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Iodine in excess in the alterations of carbohydrate and lipid metabolic pattern as well as histomorphometric changes in associated organs

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Abstract

Background: Iodine is a nonpareil constituent of thyroid hormones (THs) and a prime regulator of thyroid gland functioning. Although essential at recommended levels for the prevention of iodine deficiency disorders (IDDs), exposure to excess iodine reportedly causes hypothyroidism, hyperthyroidism, and several other emerging deleterious impacts. The objective of the present study is to explore the influence of excess iodide exposure on carbohydrate and lipid metabolism along with the histoarchitecture of certain associated organs such as the pancreas, liver, kidney, and skeletal and cardiac muscle because information on those aspects was found to be scanty.
Methods: Twelve rats were taken, six were fed with iodine through gavage at a dose of 3.5 mg potassium iodide (KI)/100-g body weight, which corresponded to 500 times of the physiological daily dosage of iodide for a period of 60 days, while the other six formed the control group.
Results: KI-treated rats presented high body weight and urinary iodine with low TH levels, suggesting a primary thyroid dysfunction. There was an increase in blood glucose, cholesterol, triglycerides, low density lipoprotein (LDL), and very low density lipoprotein (VLDL), while high density lipoprotein (HDL) levels decreased. Tissue glycogen content in the liver and skeletal muscle was decreased and was increased in the heart and kidney. Histological sections of the pancreas showed a complete disruption with hardly recognizable histoarchistructure. Treated liver sections displayed the broadened central vein with degenerated hepatocytes, while skeletal muscle sections showed dissolution of muscle fibre cells linked with loss of glycogen from these organs. Histological changes in the heart include features similar to those of a fatty heart with cardiac muscles mutilation, while that of the kidney shows an increase in glomerular tuft size and Bowman's space expansion with general deterioration.
Conclusions: It may thus be concluded that excess iodine exposure for a long duration causes the development of a biochemical state of hypothyroidism. The developed hypothyroidism was found responsible for the hyperglycaemic and hypercholestromic status evident by high blood glucose and cholesterol levels and the depletion of glycogen at its storage sites in the liver and skeletal muscle but the extra deposition in the cardiac muscle and kidney; histomicrophotographs showed severe destruction of the pancreatic structure. All these alterations are conducive for the pathogenesis of cardiovascular and kidney diseases.

Keywords: excess iodine; glucose metabolism; heart; kidney; lipid profile; pancreas.

Introduction

Iodine forms a requisite substrate for the synthesis of thyroid hormones (THs) [1]. It is a trace element with a minimum daily requirement of about 150 μm, which is essential for normal TH synthesis acting as major regulator of thyroid gland functioning. In spite of being indispensable at recommended levels for the prevention of iodine deficiency disorders (IDDs), which raise important health concerns worldwide, exposure to excess iodine reportedly causes hypothyroidism, hyperthyroidism, thyroiditis, and even initiation of carcinogenesis [2]. To prevent iodine deficiency, supplementation of iodine through salt in general and/or other iodine-containing mediators resulted in excessive iodine intake by the population, particularly in environmentally iodine-replete regions, and this poses serious public health concerns such as hypothyroidism, hyperthyroidism, autoimmune thyroid diseases, endemic goiter, and even thyroid cancer [3]. Along with severe thyroidal disturbances, several other deleterious impacts of excess iodine are gradually emerging, which include reproductive disturbances, impairment of pituitary-thyroid axis, immunological alterations, and so forth [4–6].

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It is known that TH has significant roles in regulating hepatic lipid, cholesterol, and glucose metabolism and homeostasis [7]. Existent findings suggest that clinical situations such as non-alcoholic fatty liver disease and type 2 diabetes mellitus, which are allied to dysregulated hepatic metabolism, could involve transformed intracellular TH action [7]. Moreover, TH has crucial roles in lipid metabolism and regulation of metabolic genes [8]. The role of excess iodine in modulating thyroid gland functioning is now well established, and insights into its impact on the other systems including reproduction and immunity via modulating the thyroidal axis or directly are being reported. The influence of excess iodide exposure on carbohydrate and lipid metabolism and associated organs like the liver, kidney, pancreas, and skeletal and cardiac muscle, however, is not clear from the present literary picture and was investigated in the present study.

