

Altered Acetylcholinesterase and Na⁺-K⁺ATPase Activities in Different Areas of Brain in Relation to Thyroid Gland Function and Morphology Under the Influence of Excess Iodine

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ABSTRACT

Background and objectives: Thyroid hormone exercises vital role in the event of development of central nervous system in stage of fetal life, childhood, and maintains tropic action in adulthood and also in aging. Deficiency in thyroid hormones responsible for impairment of mental performance. Inadequate iodine intake was is the major health problem. Use of iodized salt is now in vogue globally including India to eradicate iodine deficiency disorders (IDDs). This salt iodization should be at par to the iodine content of environment or water. Hilly areas have low iodine content whereas in coastal areas have higher iodine content in vegetables and water; however the people of both the areas are consuming uniform iodine containing salt. Therefore there is a chance of consuming excess iodine of the people in environmentally iodine sufficient areas. So far the effect of excess iodine specially in brain in relation to thyroid gland function yet have not been extensively investigated. **Materials and method:** For the study 36 number of rats weighing 120±10g of 90±5 days old, primarily divided into two groups on the basis of the duration of treatment viz. 30 days group and 60 days group respectively and on the exposure of the dose of iodine was divided in to two sub-group, excess iodine exposed group (100HI), was gavaged orally potassium iodide (KI) at the dose of 7 mg/Kg body weight and excessive iodine group (500HI) was gavaged orally KI at the dose of 35mg/Kg body weight. **Results:** Our overall result reveal that those doses of excessive iodine causes a state of hypothyroidism as manifested by serum T3 serum T4 and TSH levels and thyroid morphology and also alters of cholinergic homeostasis in different areas of brain as revealed by acetylcholinesterase and Na⁺-K⁺ ATPase activity. **Conclusion:** Excessive iodine with higher dose and duration causes hypothyroidism which is responsible for alteration of cholinergic homeostasis in different region of brain.

Keywords: IDD, excess iodine, hypothyroidism, cerebral cortex, hippocampus, hypothalamus, cerebellum, acetylcholinesterase, cholinergic homeostasis.

INTRODUCTION

Iodine is one of the raw materials required for the production of thyroid hormones –thyroxine (T₄) and triiodothyronine (T₃), being incorporated into tyrosine residue to thyroglobulin. It is documented and accepted that iodine deficiency is associated with the development of thyroid function abnormalities¹. Iodine deficiency causes goitre, mental defects, still birth, miscarriage, cretinism². All these manifestations are collectively called iodine deficiency disorders (IDD)³. To eradicate IDD salt iodization are in vogue throughout the world including India^{4,5}. Excess iodine intake on the other hand also causes several public health problems. High iodine level in the body increases the risk of development thyroid abnormalities such as hypothyroidism, hyperthyroidism and goiter^{6,7}. The common manifestation of excess iodine are iodine induced hypothyroidism, iodine induced hyperthyroidism, thyroid cancer etc. Prolonged consumption of excess iodine may cause abortion and even still birth⁸. In hypothyroid condition brain shows many structural defects such as increased cell density in the

cerebral cortex, lowers cell numbers in hippocampus and also alters activity status of different neurotransmitters⁹⁻¹¹. The toxic effect of excess iodine has been noted both in animals and also in cell system but effect of excess iodine on thyroid gland is species specific, cytotoxic effects of iodine has been documented in rat thyrocytes but not in dog thyrocytes¹². However, the effect of iodine in excess on functional status of brain in relation to thyroid gland function has not been explored adequately. In this study the effect of iodine in excess at two different doses i.e. one within tolerable range of the thyroid and other above that level caused thyroid dysfunction were administered regularly to adult male albino rats for two different durations (30days and 60days respectively) and the functional status of the important areas of brain viz. cerebral cortex, hippocampus, hypothalamus and cerebellum as evident by acetylcholinesterase, Na⁺-K⁺ ATPase activities and functional status of thyroid gland as evident by thyroid histology, thyroid hormones profile (T₃, T₄ and TSH) were evaluated.

MATERIALS AND METHODS

Maintenance of animal

For the present study, a total of 36 adults (90±5 days) male albino rats (*Rattus norvegicus*) of Wister strain weighing about 120±10gm were obtained from M/S Chakraborty Enterprise, Kolkata (Registration No. 1443/PO/b/11/CPCSEA). The study was done following protocol of Institutional Animal Ethics Committee (IAEC), Department of Physiology, University of Calcutta (approval number IAEC/Proposal-extl-2/2010 dated 12.08.2010). The rats were maintained on a standard diet (20% protein) made of locally available wheat (70%), Bengal gram (20%), fish meal powder (5%), dry yeast powder (4%) refined til oil (0.75%), shark liver oil (0.25%), 4% non-iodized salt and water ad libitum with adequate potassium iodide¹³. The animals were acclimatized to housing condition for one week prior to experiment. They were caged in well ventilated Polypropylene cages (25°C±2°C with plenty of air and light) under 12 hours light and 12 hours dark cycle.

