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Study of effect of ethanol on antioxidant - vitamin A and C in rat liver

A. R. Singh[1], Sushil Kumar[2], Roshan Takhelmayum[2], J. N. Sihna[3]

[1]Asst. Prof, [2]Lecturer, [3]Prof and Head, Department of Biochemistry, College of Medical Sciences and Teaching Hospital, Bharatpur, Nepal.

Abstract:

Objective: *To see the effect of consumption of locally distilled alcohol (country liquor) continuously for few months on hepatic vitamin A and C status in albino rats.*

Materials and methods: *The study was conducted in 36 male wistar strain albino rats for 3-4 months old consisting six groups of six animals each.*

Results: *The first observation was weight gain among the series of alcoholic animals when compared to the control and alcoholic fed animals supplemented with vitamin A and C, p-value by T-test between the mean values of the initial weight and final weight was < 0.01 (0.006), significant.*

Conclusion: *It was found that the major effect on hepatic vitamin A and C contents were observed more distinctly in mitochondrial fractions when compared with the rest fractions. Supplementation of vitamins helped to protect loss of the vitamins which delayed the aging process at age 9-10 months in our study.*

Key words: *Albino rats, alcoholic, hepatic, vitamin A*

Introduction:

The liver injury due to acute and chronic ethanol abuse has been proved to be dependent on its oxidative effect at the cytosolic, peroxisomal and microsomal levels.[1] But despite extensive investigations, the molecular mechanism leading to the hepatic damage still needs to be clarified. Based on technologically advanced procedures, it has been demonstrated that a group of reactive species known as free radicals might be taking a major role in the pathogenesis of tissue changes during hepatic ethanol loading. A free radical has been defined as a chemical species, capable of independent existence that contains unpaired electrons. They are energetically unstable, highly reactive and short lived.[2] Drugs including alcohol may exert toxic effect by promoting free radical formation during their metabolism and a decline of some of the antioxidant defences like reduced glutathione, vitamin C, vitamin E, vitamin A, etc. thereby increasing the ratio between pro-oxidant and antioxidant reaction resulting to a condition known as oxidative stress.[3] Polyunsaturated fatty acids within the cell membranes and lipoproteins are particularly susceptible to oxidative attack often as a result of metal ion dependent hydroxyl radical formation. Long chains of lipid peroxides may be formed causing serious disruption of cell membrane

Correspondence: Dr. A. R. Singh

E-mail: dr.drarsingh@rediffmail.com

function.[4,5] Proteins exposed to free radical attack may fragment, cross link or aggregate. The consequences include interference with ion channels, failure of cell receptor, etc. Free radical damage to DNA may cause destruction of bases, deoxyribose sugar and single or double strand breaks[6] and is implicated in mutagenesis, carcinogenesis and even cell death.[7] Antioxidants delay and protect against oxidative damage produced by free radicals. Vitamin A and C belong to nutrient antioxidants. Vitamin A is a lipid soluble antioxidant and membrane bound. It can suppress free radical induced lipid peroxidation under conditions of low partial pressure of oxygen in most tissues. Vitamin C, a water soluble antioxidant which acts as free radical scavenger could improve liver functions in alcoholic patients[8,9] and that also maintains the vitamin E level. It has been reported that both vitamins may behave as pro-oxidants if aqueous phase antioxidant fall short.[10]

The present study was undertaken to see whether the nutritional antioxidants like vitamin A and vitamin C have got any definite role in checking liver injury in alcoholics.

Materials and methods:

The study was carried out in the Department of Biochemistry, Regional Institute of Medical Sciences (RIMS), Imphal, Manipur. Albino rats (Wistar strain), 3-4 months old procured from National Institute of Nutrition, Indian Council Of Medical Research (ICMR), Hyderabad reared in the Central Animal House, RIMS, Imphal were the animals used for the study. Diet chart formulation was done according to the method given for preparation of pellet diets as published in LAIIS Centre, News (Nov, 1984). Ethical clearance was obtained from the Institutional Ethical Committee for conducting the animal experiment.

Thirty six male albino rats with average mean weight of 165 gm were selected and divided into six groups. The first group was given only normal diet and served as Control No. 2. The second, third, fourth and fifth were given alcohol over and above normal diet. The sixth group was given both alcohol and nutrient antioxidant along with normal diet. An additional group (Control-1) of six albino rats (3-4 months old) with an average mean weight of 165 gm were sacrificed at the very beginning of the study for determining antioxidant levels in various subcellular fractions. Determination of antioxidant level in alcoholic group was done by sacrificing the second, third, fourth and fifth groups after one week, one month, three and six months respectively. The first (Control No. 2, C_2) and the sixth groups were sacrificed after sixth months only to determine the hepatic antioxidant levels.