Materials and methods

Reagents

Potassium iodide (KI) was procured from E-Merck, Mumbai, India. Kits were acquired from BBI solution (Cardiff, UK), MyBioSource (San Diego, CA, USA), and BioVision (CA, USA). All other reagents were procured from Sisco Research Laboratories (SRL), Mumbai, India.

Maintenance of laboratory animals

A total of 12 healthy adult (90 ± 5 days) male albino rats (*Rattus norvegicus*) of the Wistar strain weighing 110 ± 10 g were utilised in this study following the protocol of the Institutional Animal Ethics Committee (IAEC), Department of Physiology, University of Calcutta. The animals were accommodated in clean polypropylene cages and sustained in an air-conditioned animal house (temperature, 22 ± 2 °C; relative humidity, 40–60%) with constant 12:12 light-to-dark cycle. All the animals were maintained on a standardized normal diet, which contained 70% wheat, 20% Bengal gram, 5% fish meal powder, 5% dry yeast powder, 0.75% refined til oil, 0.25% shark liver oil, 4% non-iodized salt, and water *ad libitum* [4]. In addition, KI at the dose of 0.007 mg/100-g body weight (bw) was provided with the above mentioned standard diet on a regular basis [9].

Experimental design

A total of 12 rats were divided into two groups of six rats each. Group I was designated as the control group and fed with normal diet containing KI at the recommended daily level gavaged with sterile water to neutralise any stress impacts due to gavage feeding as compared to treated groups. Group II was designated as the treated group and were fed with iodine through gavage at a dose of 3.5 mg KI/100-g body weight dissolved in sterile water, which corresponded to 500 times of the physiological daily dose of iodide [4]. This dose was above the tolerable level but does not cause any toxicity, as evident by the serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) levels noted from previous studies [4].

The treatment duration for both groups amounted to 60 days, as previous studies [4] have reported changes at such lengths of time and dose. The animals were sacrificed 24 h after the last treatment, following the institutional ethical protocols. Blood samples for assays were collected from the hepatic portal vein of rats. Plasma samples were separated by centrifugation at 2000 RPM and stored at −50 °C until assayed.

Body weight

The initial body weights were noted on the first day before treatment, and the final body weight, on the day of sacrifice.

Estimation of SGOT and SGPT

SGOT and SGPT were assessed using the enzymatic kit of BBI solution (Cardiff, UK) as per the manufacturer's protocol.

Estimation of iodine in urine and different organs

A quantified amount of urine sample was digested in alkaline medium followed by ashing in Muffle furnace (Cheminco, India) at 600 °C, and iodine was estimated by its catalytic action on the reduction of ceric ion (Ce^{++}) to cerous ion (Ce^{3+}), conserving all internal quality control [10]. Tissue iodine was evaluated as per the method of Karmarkar et al. [10], with trivial adjustments.

Assay of serum T_3 and T_4

Serum total T_3 and T_4 were assayed using ELISA kits procured from RFCL Limited, India (Code no. HETT 0854 for T_3 and HETF 1208 for T_4).

Estimation of blood glucose level

Blood glucose was monitored by using "glucotide whole blood glucose meters" using Accu-Chek Go (Roche) glucometer. Glucotide test strips are specific for glucose. A drop of blood from the tail vein of the rat before sacrifice was applied to the Glucotide test strip. The test reaction is based on the hexokinase method using dry reagent technology and provides a quantitative measurement of glucose in whole blood [11].

Measurement of tissue glycogen levels

Tissue glycogen from the liver, kidney, heart, and skeletal muscle was measured using a kit (Catalog K646-100) obtained from BioVision, CA, USA, as per the instructions of the manufacturer.