Animal grouping and experimental design for the study

At first 36 number of rats primarily divided into two groups on the basis of the duration of treatment viz. 30 days group and 60 days group respectively. Again each group was further divided in to two sub-group, excess iodine group (100HI), and excessive iodine group (500HI). Therefore total animals groups were: control 30D, 100HI 30D, 500HI 30D and control 60D, 100HI 60D, 500HI 60D. Control animals fed with normal diet and 100HI 30D group was fed potassium iodide (KI) through gavage at a dose of 0.7 mg/100 g body weight for a period of 30 days, 500HI 30D group was fed KI through gavage a dose of 3.5 mg/100 g body weight for a period of 60 days, 100HI 60D group was fed KI through gavage at a dose of 3.5 mg/100 g body weight for a period of 60 days, 500HI 60D group was fed KI through gavage at a dose of 3.5 mg/100 g body weight for a period of 60 days. In this study KI was administered at such a dose that excess iodine corresponds to 100 times (100HI) and excessive iodine corresponds to 500 times (500HI) higher than its physiological daily dose²⁷. Both the treated and control groups of animals were sacrificed by decapitation on the next day of last treatment following ethical protocol.

Separation of serum

Blood sample for hormone assay were collected from the hepatic portal vein of rat. Plasma from blood samples were separated out by centrifugation at 2000 RPM and was stored at -50°C until assay of the same.

Preparation of brain cytosolic fraction

Brain was dissected into four gross anatomical regions - cerebral cortex, hippocampus, hypothalamus and cerebellum carefully according to stereotaxic atlas of Paxinos and Watson¹⁴, were taken in ten times ice-cold phosphate buffer saline pH7.4 (PBS), homogenized and centrifuged at 1300g for 10 min. The resulting pellet consisting of nuclear and cellular materials was discarded. The supernatant was pulled and spun at 17000 g for 15 minutes and cytosolic portion was aliquoted and stored at -50°C for further analysis.

Body and brain weights

The body weights of the animals were recorded on the first day before treatment (initial) and on the day of sacrifice (final). Body weight was averaged for each week until the end of the treatment. The whole body weight and the weight of different areas of brain were measured to the nearest gram.

Analysis of urine sample

Urinary iodine was determined by the arsenite method following dry ashing in presence of potassium carbonate maintaining internal quality control having a known concentration range of iodine content with each batch of test samples. In this method iodine content in urine samples were estimated by drying urine at 600° C in presence of potassium carbonate and the iodine present in the ash was measured by ceric-arsenite system¹⁵.

Estimation of SGOT and SGPT

SGOT and SGPT activity of both in serum was estimated using enzymatic kit of BBI Solution (Cardiff, UK) following manufacturer's protocol.

Protein Estimation

Protein content of the sample was determined by method of Bradford using BSA (Sigma Chemical Company, USA) as standard¹⁶.

ELISA of Serum T3, T4, and TSH

Serum total T3 and T4 were assayed using ELISA kits obtained from RFCL Limited, India (Code no. HETT 0854 and HETF 1208, respectively) according to the manufactures instruction. Serum TSH was assayed using ELISA kit obtained from Cusabio Biotech Co. Ltd.China (Lot no. C0710270665) according to the manufactures instruction.

Brain Na⁺-K⁺ ATPase assay

Synaptosomal Na⁺-K⁺ ATPase was assayed in reaction mixture containing (i) 30 mM imidazole, 130 mM NaCl, 20 mM KCl, 4mM MgCl₂ and (ii) 30 mM Imidazole, 4mM MgCl₂ and 1mM ouabain (Sigma Chemical Co. USA) at pH 7.4 for 60 min at 0°C and stopped by addition of 0.1 ml 20 % SDS after 10 min. The inorganic phosphate (Pi) liberated was determined spectrophotometrically at the absorbance of 850 nm¹⁷.

Determination acetylcholinesterase activity

Acetylcholinesterase (AChE) activity was determined in brain regions using acetylthiocholine iodide as a substrate according to the method of Ellman et al. (1961)¹⁸. In this method AChE in samples hydrolyzes acetylcholine iodide into thiocholine and butyric acid. The thiocholine reacts with 5,5'-dithiobis(2-nitrobenzoic acid) to form 5-thio-2-nitrobenzoic acid. The yellow colour developed was measured spectrophotometrically at 412 nm¹⁸.

Histopathology of thyroid gland

After sacrifice the thyroid gland of all animals was dissected out and fixed in buffered formalin. The tissues were processed in the usual way for paraffin embedding and sections were stained with Hematoxylin and Eosin (HE) for histopathology (400X). Tissue samples were processed by conventional paraffin processing method, 5 µm section were stained with haematoxylin and eosin and examined in a light microscope.

Statistical Analysis

Table 1: Alteration in the body weight (g) in experimental group of animals subjected to excess (100 HI) and excessive (500HI) iodine for different duration 30 days and 60days respectively.

