Effect of Zinc Supplementation on ^{131}I Uptake Studies in Alcoholic Rats

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***Abstract*---**Zinc is a powerful antioxidant and an essential trace element which plays important role in treating adverse effects of alcohol. Present study aimed at investig1ating the effect of zinc under alcoholic intoxication in thyroid. Male Wistar rats were divided into four groups; Normal control, Ethanol treated, Zinc treated and Zinc+Ethanol treated. 3 ml of 30% ethanol was given orally to ethanol and zinc + ethanol treated animals daily for 2,4 and 8 weeks. Zinc ($ZnSO_4.7H_2O$) was given at a dose of 227 mg/L mixed with drinking water of the animals. Ethanol feeding resulted in an increase in 2 hour and 24 hour thyroidal ^{131}I uptake and decrease in biological and effective half lives of ^{131}I. Zinc supplementation could restore above mentioned parameters which were altered under ethanol intoxication. Therefore, it appears that zinc has somewhat protective potential in normalizing some of the damage caused in thyroid functions following ethanol administration.

***Keywords*---**Zinc, Ethanol, Half life, Thyroid ^{131}1- uptakes studies

I. Introduction

ALCOHOL influences virtually every organ directly or indirectly and produces detrimental effects on human systems [1]. Alcohol induces injury to gastrointestinal mucosa, liver, brain, and pancreas and causes several other hormonal disorders. Many investigators have examined the effects of ethanol on thyroid functions in patients with chronic alcoholism or in animals with ethanol feeding [2]. The interaction of thyroid and alcohol includes alteration in thyroid hormone metabolism, iodine uptake, binding by plasma protein and thyrotropin releasing and stimulating hormone levels [3]. A direct effect of ethanol on intracellular thyroid hormone metabolism and/or function seems conceivable [4]. However, the effects of ethanol on endocrine physiology, mainly the thyroid functions, are diverse and are not well understood.

Due to its ability to diffuse across all biological membranes, ethanol exerts its effects on the absorption of various exogenous compounds and heavy metals [5].The implication of essential trace elements in endocrinological processes mainly thyroid function, have been reviewed [6]. Most concerned elements in this field are iodine, selenium, copper and also zinc.

Zinc is one of the major trace elements, which has a clearly defined role in thyroid hormone metabolism. However, the exact mechanism of action of zinc and it's exact role in regulating thyroid hormone metabolism is not well understood. Alcoholics are often characterized by hyperzincuria and hypozincemia having increased urinary zinc and decreased serum zinc along with low zinc level in the body tissue [7]. This derangement of zinc metabolism may be a possible factor responsible for deleterious effects of chronic alcoholism.

Therefore, information on thyroid functions shall be required for better therapeutic management in such adverse conditions. So the present study was undertaken to investigate the efficacy of zinc in regulating thyroid functions under conditions of ethanol intoxication.

II. Methods

Male Wistar rats weighing 150-195 g were procured from the Central Animal House of Panjab University, Chandigarh. The principles of animal care as laid down by the National Institute of Health were strictly followed. The animals were acclimatized in polypropylene cages in the departmental animal house under hygienic conditions for 1 week before being subjected to various treatment schedules. The animals had free access to food and water throughout the study.

The animals were randomly segregated viz., control, ethanol- treated, zinc treated and zinc + ethanol treated (combined treatment group). 3 ml of 30% ethanol was administered orally daily to the ethanol- treated group for a period of 8 weeks. Zinc sulfate ($ZnSO_4.7H_2O$) at a dose level of 227 mg/L was mixed in the drinking water of the rats of the zinc-treated group [3]. The animals of the combined treatment group were administered ethanol as given in ethanol- treated group and zinc as given in the zinc group.

Thyroid radioiodine uptake measurements: At the end of each treatment schedule, an amount of 0.37 MBq (carrier-free) of ^{131}I (BRIT-BARC, Mumbai, India) was given intraperitoneally to each animal, ^{131}I uptake measurements over the thyroid were performed at 2 h, 24 h and, thereafter, daily at 24-h intervals, for a total duration of 10 days by using the IAEA (International

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Atomic Energy Agency, Vienna, Austria) recommended well-type gamma-sensitive probe (ECIL, Hyderabad, India).