Assay of lipid profile parameters

Serum total cholesterol level: The total serum cholesterol was estimated using ELISA kit (MBS2600008) from MyBioSource, San Diego, CA, USA, following the manufacturer's procedure.

Serum HDL cholesterol level: The serum HDL cholesterol was assayed using ELISA kit (MBS704516) from MyBioSource, San Diego, CA, USA, as per manufacturer's procedure.

Serum LDL cholesterol level: The serum LDL cholesterol was assayed using ELISA kit (MBS2508037) from MyBioSource, San Diego, CA, USA, as per manufacturer's protocol.

Serum VLDL cholesterol level: The serum VLDL cholesterol was assayed using ELISA kit (MBS2501535) from MyBioSource, San Diego, CA, USA, as per manufacturer's protocol.

Serum triglyceride level: The serum triglyceride level was assayed using ELISA kit (MBS005097) from MyBioSource, San Diego, CA, USA, as per manufacturer's protocol.

Histological and histomorphometric study

Instantly after the sacrifice, the liver, pancreas, heart, kidney, and skeletal muscle of control and experimental animals were collected to study the morphological changes, if any, after exposure to iodine in excess. They were fixed in Bouin's fluid followed by dehydration and embedding in paraffin. Five (5)-µm-thick paraffin sections of each of these organs were taken onto slides and subsequently stained with haematoxylin and eosin (H and E). Each slide was examined under a bright field microscope (model: EVOS XL Core Imaging System, Life Technologies) at a magnification of 20× for apparent histological changes.

To get a better understanding of the organs after excess iodine exposure, details of the cellular structure rather than semiquantitative analysis have been investigated. Sizes of hepatocytes with a clearly visible nucleolus were measured from four different fields of indiscriminately selected sections of the liver. Two diameters at right angles to each other, passing through the center of each cell, were measured. One was considered the long diameter, and the other, the short diameter. The long and short diameters of the nucleus of the hepatocytes with a clearly visible nucleolus were measured. The sizes of central veins were measured by recording two diameters at right angles to each other [12]. The number of pancreatic islets and β-cells was estimated with light microscopy utilising point counting with a 20× objective. For each section, 15 fields of view were analysed at a go. The density of β-cells and islets was calculated as the ratio of β-cells/islets to pancreatic tissue and expressed as a percentage; product of density and pancreatic weight were utilized to obtain β-cell/islet mass [13].

In the kidney sections to study the changes if any between the control and treated, the area of each glomeruli in the fields was measured using ocular micrometer [14].

Skeletal muscle and cardiac muscle test areas were measured with a 20× objective using the Image-Pro Plus software (Rockville, MA, USA). To measure these areas, a circular area was selected with the aid of this software, in which the nuclei were counted. Following this procedure, the area of the respective muscle cells was calculated by dividing the total selected circular area by the total number of nuclei present in that area [15].

Measurement of total oxidant and antioxidant status

Measurement of the TAS: Serum TAS levels were estimated by Erel's method, wherein the antioxidative effect of the sample against the potent free radical reactions, which is initiated by the produced hydroxyl radical, is measured. The results are expressed as µmol Trolox Eq/L [16].

Measurement of the TOS: Serum TOS values were also determined through a method developed by Erel. The assay is calibrated with hydrogen peroxide (H_2O_2), and the results are expressed in terms of micromolar hydrogen peroxide equivalent per litre (µmol H_2O_2 Eq/L) [16].

Calculation of OSI: OSI was defined as the ratio of the TOS level to TAS level. Specifically, OSI (arbitrary unit) = TOS/TAS.

Statistical analysis

Results were expressed as mean ± standard deviation (SD). The significance of difference between the control and treated groups was estimated using Student's two tail 't'-test. In all data sets, a value of $p < 0.05$ was considered as statistically significant. Statistical analyses were performed using GraphPad Prism 6 for Windows (GraphPad Software Inc., USA) and MS-Office Excel 2010 (Microsoft Corporation, WA, USA).