Parameters Studied	30days treatment			60days treatment		
	Control	100HI 30D	500HI 30D	Control	100HI 60D	500HI 60D
Body weight(initial)	122.5±4.39	124.67±2.36	121.25±3.7	125.67±2.18	123.42±2.94	127.50±2.18
Body weight(final)	140.33±3.66	135.5±6.9	129.58±5.05	161.50±4.98	147.5±5.43	142.25±4.33
Gain in body weight (%)	14.56	8.69	6.87	28.51	16.27	11.57

Table 2: Alteration of urinary iodine excretion (µg/dl) pattern under the influence of excess (100HI) and excessive (500HI) iodine for different time duration.

30 days treatment			60 days treatment		
Control 30D	100HI 30D	500HI 30D	Control 60D	100HI 60D	500HI 60D
29.95±3.28	40.23±4.78 ^a	97.28±4.59 ^a	32.5±3.6	48.93±3.5 ^{a,b,c,d}	113.7±5.95 ^{a,b,c,d}

Table 3: Alteration in the SGOT and SGPT values of experimental animals subjected to excess (100HI) and excessive (500HI) iodine under different durations of time.

Parameters Studied	30 days treatment			60 days treatment		
	Control	100HI 30D	500HI 30D	Control	100HI 60D	500HI 60D
SGOT (IU/L)	21.34±0.59	23.51±0.95	24.28±1.67	24.2±1.58	24.38±1.19	27.16±1.01
SGPT (IU/L)	21.52±2.02	22.49±1.94	22.93±1.07	23.85±1.69	25.43±1.96	27.82±1.79

Table 4: Changes in relative thyroid gland weight (mg/100 gm) in experimental animals subjected to excess (100HI) and excessive (500HI) iodine for different durations.

30 days treatment			60 days treatment		
Control 30D	100HI 30D	500HI 30D	Control 60D	100HI 60D	500HI 60D
7.55±0.97	7.94±0.87	9.89±0.93 ^a	7.64±0.75	10.69±0.92 ^{a,b,c,d}	11.13±0.07 ^{a,b,c,d}

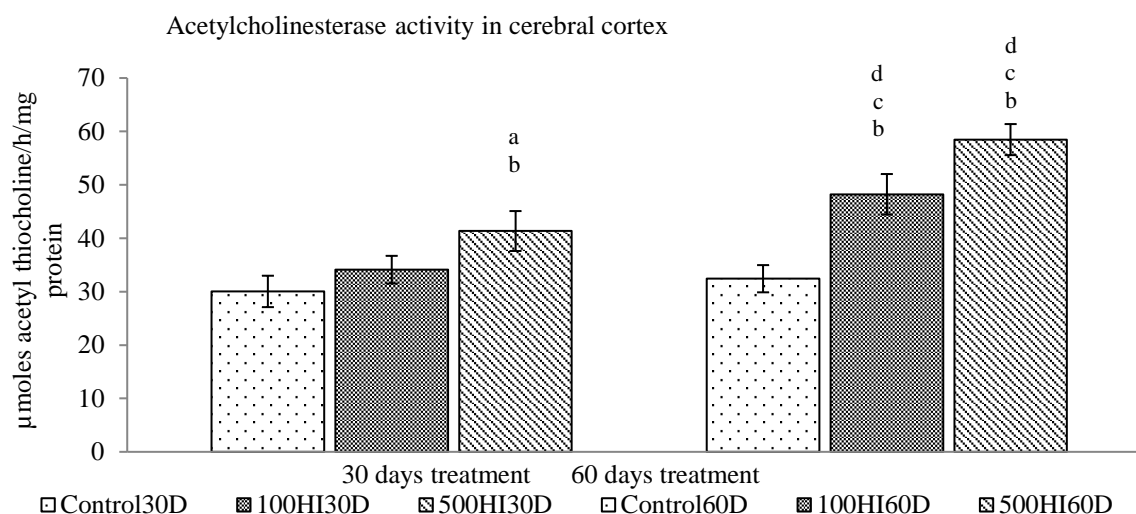


Figure 1: Effect of excess iodine (100HI KI at dose of 0.7 mg KI/ 100 g body weight) and excessive iodine (500HI KI at dose of 3.5 mg KI/ 100 g body weight) on AChE activity in cerebral cortex of adult male rats. Each bar denotes mean ± SD of six animals per group. Mean values are significantly different by ANOVA at P<0.05. Control 30D versus 100 HI 30D versus Control 60D (not significant)

^adenoted significant when compared with Control 30D, ^bdenoted significant when compared with Control 60D. ^cdenoted significant when compared with 100HI 30D. ^ddenoted significant when compared with 500HI 30D.

Results were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) with Tukey's post hoc test was done for determination of level of significance in different control and experimental groups of animals. A value of *p* <0.05 was interpreted as statistically significant. Statistical analysis was done using Origin 8 (Origin Lab Corporation, Northampton, USA)

and MS-Office Excel 2010 (Microsoft Corporation, Washington, USA) software packages.