During the course of recording radioactivity, five sets of measurements/ counts over the thyroid were taken on each animal in order to minimise the statistical error (SE). The SE for the count rate/s for each animal was calculated to be 5%. The standard activity of ^{131}I (equivalent to that injected in each animal) was also measured to account for the physical decay of the radioisotope and possible instrumental error, during the study and to calculate percentage uptakes of ^{131}I by the thyroid at 2 h and 24 h

To determine the biological half-life (T_{biol}) in the thyroid, the percentage of ^{131}I uptake values at different time intervals from 24-h onward were calculated by taking the 24-h uptake as 100%.

The per cent thyroidal ^{131}I uptake values were plotted (y-axis – log scale) as a function of time (x-axis – linear scale) on a semi-log paper. Further, the T_{biol} of ^{131}I was interpolated from the semi-log plot and was calculated by taking the difference on the x-axis of any two points, where the percentage uptake was being bisected [8].

Estimation of iodoaminoacids**:** Iodoaminoacids were separated from the thyroid gland of rats at the end of the experiment in normal controls and all the treated groups. Iodoaminoacids were separated following the method of Mouriz et al. [9].

Statistical analysis: The statistical significance of the values has been determined using analysis of variance (ANOVA) followed by Newman Keul's test and the determinations are represented as Means ± S.D.

III. Results

Ethanol feeding caused a statistically significant decrease ($p<0.01$) in zinc levels in the serum as compared to the normal controls. However, zinc administration caused a statistically significant increase ($p<0.05$) in zinc levels as compared to the normal controls. Co-administration of ethanol and zinc did not result in any significant change in zinc levels in comparison with the normal controls but showed a significant increase in comparison with ethanol fed group ($p<0.001$) (TABLE I).

Ethanol consumption significantly increased the thyroidal 2h and 24h ^{131}I uptake only after 4 weeks ($p<0.05$) as compared to the normal controls. Zinc supplementation alone was found to increase 2h ($p<0.05$) and 24h ($p<0.01$) thyroidal ^{131}I uptake significantly only after 4 weeks and 2h uptake after 8 weeks ($p<0.05$) as compared to the normal controls, thus indicating that zinc may be playing a protective role in improving thyroid function. On the other hand, when zinc was administered along with ethanol, the 2h ($p<0.05$) and 24h ($p<0.001$) of ^{131}I uptake in thyroid was found to be increased significantly after 4 weeks as compared to normal control rats. However, the uptake was found to decrease significantly ($p<0.01$) when compared with ethanol fed rats, and 2h uptake also showed the same trend (TABLE II and III). Thus combined ethanol and zinc treatment showed significant elevation in ^{131}I uptake after 4 weeks in comparison to normal controls. However, this uptake was less as compared to ethanol fed rats

Zinc treatment as well as ethanol treatment showed statistically significant decrease in T_{biol} of ^{131}I only after 4 weeks ($p<0.01$) in comparison with normal controls. Similar trend was observed in the effective half-life of ^{131}I after all the treatment durations as was found in case of the biological half- life of ^{131}I (TABLE V). Zinc supplementation resulted in a significant decrease in biological and effective half-lives of ^{131}I after 4 weeks. T_{biol} of ^{131}I was found to be increased significantly ($p<0.001$) after 2 weeks of combined ethanol and zinc treatment when compared to normal controls. When combined ethanol and zinc treated rats were compared with ethanol treated ones, the T_{biol} was found to be elevated significantly after 2 ($p<0.01$) and 8 ($p<0.05$) weeks (TABLE IV).

DIT, MIT, T_4 and T_3 were found to be reduced following ethanol feeding in comparison with the normal controls, Only Iodide was found to be significantly increased ($p<0.001$) after 8 weeks of zinc supplementation as compared to normal controls. Combined ethanol feeding and zinc supplementation caused a significant reduction in DIT ($p<0.01$) and T4 ($p<0.05$), significant elevation in iodide ($p<0.001$) when comparison was made with the normal control group. On the other hand, iodide was found to be increased significantly ($p<0.01$) when compared with ethanol fed rats (TABLE VI).

