incidence of fetal alcohol syndrome and prevalence of alcohol-related neurodevelopmental disorder. Teratology. 1997;56:317-26.
11. Jacobson JL, Jacobson SW, Sokol RJ, Ager JW Jr. Relationship of maternal age and pattern of drinking to functionally significant cognitive deficit in infancy. Alcohol Clin Exp Res. 1998; 22:345-51.
12. Ladue RA, Streissguth AP, Randels SP. Clinical considerations pertaining to adolescents and adults with fetal alcohol syndrome.

13. Ostrowska J,uczaj W , Kasacka I , Róaski A, Skrzydlewska E, Ostrowska, W. et al. Green tea protects against ethanol-induced lipid peroxidation in rat organs. Alcohol. 2004;32(1):25-32.
14. Kato S, Kawase T, Alderman J, Inatomi N, Lieber CS. Role of xanthine oxidase in ethanol-induced lipid peroxidation in rats. Gastroenterology. 1990;98:203-10
15. Norman R. Alcohol and antioxidant systems. Alcohol. 1994;29:513-22.
16. Lieber CS. Alcohol and the liver: Metabolism of alcohol and its role in hepatic and extrahepatic diseases. Mt Sinai J. Med. 2000;67:84-94.
17. Panchenko, LF, Pirozhkov SV, Popova SV. Effect of chronic ethanol treatment on peroxidation acetyl-CoA oxidase activity and lipid peroxidation in rat liver and heart. Experientia. 1987;43:580-581.
18. Lieber CS. Alcohol and the liver: metabolism of alcohol and its role in hepatic and extrahepatic diseases. The Mount Sinai Journal of Medicine. 2000;67:84–94.
19. Franco CP, Akamine EH, Di Marco GS, Casarini DE, Fortes ZB; Tostes RCA., et al. NADPH oxidase and enhanced superoxide generation in intrauterine undernourished rats: involvement of the renin-angiotensin system. Cardiovasc Res. 2003:59;767–775.
20. Peterside IE, Selak MA,Simmons RA. Impaired oxidative phosphorylation in hepatic mitochondria in growth-retarded rats. *Am J* Physiol Endocrinol Metab. 2003; 285:E1258–E1266.
21. Selak MA, Storey BT, Peterside I,Simmons RA. Impaired oxidative phosphorylation in skeletal muscle of intrauterine growth-retarded rats. Am J Physiol Endocrinol Metab. 2003;285:E130–E137.
22. Kartha R, Krishnamurthy S. Factors affecting invitro lipid peroxidation in rat brain homogenates. Ind J Physiol Pharmacol. 1978;22(1):44-52.
23. K. Muthusami, P. Chinnaswamy. Effect of chronic alcoholism on male fertility hormones and semen quality. *Fertility and Sterility*, Volume 84, Issue 4, 919-924.
24. K. Doreswamy and Muralidhara. Genotoxic consequences associated with oxidative damage in testis of mice subjected to iron intoxication. Toxicology. 2005; 206(1):169-178.
25. Zhu Q, Meisinger J, Emanuele NV, Emanuele MA, LaPaglia N, Van Thiel DH.Ethanol exposure enhances apoptosis within the testes. Alcohol Clin Exp. 2000;24(10):1550-1556.
26. Ganaraja B, Crystal D D'Souza, Vijayalakshmi BM, Nayanatara AK, Ramesh Bhat M, Ramaswamy C. Use of vitamin C on effect of ethanol induced lipid peroxidation in various tissues, sperm count & morphology in the wistar rats. Journal of Chinese clinical medicine. 2008;3 (11):1-5.
27. M Maneesh1, H Jayalekshmi1, Sanjiba Dutta, Amit chakrabarthi, Vasudevan DM Role of oxidative stress in ethanol induced germ cell apoptosis an experimental study in rat. Indian Journal of Clinical Biochemistry. 2005;20(2):62-67.