RESULTS

100HI 30D KI at a dose of 0.7mg/100 g body weight for 30days treatment and 500HI 30D KI at dose of 3.5 mg KI/100g body weight for 30 days, 100 HI 60D KI at dose

Table 5: Alteration in serum T3, T4 and TSH levels of experimental animals subjected to excess iodine (100HI) and excessive iodine (500HI) for different time durations.

Parameter Studied	30 days treatment			60 days treatment		
	Control 30D	100HI 30D	500HI 30D	Control 60D	100HI 60D	500HI 60D
Serum T3 (ng/ml)	2.5±0.13	2.14±0.12 ^a	0.51±0.09 ^{ac}	2.54±0.17	2.21±0.08 ^{abd}	0.34±0.07 ^{abc}
Serum T4 (µg/dl)	5.44±0.3	5.66±0.33	6.57±0.29 ^a	5.69±0.19	7.37±0.20 ^{a,b,c,d}	7.89±0.16 ^{a,b,c,d}
Serum TSH (µIU/ml)	0.15±0.01	0.17±0.01	0.27±0.01 ^a	0.16±0.01	0.34±0.01 ^{b,c,d}	0.48±0.01 ^{b,c,d}

Table 6: Alteration in weight (gm) of different areas of brain in experimental animals subjected to excess (100HI) and excessive (500HI) iodine under different time durations.

Areas of brain	30 days treatment			60days treatment		
	Control 30D	100HI 30D	500HI 30D	Control 60D	100HI 60D	500HI 60D
Cerebral cortex	0.819±0.0047	0.823±0.0048	0.775±0.0051 ^a	0.822±0.006	0.796±0.0047 ^{a,b,c,d}	0.773±0.0057 ^{a,b,c}
Hippocampus	0.18±0.0057	0.173±0.0133	0.166±0.0098	0.187±0.0049	0.158±0.0067 ^{b,c,d}	0.15±0.0039 ^{b,c,d}
Hypothalamus	0.033±0.0073	0.03±0.0067	0.027±0.0039	0.034±0.0061	0.029±0.003	0.025±0.0049
Cerebellum	0.259±0.0086	0.256±0.0062	0.237±0.0052 ^a	0.255±0.0063	0.229±0.0066 ^{a,b,c,d}	0.205±0.0044 ^{a,b,c,d}

Table 7: Alteration of Na⁺-K⁺ ATPase activity in different areas of brain in experimental animals subjected to excess (100HI) and excessive (500HI) iodine for different durations.

Areas of brain	30 days treatment			60 days treatment		
	Control 30D	100HI 30D	500HI 30D	Control60D	100HI 60D	500HI 60D
Cerebral cortex	3.12±0.63	2.83±0.98 ^a	2.42±0.72 ^a	3.15±0.98	1.79±0.67 ^{a,b,c,d}	0.682±0.23 ^{a,b,c,d}
Hippocampus	2.89±0.57	2.81±0.45	1.54±0.64 ^a	2.89±0.68	1.06±0.49 ^{b,c,d}	0.519±0.34 ^{a,b,c,d}
Hypothalamus	2.29±0.54	2.35±0.52	2.47±0.89	2.33±0.59	2.37±0.83	2.48±0.65
Cerebellum	1.84±0.73	1.86±0.57	1.98±0.78	1.89±0.45	1.99±0.28	2.02±0.54

of 0.7mg/100 g body weight for 60 days, 500 HI 60D KI at dose 3.5 mg KI body weight for 60days. Data is presented here as mean±SD, n=6. 100HI 30D at a dose of 0.7mg KI/100g body weight for 30Days, 500HI 30D at dose of 3.5 mg KI/ 100g body weight for 30 days. 100 HI 60D at a dose of 0.7mg KI/100g body weight for 60 days and 500HI 60D at dose of 3.5 mg KI/ 100g body weight for 60 days. Data presented as mean±SD, n=6. Values bearing superscripts are significantly different by ANOVA P<0.05. ^a denoted significant when compared with 30 days control (Control 30D). ^b denoted significant when compared with 60days control (Control 60D). ^cdenoted significant when compared with 100HI 30D. ^ddenoted significant when compared with 500HI 30D. 100HI 30D at a dose of 0.7mg KI/100g body weight for 30Days, 500HI 30D at dose of 3.5 mg KI/ 100g body weight for 30days. 100HI 60D at a dose of 0.7mg KI/100g body weight for 60Days and 500 HI 60D at dose of 3.5 mg KI/ 100g body weight for 60 days. Data presented as mean±SD, n=6. All the values were statistically insignificant at p<0.05. 100HI 30D at a dose of 0.7mg KI/100g body weight for 30 days, 500HI 30D at dose of 3.5 mg KI/ 100g body weight for 30days. 100HI 60D at a dose of 0.7mg KI/100g body weight for 60 days and 500HI 60D at dose of 3.5 mg KI/