IV. Discussion

Reduced serum zinc concentrations upon ethanol feeding in the present study are in agreement with the observations of other workers [10]. These lowered serum zinc levels can be explained by an increased ethanol-induced urinary zinc excretion. Inadequate zinc ingestion by the chronic alcoholics has also been postulated [11]. This may reflect the mobilization of zinc from tissues such as erythrocytes by increased catabolism due to excess thyroid hormones [12]. Lowered zinc concentration as a result of ethanol feeding could also be due to some alteration in the transport or metabolism of zinc in toxic conditions afforded by ethanol. The high turnover of zinc following its supplementation may be related to the increased induction and mobilization of metallothionein as reported by other workers [13]. Restoration of normal zinc levels upon zinc supplementation to ethanol fed rats confirm that body zinc content has a direct bearing on dietary zinc levels.

Ethanol consumption significantly increased the thyroidal 2h and 24h ^{131}I uptake only after 4 weeks as compared to the normal controls. This is in agreement with

the study of earlier workers too [13]. The net increase in thyroidal ^{131}I uptake in rats treated with ethanol could be due to either by increased intrathyroidal availability of trapped iodine or by increased discharge of thyroid hormones [14]. Combined ethanol and zinc treatment showed significant elevation in ^{131}I uptake after 4 weeks in comparison to normal controls. However, this uptake was less as compared to ethanol fed rats which suggest that zinc may be playing an important role in regulating the thyroid ^{131}I uptake. Zinc has earlier been shown to bind with thyroid hormone receptors and improves thyroid function in hypozincemics [15]. The decrease in biological and effective half- lives of ^{131}I was significantly less after 4 weeks of ethanol feeding suggest increased turnover of ^{131}I. Moreover, ethanol feeding for 4 weeks has also shown increase in thyroidal ^{131}I uptake which may corroborate the increased requirement of iodine by the body and hence the reduced biological and effective half-lives of iodine. Further, reduced biological and effective half-lives of iodine in the ethanol treated animals as compared to that of the normal controls indicates its increased turnover which may be explained on the basis of some reports which indicate hyperthyroidism following alcohol ingestion [16]. Zinc supplementation resulted in a significant decrease in biological and effective half-lives of ^{131}I after 4 weeks. This could be due to increased ^{131}I uptake as observed in the present study and hence increased turnover of ^{131}I in the thyroid. When combined ethanol and zinc treated rats were compared with ethanol treated ones, the T_{biol} was found to be elevated significantly after 2 and 8 weeks thus suggesting that zinc is playing a protective role under these conditions by normalizing the reduced values of biological and effective half-lives.

Only Iodide was found to be significantly increased after 8 weeks of zinc supplementation as compared to normal controls which might be due the dominant effect of zinc over ethanol as zinc treatment alone indicated significant rise in iodide [17]. Combined ethanol feeding and zinc supplementation caused a significant reduction in DIT and T4, significant elevation in iodide as compared to the normal control group. This could be due to the cumulative effect of both ethanol as well as zinc.

Thus, it is concluded from the study that zinc is a powerful modulator of several physiological functions and it has somewhat potential in alleviating some of the altered thyroidal functions following ethanol administration. It is evident that the work presented here may have both practical and theoretical implications in that the resulting biochemical and clinical modifications can be prevented by adequate supplementation of zinc. So the possible design of drugs is required which may specifically improve the thyroid functions under such conditions.

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TABLE I
EFFECT OF ZINC ON SERUM ZINC LEVELS FOLLOWING ZINC ADMINISTRATION TO ETHANOL TREATED RATS

Groups	Zinc levels (µg/g)
I Control	35.31 ± 2.90
II Zinc	40.29 ± 0.73[a]
III Ethanol	26.84± 1.94 [b]
IV Ethanol +Zinc	38.06± 1.83 [r]

Values are expressed as Means± SD of 6 to 8 animals.
[a]p< 0.05, [b]p< 0.01 by Newman – Keuls test when the values of group II, III and IV are compared with those of group I.
[r]p< 0.001 by Newman – Keuls test when the values of group IV are compared with those of group III.

TABLE II
EFFECT OF ZINC ON 2H THYROIDAL 131I UPTAKE AFTER DIFFERENT DURATIONS ON ETHANOL FED RATS

Groups	Duration of Treatments		
	2 weeks	4 weeks	8 weeks
I Control	21.44 ± 5.81	24.96 ± 8.61	31.31 ± 5.57
II Ethanol	19.76± 5.57	44.37± 7.08 [c]	36.56± 2.22
III Zinc	18.61 ± 4.85	31.58 ± 6.43 [a]	38.15 ± 4.58 [a]
IVEthanol +Zinc	23.53± 3.74	34.74± 3.52 [a,q]	30.58± 2.81 [p]

Values are expressed as Means± SD of 6 to 8 animals.
[a]p< 0.05, [c]p< 0.001 by Newman – Keuls test when the values of group II, III, IV, are compared with those of group I.
[p]p< 0.05, [q]p< 0.01 by Newman – Keuls test when the values of group IV, are compared with those of group II.