Results

Body weight

Alterations in body weight of control and excess-iodide-treated animals after 60 days of treatment are documented in Table 1. The control animals showed a net body gain

Table 1: Alterations in the body weight (g) and organ weight of experimental animals subjected to excessive iodine for 60 days.

Parameters	Control (60 days)	Excess iodine treated (60 days)
Body weight (initial), g	120 ± 6.25	121.08 ± 7.21
Body weight (final), g	149.8 ± 8.55	159.63 ± 6.12
Percentage gain in body weight	24.16%	31.83%[a]
Pancreas, g	0.478	0.346[a]
Kidney, g	1.805	1.457[a]
Heart, g	0.701	0.583[a]

Data are presented as mean ± SD, n = 6. Values bearing superscripts are significantly different by t-test; $p < 0.05$.

weight of 24.16%, while the treated animals gained body weight was up to 31.83%.

Urinary iodine excretion pattern and serum GOT and GPT activity

Urinary iodine content in treated animals after 60 days was 120.56 ± 2.93 μg/dL, while that of the control group was 33.24 ± 3.23 μg/dL, demonstrating a significant increase ($p < 0.05$) in urinary iodine content in the excess-iodine-administered groups of animals compared to control animals, as shown in Table 2. The serum GOT and GPT levels were elevated but not significantly in the experimental group as compared to the control group represented in Table 2.

Changes in food consumption pattern

Food consumption pattern was noted in each group for the entire period of study, and it was observed that feeding amounts remained almost similar with non-significant changes in experimental group in view of control group, as seen in Table 3.

TH profiles and iodine concentration in different organs

Serum T_3 of control group was 2.84 ± 1.27 ng/dL, while it decreased significantly ($p < 0.05$) to 0.87 ± 2.98 ng/dL in excess-iodine-treated group. Serum T_4 level in experimental animals increased significantly ($p < 0.05$) to 7.15 ± 1.64 μg/dL in comparison to 5.18 ± 1.86 μg/dL of control animals (Table 4).

Iodine concentrations in different organs of excess-iodine-treated animals were found be significantly higher ($p < 0.05$) than those of control animals, as shown in Table 4.

Blood glucose levels

Blood glucose levels increased significantly in experimental animals as compared to those of control animals (Figure 1A).

Tissue glycogen concentrations

Tissue glycogen levels of experimental animals treated with excess iodine declined significantly in the liver and skeletal muscles but increased significantly in the kidney and heart as compared to those of control animals (Figure 1B).

Lipid profile parameters

Serum cholesterol levels including total cholesterol (Figure 2A), VLDL (Figure 2D), and LDL (Figure 2B) increased significantly in experimental animals, while HDL (Figure 2C) levels decreased significantly in control animals. Serum triglycerides level also increased significantly in treated animals as compared to that of control animals (Figure 2E).

Table 2: Alterations in the urinary iodine (μg/dL), SGOT (IU/L) and SGPT (IU/L) of experimental animals subjected to excessive iodine for 60 days.

Parameters	Control	Excess iodine treated
Urinary iodine, μg/dL	33.24 ± 3.23	120.56 ± 2.93[a]
SGOT, IU/L	23.22 ± 1.23	25.52 ± 1.91
SGPT, IU/L	21.95 ± 1.11	22.43 ± 0.87

Data are presented as mean ± SD, n = 6. Values bearing superscripts are significantly different by t-test; $p < 0.05$.

Table 3: Food consumption pattern of experimental animals subjected to excessive iodine for 60 days.

Parameters	Control	Excess iodine treated
Food intake/day/rat, g	25 ± 1.6	25 ± 2.1

Data are presented as mean ± SD. Values bearing superscripts are significantly different by t-test; $p < 0.05$.