100g body weight for 60days. Data presented as mean±SD, n=6. Values bearing superscripts are significantly different by ANOVA P<0.05. ^a denoted significant when compared with 30 days control (Control 30D). ^b denoted significant when compared with 60days control (Control 60D). ^cdenoted significant when compared with 100HI 30D. ^ddenoted significant when compared with 500HI 30D. Effect of excess iodine on serum T3, T4 and TSH of adult male rats. Values were expressed as mean±SD, n=6. 100HI 30D (0.7mg KI/100g body weight) and 500HI 30D (3.5 mg KI/ 100g body weight). Mean values are significantly different by ANOVA at P<0.05. ^a denoted significant when compared with Control 30D, ^bdenoted significant when compared with Control 60D. ^cdenoted significant when compared with 100HI 30D. ^ddenoted significant when compared with 500HI 30D. 100HI 30D KI at a dose of 0.7mg/100 g body weight for 30Days treatment and 500HI 30D KI at dose of 3.5 mg KI/100g body weight for 30Days, 100HI 60D KI at dose of 0.7mg/100 g body weight for 60Days, 500HI 60D KI at dose 3.5 mg KI body weight for 60Days. Data is presented here as mean±SD, n=6. Mean values are significantly different by ANOVA at P<0.05. ^adenoted significant when compared with Control 30D, ^bdenoted significant when compared with Control 60D,

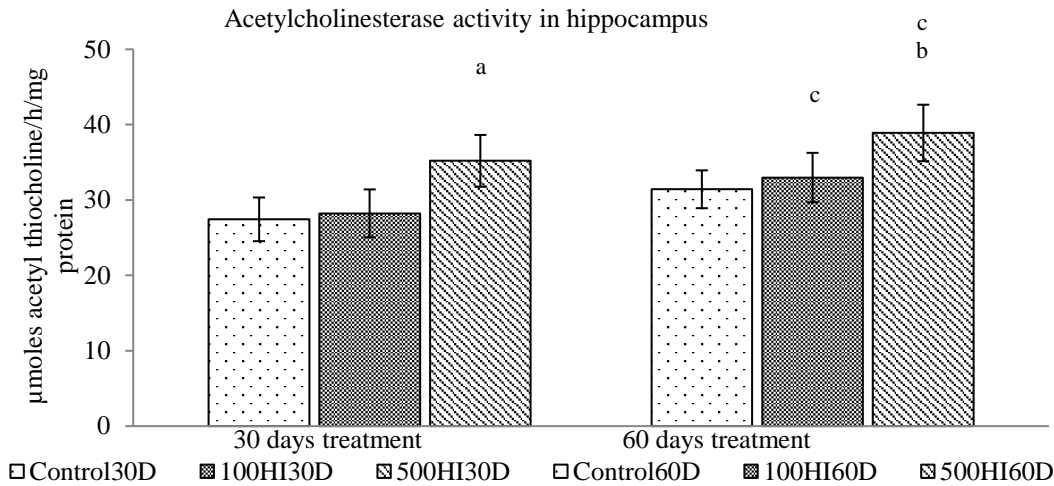


Figure 2: Effect of excess iodine (100HI KI at dose of 0.7 mg KI/ 100 g body weight) and excessive iodine (500HI KI at dose of 3.5 mg KI/ 100 g body weight) on AChE activity in hippocampus of adult male rats. Each bar denotes mean \pm SD of six animals per group. Mean values are significantly different by ANOVA at $P < 0.05$.

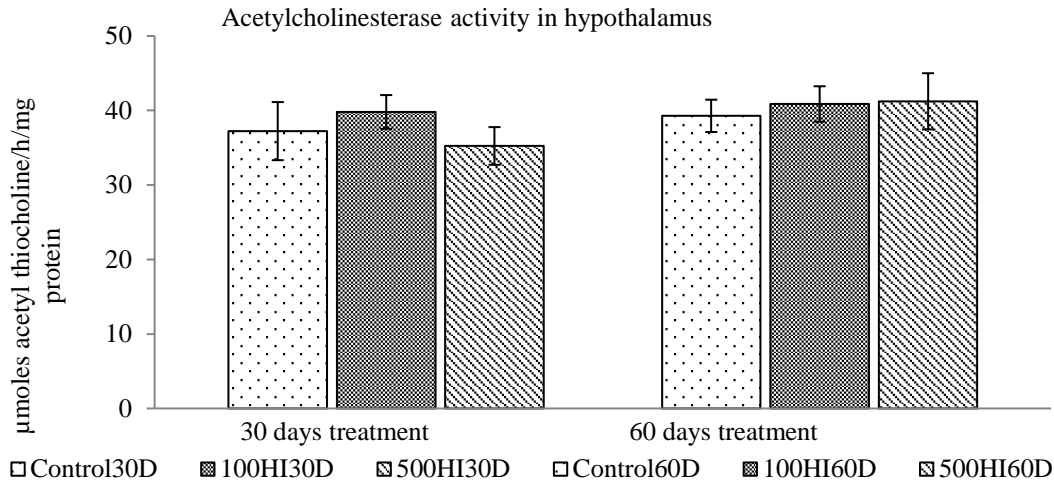


Figure 3: Effect of excess iodine (100HI KI at dose of 0.7 mg KI/ 100 g body weight) and excessive iodine (500HI KI at dose of 3.5 mg KI/ 100 g body weight) on AChE activity in hypothalamus of adult male rats. Each bar denotes mean \pm SD of six animals per group. Mean values are significantly different by ANOVA at $P < 0.05$. Control 30D versus 100HI 30D versus 500HI 30D nonsignificant and Control 60D versus 100HI 60D versus 500HI 60D nonsignificant.