All the chemicals and reagents used for the study were of analytical grade and alcohol used for feeding animals was collected from a local distiller. This sample contains 37.95% alcohol as per analysis method given by Department of Food and Technology and Biochemical Engineering, Jadavpur University, Calcutta. Four hundred I.U. of vitamin A (Retinol procured from Eupharma Lab. Ltd. Mumbai), and 4 mg of vitamin C (Ascorbic acid from ABBOTT Lab. India) were supplemented to the sixth group per day per animal.

For collection of rat liver, the abdomen was cut and tissue was dissected and then a homogenate was prepared in 20% 0.25 M sucrose solution using Potter Elvehjem type homogenizer. Differential centrifugation of the homogenate was done in high speed refrigerated centrifuge machine (Beckman's Avanti-30) to separate the various sub-cellular fractions. All the sub-cellular fractions and 15000 X g, 1 hr supernatant were used

for study of antioxidant levels in them. Methods of Natelson S[11] were used to estimate the levels of vitamin A and vitamin C.

Results:

In table 1, the sixth group and the first group (C_2) show mean body weight of 201 and 204 gm respectively showing a weight gain of 36 gm and 39 gm within a span of six months. The alcoholic group, on the other hand showed a better rate of weight gain showing the increase of 15 gm, 30 gm, 39 gm and 75 gm when recorded after one week, one month, three months and six months respectively.

Table 1. Comparative body weight changes in different groups of animals.

Animal groups/ duration	Initial weight in grams (mean)	Final weight in grams (mean)	Weight changes in grams (mean)
1 week ALC (2nd gr)	165	180	15
1 month ALC (3rd gr)	165	195	30
3 months ALC (4th gr)	165	204	39
6 months ALC (5th gr)	165	240	75
6 months ALC+AO (6th gr)	165	201	36
6 months Control- C_2 (1st gr)	165	204	39

p-value by T-test between mean values of initial weight and final weight <0.01 (0.006), significant. ALC= Alcoholic and AO= Antioxidant

Table 2 shows vitamin A distribution in all the subcellular fractions though the nuclear fraction and light mitochondrial fraction show slightly higher level. Effect of alcohol loading in the level of vitamin A can be seen in the subcellular fractions are all significant. Heavy mitochondrial fraction shows the greatest fall (p <0.001). Rearing the animal for six months showed decrease in the level of vitamin A in nuclear fraction, heavy and light mitochondrial fractions in the first (Control 2, C_2) and sixth groups (alcohol and antioxidant).

Table 2: Comparative study of vitamin A concentration in various subcellular fractions of alcoholic rat liver.

S.F.	Control-1 Mean±SD	Alcohol 1 week Mean±SD	Alcohol 1 month Mean±SD	Alcohol 3 months Mean±SD	Alcohol 6 months Mean±SD	Alcohol Antioxidant 6 months Mean±SD	Control C_2 Mean±SD
H	68.80±5.00	71.35±0.74	66.75±1.68	60.20±2.62*	52.27±2.99*	76.62±2.98**	68.67±0.79
N	18.36±0.99	18.35±0.63	14.45±1.73*	14.53±1.69*	13.83±1.47*	22.40±2.60*	17.00±1.81
M_1	16.67±0.47	17.14±0.38	12.76±1.39**	12.50±1.41**	12.16±1.39**	20.69±2.59*	14.18±1.51*
M_2	18.28±0.34	18.11±0.43	14.27±1.74**	14.26±1.74*	13.77±1.52**	17.05±1.02	17.09±0.74*
Sup	15.31±0.67	15.88±1.03*	13.67±1.95	12.82±1.35*	12.73±1.29*	17.46±0.52**	15.17±0.85

Values expressed as mg/G liver. *p <0.05 **p <0.001 S.F. = Subcellular fraction, H= Homogenate, N= Nuclear fraction, M_1= Heavy mitochondrial, M_2= Light mitochondrial, Sup= Supernatant.

The quantity of vitamin C recovered as sum of all the fractions seems to be much higher than that of the whole homogenate. The recovery is higher in the soluble fraction than that of the whole homogenate. On alcohol loading, vitamin C level decreases in the soluble fraction and on antioxidant supplementation in the sixth group, the level of vitamin C increases significantly in 15000 X g, 1 hr. supernatant. Sacrificing the animals after 6 months decreases in level of vitamin C in 15000 X g, 1 hr supernatant and the decrease is similar in both alcoholic and non alcoholic groups, suggesting a negligible role of alcohol in changing the hepatic vitamin C level (Table 3). The changes must be simply because of aging.