Table 4: Alterations in serum T_3 levels (ng/dL), T_4 levels (μg/dL), and iodine concentration in different tissues (mg/100 g) of experimental animals subjected to excessive iodine for 60 days.

Parameters	Control	Excess iodine treated
T_3 levels, ng/dL	2.84 ± 1.27	0.87 ± 2.98[a]
T_4 levels, μg/dL	5.18 ± 1.86	7.15 ± 1.64[a]
Thyroidal iodine concentration	1.54 ± 0.98	2.04 ± 0.83[a]
Pancreatic iodine concentration	0.54 ± 0.11	1.02 ± 0.43[a]
Liver iodine concentration	1.23 ± 0.74	1.45 ± 0.82[a]
Cardiac iodine concentration	1.97 ± 0.28	2.26 ± 0.96[a]
Renal iodine concentration	2.08 ± 0.72	3.12 ± 0.52[a]
Skeletal muscle iodine concentration	1.63 ± 0.56	2.21 ± 0.42[a]

Data are presented as mean ± SD, n = 6. Values bearing superscripts are significantly different by t-test; $p < 0.05$.

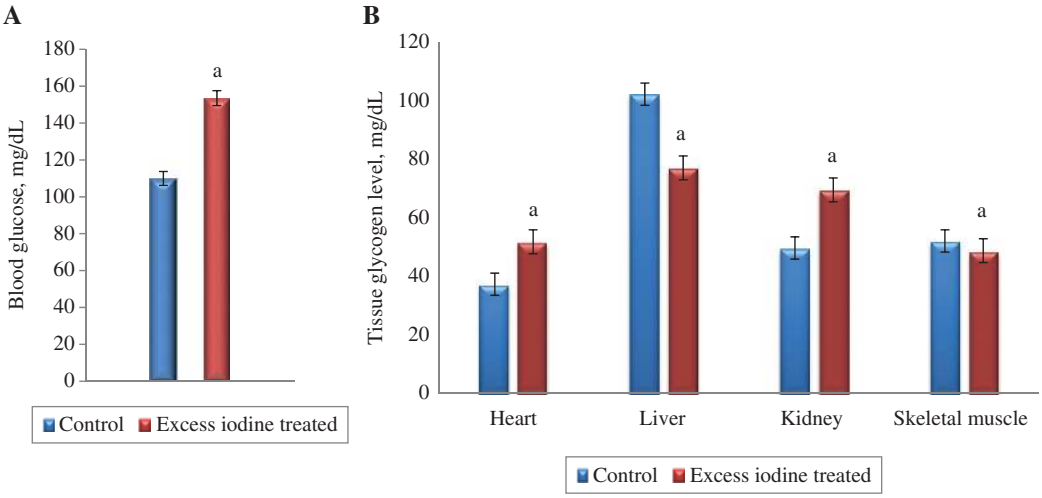

Figure 1: Marked alteration in carbohydrate metabolic pattern was observed as evident by increased blood glucose level and tissue specific changes in glycogen concentration as compared to control.
(A) Alterations in blood glucose level of experimental animals subjected to excessive iodine (3.5 mg KI/100 g bw) for 60 days and
(B) alterations in tissue glycogen content of experimental animals subjected to excessive iodine (3.5 mg KI/100 g bw) for 60 days.
Data are presented as mean ± SD, n = 6. Values bearing superscripts are significantly different by t-test; $p < 0.05$.

Histopathological and histomorphometric changes

Liver

Control liver at 20× (Figure 4A) section showed the presence of central vein, normal hepatocytes with well-placed sinusoids, while excess-iodine-treated liver sections (Figure 4B) in comparison displayed broadened central vein, cytoplasmic vacuolations, and degenerated hepatocytes including degranulated nucleus. Increase in hepatocyte diameter along with increase in central vein diameter in treated animals as compared to normal was observed (Table 5).