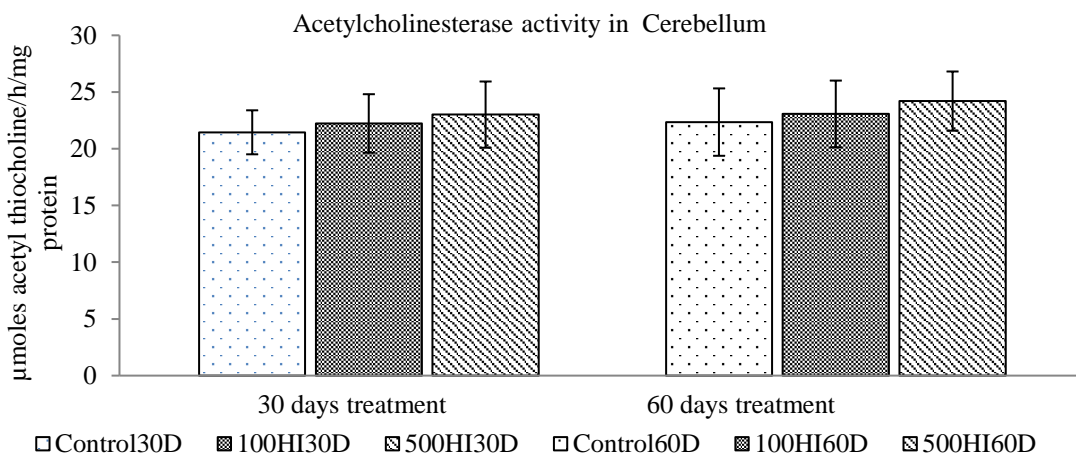


Figure 4: Effect of excess iodine (100HI KI at dose of 0.7 mg KI/ 100 g body weight) and excessive iodine (500HI KI at dose of 3.5 mg KI/ 100 g body weight) on AChE activity in cerebellum of adult male rats. Each bar denotes mean \pm SD of six animals per group. Mean values are significantly different by ANOVA at $P < 0.05$. Control 30D versus 100HI 30D versus 500HI 30D nonsignificant and Control 60D versus 100HI 60D versus 500HI 60D nonsignificant.

^cdenoted significant when compared with 100HI 30D. ^ddenoted significant when compared with 500HI 30D. 100HI 30D at a dose of 0.7mg KI/100g body weight for 30 days, 500HI 30D at dose of 3.5 mg KI/ 100g body weight for 30 days. 100HI 60D at a dose of 0.7mg KI/100g body weight for 60 days and 500HI 60D at dose of 3.5 mg KI/ 100g body weight for 60 days. Data presented as mean±SD, n=6. Values bearing superscripts are significantly different by ANOVA P<0.05. ^a denoted significant when compared with Control 30D, ^bdenoted significant when compared with Control 60D, ^cdenoted significant when compared with 100HI 30D. ^ddenoted significant when compared with 500HI 30D.

Acetylcholinesterase activity in different areas of brain under the influence of excess iodine.

Control 30D versus 100 HI 30D versus Control 60D versus 100HI 60D (not significant) ^a denoted significant when compared with Control 30D, ^bdenoted significant when compared with Control 60D. ^cdenoted significant when compared with 100HI30D.

Histopathology of thyroid gland

DISCUSSION

To evaluate the effect of prolonged increased iodine intake in different areas of brain of adult rats on cholinergic activity, potassium iodide (KI) at higher doses (100 HI and 500 HI) was administered regularly through gavage for different durations (30 days and 60 days respectively). In the experimental animals supplemented with excess iodine, iodine nutritional status was determined measuring urinary iodine content, while toxicity level was analyzed by measuring SGOT and SGPT activities and thyroid functional status was studied as evident by thyroid hormone profiles and thyroid morphology and cholinergic activity status of brain was evaluated as evident by acetylcholinesterase and sodium potassium ATPase activity in different areas of brain viz. cerebral cortex, hippocampus, hypothalamus and cerebellum. Iodine nutritional status of the body is generally evaluated by measuring the urinary iodine concentration as nearly 90% of the body's iodine is excreted through urine¹⁹. Urinary iodine concentration is the key marker of iodine nutritional status thereby helps to identify iodine deficiency or iodine excess²⁰⁻²². In this study iodine nutritional status of the experimental animals after administration of iodine in excess for different durations was evaluated measuring urinary iodine concentration following ceric-arsenite method. It has been found that urinary iodine concentration was increased in a dose dependent manner. Alteration of body weights after administration of excess (100HI) and excessive iodine (500HI) for different durations has been presented in Table 1. Intake of excess iodine significantly reduced the net body weight gain of the experimental group of animals depending on the dose and duration in comparison to the control group. The rate of decrease in body weight gain percentage is consistent with earlier investigations, where excess iodine was found to be involved with altered metabolic condition²³⁻²⁵. This may be due that the excess iodine causes hypothyroidism evident