TABLE III
EFFECT OF ZINC ON 24H THYROIDAL 131I UPTAKE AFTER DIFFERENT DURATIONS ON ETHANOL FED RATS

	Percentage Uptake of ^{131}I at 24 h		
Groups	Duration of Treatments		
	2 weeks	4 weeks	8 weeks
I Control	43.48 ± 3.23	46.09 ± 9.59	49.01 ± 8.71
II Ethanol	39.39± 3.61	67.72± 2.92 [c]	53.23± 4.14
III Zinc	39.19 ± 2.40	54.57 ± 4.65 [b]	51.65 ± 7.81
IV Ethanol +Zinc	37.37± 6.41	57.58± 3.52 [c,q]	46.09± 7.35

Values are expressed as Means± SD of 6 to 8 animals.
bp< 0.01, cp< 0.001 by Newman – Keuls test when the values of group II, III, IV, are compared with those of group I.
qp< 0.01 by Newman – Keuls test when the values of group IV are compared with those of group II .

TABLE IV
EFFECT OF ZINC ON BIOLOGICAL HALF LIVES OF 131I IN THYROID ON ETHANOL FED RATS.

	T_{biol} (hours)		
Groups	2 weeks	4 weeks	8 weeks
I Control	108 ± 15.50	111 ± 10.45	98 ± 20.09
II Ethanol	119± 11.71	73 ± 19.02 [b]	95± 14.09
III Zinc	115 ± 9.35	78 ± 13.45 [b]	83 ± 14.67
IV Ethanol +Zinc	145± 7.04 [c,q]	98± 22.47	116± 13.37 [p]

Values are expressed as Means± SD of 6 to 8 animals.
[b]p< 0.01, [c]p< 0.001 by Newman – Keuls test when the values of group II, III, IV, are compared with those of group I.
[p]p< 0.05, [q]p< 0.01 by Newman – Keuls test when the values of group IV, are compared with those of group II .

TABLE V
EFFECT OF ZINC ON EFFECTIVE HALF LIVES OF 131I IN THYROID OF ETHANOL FED RATS

	T_{eff} (hours)		
Groups	2 weeks	4 weeks	8 weeks
I Control	70 ± 6.37	69 ± 4.24	62 ± 8.67
II Ethanol	75± 4.46	51 ± 9.64 [b]	61± 6.31
III Zinc	74 ± 3.66	54 ± 6.82 [b]	55 ± 8.41
IV Ethanol +Zinc	84± 2.29 [c,q]	63± 9.14 [p]	70± 5.34 [p]

Values are expressed as Means± SD of 6 to 8 animals.
[b]p< 0.01, [c]p< 0.001 by Newman – Keuls test when the values of group II, III, IV, are compared with those of group I.
[p]p< 0.05, [q]p< 0.01 by Newman – Keuls test when the values of group IV, are compared with those of group II .

TABLE VI
EFFECT OF ZINC ON THE DISTRIBUTION OF IODOAMINOACIDS AND IODIDE IN THYROID OF ETHANOL FED RATS.

Groups	DIT	MIT	IODIDE	T_4	T_3
I Control	30.96 ± 3.91	17.15 ± 4.52	22.28 ± 3.94	19.23 ± 2.20	12.19 ± 1.27
II Ethanol	27.57± 5.29	19.45 ± 3.36	24.15± 4.00	16.11± 2.69	10.86 ± 0.95
III Zinc	26.33 ± 3.16	21.87 ± 2.45	32.55 ± 3.45 [c]	18.64 ± 2.38	11.78 ± 1.21
IV Ethanol +Zinc	21.71± 2.81 [b]	22.83± 3.03 [a]	32.71± 3.86 [c,q]	14.12± 2.90 [a]	10.89± 1.97

Values are expressed as Means± SD of 6 to 8 animals.
[a]p< 0.05, [b]p< 0.01, cp< 0.001 by Newman – Keuls test when the values of group II, III, IV, are compared with those of group I.
[q]p< 0.01 by Newman – Keuls test when the values of group IV, are compared with those of group II .

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