Pancreas

Control pancreas at 20× (Figure 4C) showed normal centroacinar cells and islets of Langerhans, while treated pancreas sections (Figure 4D) showed focal necrosis of acini with total disruption of the entire morphology and hardly recognizable histoarchistructure. Significant changes in islet density as well as quantity along with β cell were noted between the two groups (Table 5).

Kidney

Excess-iodine-treated sections at 20× (Figure 5B) showed an increase in glomerular tuft size and Bowman's space expansion with overall swelling and deterioration of tubules along with inflammation in blood vessels and fat deposition in view of the control kidney section (Figure 5A), which shows normal size of glomerulus and Bowman capsules including regular blood vessels and tubules. Morphometric changes in the area, width, and length of both glomerulus and Bowman's capsule of treated group compared with control were observed (Table 5).

Skeletal muscle

The control animals' skeletal muscle fibres at 20× (Figure 5C) were of relatively uniform size and shape, with nuclei located at the periphery of the individual muscle cells (myofibres) with definite bundle structure, while for the excess-iodine-treated animals' sections (Figure 5D), there was a dissolution of muscle fibre cells, nuclear shrinkage, and muscle bundle fracture. Decrease in fibre diameter and nuclei/field view was found in excess-iodine-treated group (Table 5).

Heart

Histological changes in the heart of excess-iodine-treated animals at 20× (Figure 5F) include features similar to that of fatty heart with augmented hyalinization, cardiac muscles mutilation, nuclei loss, and necrosis of muscle fibres in comparison to regular cardiac muscle structure