from the T3, T4 and TSH level of the experimental animals²⁶, which is responsible for the decrement of gain in body weight percentage²⁷. Alteration in weight of different areas of brain has been presented in Table 6. Complete brain development takes place in rat at the age 2-3 weeks of age²⁸. In this study adult rats were used in the age group of 90±5 days. So no further change in weight of different areas of brain was observed in 30 days excess and excessive iodine treatment group of experimental animals. But when the duration of treatment was increased, the weight of the different areas of the brain was decreased. Iodine induced hypothyroidism after the supplementation of excess iodine (500HI) was possibly responsible for decrease of brain weight significantly also observed by different workers²⁹. In the present study no significant change has been found in serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) in both the 100HI and 500HI groups against their respective control. Under the influence of any external substances liver abundantly exposed to the adverse effects of those compounds and is prone to hepatotoxicity and SGOT and SGPT is important marker of hepatotoxicity³⁰. In this study no such increment in SGPT and SGOT activities were noted. Therefore under the influence of excess iodine as used in this study no toxicity was developed. An appreciable fall in serum T3 and T4 levels were observed in 500HI 30D, 100HI 60D and 500HI 60D groups in respect to corresponding control group. In the euthyroid state at normal iodine concentration, the rate of conversion of inorganic iodine from iodine pool to the organic iodine is great for the prevention of attainment of maximal iodine concentration^{31,32}. However administration of excess iodine shows a biphasic response in thyroid gland; first it increases and then decreases organic iodide and thyroid hormone synthesis, the mechanism as mentioned is referred as Wolff-Cheekoff effect^{31,33,34}. Dysfunction of thyroid gland is well documented in human associated with the use of iodine rich contrast agents^{35,36}. No histopathological change in thyroid gland was noted in 100HI 30D group as thyroid gland can tolerate excess iodine even when the factor increases 100 times of its daily dose³⁷. But when the duration or dose of treatment increased, Wolff-Cheekoff block is withdrawn, Sodium Iodide Symporter (NIS) is down regulated and thyroid intracellular iodine drops^{38,39}. In higher concentration iodine could bind to organic compounds and interfere with metabolic processes and responsible for necrotic and apoptotic change of thyrocytes⁴⁰⁻⁴². Histopathological studies in 100HI treated for 60 days showed hyperplasia and necrosis, in 500HI 30D group hypertrophy of colloid follicles and in 500HI 60D group atrophy of thyroid follicles were observed. All these observations suggest that excess iodine developed a state of disfunctioning thyroid as well as hypothyroidism. On the contrary serum TSH level also significantly increased in 100HI 60D and 500HI 60D group with respect to corresponding control group. In our study T3 and T4 levels were reduced and TSH level was increased, developing a state of biochemical primary

hypothyroidism⁴³. Passage of any substances including hormone from blood to brain is regulated by blood brain barrier and blood-cerebrospinal fluid barrier³⁵. Membrane transporter MCT8 and MCT10 are specified for the transport of iodothyronines, with slightly higher affinity for T3 than T4 and mediates both influx and efflux of the hormones^{36,44}. The mode of action of thyroid hormones on brain is through gene expression but non-genomic action also has been proposed⁴⁵. A balance between the rate of synthesis and the rate of release and utilization of a neurotransmitter is the main regulatory mechanism of neurotransmission. Acetylcholinesterase (AChE) plays an essential role in the mechanism of cholinergic neurotransmission by rapidly splitting Ach into acetate and choline^{46,47}. In brain, a significant decrease of choline glycerophospholipids concentration found in hypothyroid condition which leads to diminution in reservoir of membrane choline^{48,49}. The decreased membrane choline supply is reflected by reduction in biosynthesis and concomitant release of Acetylcholine (Ach) from biologically active neurons and thereby enhances acetyl cholinesterase (AChE) activity⁵⁰. A state of hypothyroidism is responsible for alteration of many neurochemical parameters. An increase in AChE activity caused by excess iodine may lead to a reduction of cholinergic neurotransmission efficacy due to a decrease in Ach level in the synaptic cleft. Decreased Ach level in the synaptic cleft causes progressive cognitive impairment, especially in the young adult was suggested due to increase of synaptosomal membrane sphingomyelin. Increased AChE level participates in apoptosis by promoting or suppressing cell death⁵¹. For the supplementation of excess iodine there develops a biochemical state of hypothyroidism that in turn increased acetylcholinesterase activity markedly in cerebral cortex and hippocampus but