Table 3: Comparative study of vitamin C concentration in various subcellular fractions of alcoholic rat liver.

S.F	Control-1	Alcohol			
	Mean±SD	1 week Mean±SD	1 month Mean±SD	3 months Mean±SD	
H	201.17±6.65	189.33±4.84*	189.33±4.84**	186.50±3.45*	1
N	44.95±2.44	44.70±2.51	43.75±0.76	43.75±0.76*	
M_1	43.47±1.00	43.47±1.00	43.75±0.76	43.75±0.76	
M_2	43.47±2.07	43.75±0.76	44.70±2.51	44.70±2.51	
Sup	220±10.73	206.33±4.97*	207.33±2.51	267.33±13.54*	2

Values expressed as mg/G liver. *p <0.05 **p <0.001 S.F. = Subcellular fraction, H= Homogenate, N= Nuclear fraction, M_1= Heavy mitochondrial, M_2= Light mitochondrial, Sup= Supernatant.

Discussion:

In this study, we found that alcoholic animals had a better weight gain when compared to control and alcoholic groups supplemented with antioxidant vitamins. Alcohol when given in reduced dose, instead of causing any harmful hepatic changes, might have simply stimulated the whole organ system thereby increasing different vital functions leading to better appetite and food intake. This may be one of the possible reasons of getting higher weight gain in alcoholic group.[12] The rapid increase in weight may not be a good sign of healthiness because most of chronic alcoholics are always on higher side of expected normal weight. The cause of the increased body weight may be due to increased deposits of hepatic lipids and also other adipose tissues. Alcohol has extra calories of its own and if taken regularly becomes an appetizer.

The fall in vitamin A content in the alcoholic liver may be due to its utilisation in trying to control the ethanol mediated free radical generation. The low recovery from the subcellular fractions may be explained on the basis of the loss of certain naturally occurring protective antioxidant, after cell fractionation which in turn leading to the mobilisation of more of the vitamin A from the hepatic store in the alcoholic animals. Due to the lack of ethanol mediated free radical

generation in non alcoholic animals (control), naturally occurring protective antioxidants may still be present unaffected and thus recovery after cell fractionation may still be maintained at 100%. The complete recovery of vitamin from the subcellular fractions in alcoholic animals supplemented with the antioxidant vitamin shows the importance of vitamin A in counteracting the generation of free radicals or quenching the free radicals already generated.

One week ethanol feeding seems, to have no impact on the antioxidant status of the animal. Only light mitochondrial fraction shows just significantly decreased level. Maria et al, 1982 reported the changes in the structure of mitochondria in ethanol fed rats.[13] Further, one month of ethanol loading, affected all the subcellular fractions. The homogenate however, still maintain the vitamin level suggesting the presence of naturally occurring protective antioxidant. The sudden significant decrease of the vitamin level in the subcellular fractions explains the loss of the said natural antioxidant during the cell fractionation initiated by the ethanol mediated free radicals. After three months the alcoholic animals show the same pattern of changes as that of one month already discussed. Here the only change is that homogenate show a significant decrease in its vitamin A content. After six months of ethanol loading, the light mitochondrial fraction shows a further fall in hepatic vitamin A content supporting the earlier views that a drastic structural change may be related to its increase fall of the vitamin.

When antioxidant vitamin is given along with the alcohol for six months, the antioxidant status in the hepatic subcellular fractions show a dramatic improvement. All the subcellular fractions shows significantly increased level of the vitamin except that of light mitochondrial which has been considered as the most sensitive fraction in terms of its capacity to hold this vitamin because of its ethanol sensitive structural changes, the extent of the decrease however, is not significant. In short, a conclusion may be drawn for the antioxidant vitamin A as having an important role in checking the level of hepatic antioxidant levels during ethanol loading thereby helping in the prevention of ethanol mediated hepatic injury. Aging also seems to affect the vitamin A status of two mitochondrial fractions sparing rest of the fractions.

Vitamin C is mainly recovered from soluble fraction and is not affected by the progress of alcohol loading. The aging process seems to be the major factor in changing vitamin levels in soluble fraction of the cells. The study reaffirms that antioxidant supplementation seems to be useful in maintaining the vitamin levels affected by process of aging and alcohol.

Conclusion:

From all the findings, it is suggested that antioxidant vitamin supplements will be beneficial to alcoholic population but for it to be recommended, a thorough trial study in human alcoholics with a well control dietary chart and proper assessment of health status at different stages of aging be very much needed.

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