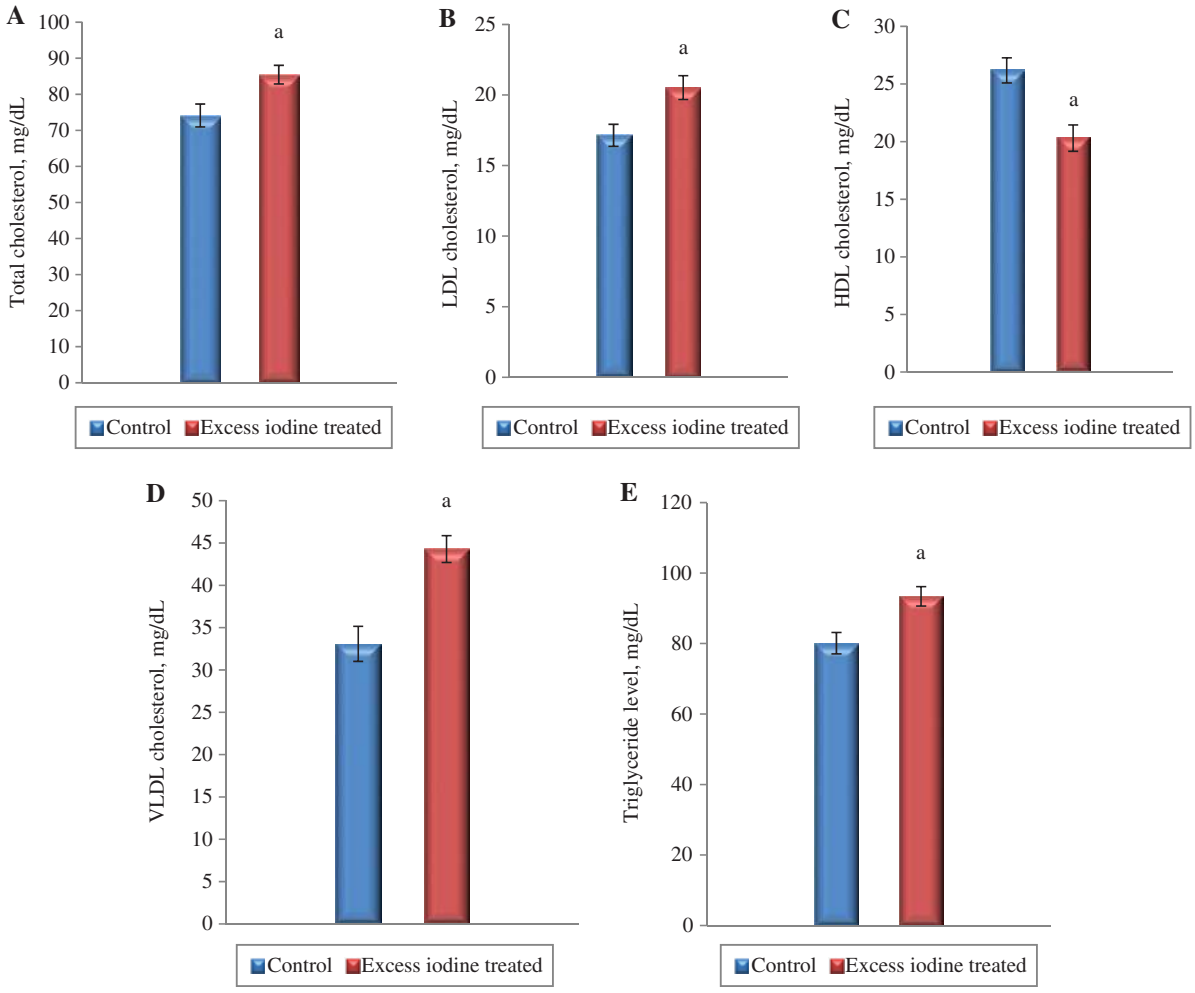

Figure 2: Excess iodine increased the levels of bad cholesterol and triglyceride but decreased good cholesterol.
Effect of treatment of excess iodine at a dose of 3.5 mg KI/100 g bw for 60 days on (A) total cholesterol level, (B) LDL cholesterol level, (C) HDL cholesterol level, (D) VLDL cholesterol level, and (E) serum triglyceride level. Data are presented as mean ± SD, n = 6. Values bearing superscripts are significantly different by t-test; $p < 0.05$.

and nuclei distribution of control animals (Figure 5E). Decline in nuclei/field view associated with diminished fibre diameter was observed (Table 5).

Oxidant-antioxidant status

An increase in oxidant level with decreased antioxidant level leading to an impaired oxidative state was found in the treated animals as compared with the control animals (Figure 3).

Discussion

The deleterious impacts of excess iodine intake in this era of post salt iodization phase especially in iodine-replete regions are currently being investigated worldwide, yielding reports of impairment of thyroid functions, carcinogenesis, reproductive malfunctions, to mention some [17]. TH impairment is closely linked with the metabolic processes as TH regulates cholesterol and carbohydrate metabolism through direct actions on gene expression as well as cross-talk with other nuclear receptors [18]; however, the impacts on those metabolic aspects remain unexplored under the effects of excess iodine. The present study therefore evaluates the effect of exposure to excess iodide for 60 days on carbohydrate and lipid metabolism at relatively high doses of 3.5 mg potassium iodide (KI)/100-g body weight, which corresponds to 500 times the recommended daily dose of iodide. On treating animals with a daily dose of iodide as mentioned, serum toxicity markers SGOT and SGPT increased slightly but not significantly, indicating non-severe toxicity after such administration.