not so much in the hypothalamus and cerebellum. The present study has confirmed the involvement of thyroid hormones in regulating the activity of cholinergic system of the different areas of brain in adult rats. Na⁺-K⁺ATPase is a protein responsible for maintaining resting cell membrane potential, abundant in brain and plays a major role in neuronal activity^{52,53}. The Na⁺-K⁺ATPase activity was inhibited significantly in cerebral cortex and hippocampus of excess iodine treated animals but no such significant change was noted in hypothalamus and cerebellum. Excess iodine treatment has reduced the Na⁺-K⁺ATPase activity in major areas of brain by reducing thyroid hormone level thereby developing a state of hypothyroidism. The reduced thyroid hormone level is responsible for decreased of Na⁺-K⁺ATPase activity in a selective way. Na⁺-K⁺ATPase having two subunits namely alpha and beta. T3 regulates the concentration of mRNA expression of the enzyme subunit alpha and beta by binding of T3 to nuclear receptors. So in hypothyroid condition where T3 concentration was significantly lower synthesis of Na⁺-K⁺ATPase was downregulated^{54,55}. The variation in inhibition of Na⁺-K⁺ATPase activity in different brain areas is due to area wise distribution of thyroid hormone receptors. The distribution and density of receptors in the different areas of brain is an important factor in maintaining the crucial role of thyroid hormone in physiology of nervous system. It has been found that cerebral cortex and hippocampus has higher level of thyroid hormone receptors while other region like hypothalamus has a lower density of these receptors⁵⁶. It has been reported that inhibition of Na⁺-K⁺ATPase activity in brain causes edema and cell death in the central nervous system and interferes with learning and memory processes⁵⁷. It has also been reported that Na⁺-K⁺ATPase activity is selectively reduced in patients with cognitive

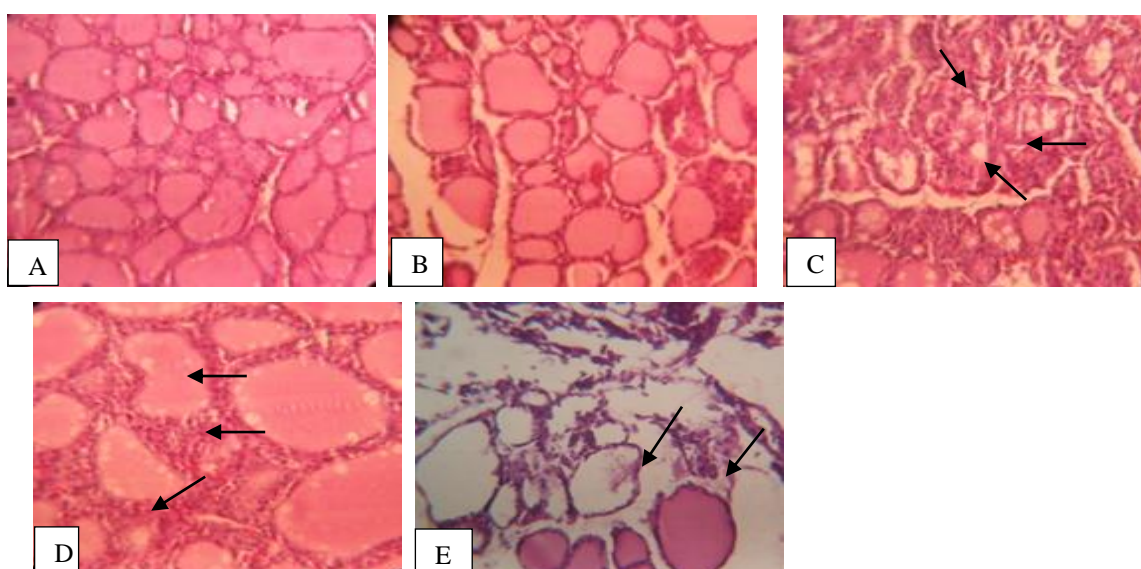


Plate 1: Microphotograph of HE stained section of thyroid gland (x400) of (A) control follicles are normal in shape and size and colloid content is normal, (B)100HI 30D (KI at a dose of 0.7mg/100g body weight for 30 days), follicles are normal in shape and size and colloid content is normal, (C) 500HI 30D (KI at a dose of 3.5mg/100g body weight 30 days) hyperplasia and necrosis of thyroid follicle (D) 100HI 60D (KI at a dose of 0.7mg/100g body weight for 60 days) thyroid gland hypertrophy and scalloping of colloid (E) 500HI 60D (KI at a dose of 3.5mg/100g body weight for 60 days) atrophy of thyroid follicle.

deficits, such as Alzheimer's disease⁵⁸⁻⁶⁰. Earlier work shows that hypothyroidism is responsible for depressive illness, unipolar depression and mental dysfunction⁶¹. The cholinergic pathways in the CNS are also known to participate in various cognitive and memory functions^{62,63}. Therefore excess iodine induced hypothyroidism may be responsible for such consequences in brain function.

CONCLUSION

In summary overall results reveals that iodine in excess that is 500 times more than of its adequate requirement supplemented for short (30 days) and long (60 days) duration and even 100 times more than of its adequate requirement for long (60 days) duration induces a state of biochemical as well as morphological hypothyroidism which causes an imbalance in cholinergic homeostasis that might be considered as an important pathophysiological mechanism for alteration of brain function under such situation.

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