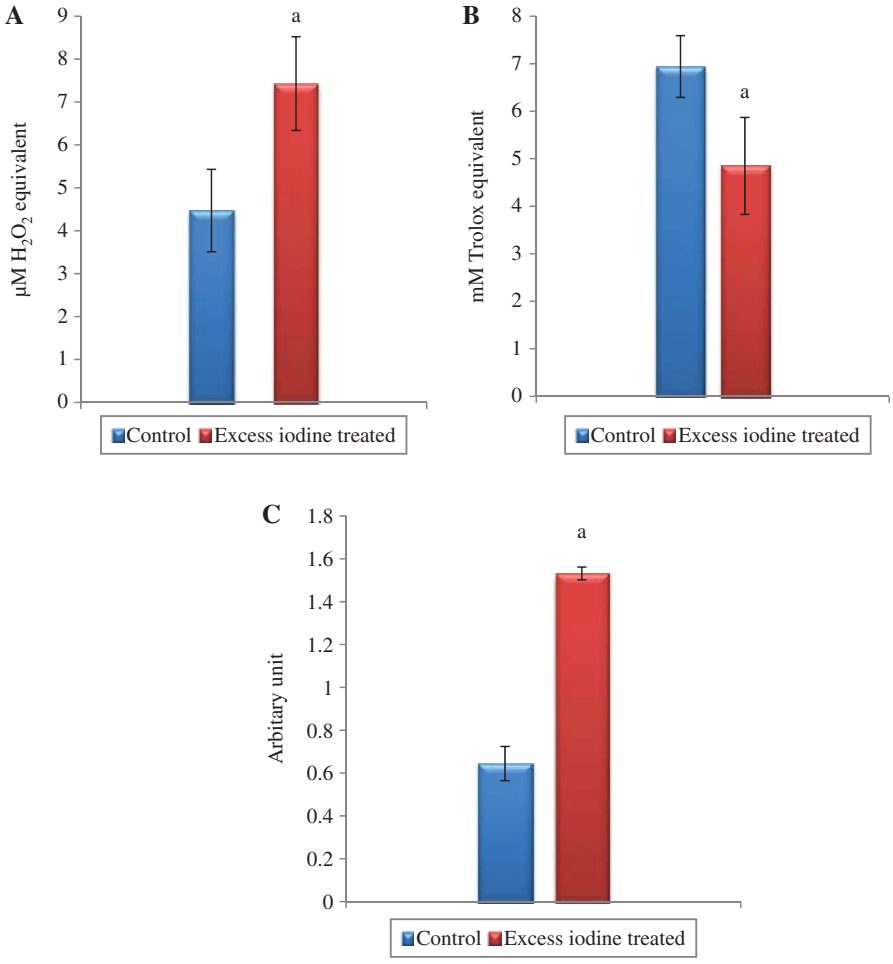

Figure 3: Excess iodine impairs normal oxidant/anti oxidant status.
(A) Alterations in total oxidant status (TOS) of experimental animals subjected to excessive iodine (3.5 mg KI/100 g bw) for 60 days and (B) alterations in total antioxidant status (TAS) of experimental animals subjected to excessive iodine (3.5 mg KI/100 g bw) for 60 days. (C) Alterations in oxidative status index (OSI) of experimental animals subjected to excessive iodine (3.5 mg KI/100 g bw) for 60 days. Data are presented as mean ± SD, n = 6. Values bearing superscripts are significantly different by t-test; $p < 0.05$.

Body weight gain percent in excess-iodine-treated animals increased significantly as compared to control animals in corroboration with previous studies of our laboratory using similar dose [4]. Such weight gain, along with an increase in thyroid gland weight, has been commonly associated as a preliminary but positive sign of induction of hypothyroidism in preceding studies [19]. T_3 and T_4 levels were measured to scrutinise the thyroid status, and T_3 levels in excess-iodide-exposed animals were found to be significantly lowered, while T_4 levels were significantly increased in comparison to the control animals. High expression and activity of sodium-iodide symporter (NIS), which accumulates iodine in tissues primarily in the thyroid gland, have also been reported in metabolic tissues including gastric mucosa, stomach, salivary glands, while low levels of NIS have also been detected by immunohistochemistry [20] and/or RT-PCR [21] in other extrathyroidal tissues like small intestine, colon, rectum, pancreas, kidney, bile duct, lung, heart, placenta [22], as well as in preneoplastic stages of liver carcinogenesis and in human cholangiocarcinoma [23]; thus, iodine accumulation in various tissues associated with carbohydrate and lipid metabolism was measured. Greater iodine accumulation was found in excess-iodide-administered animals compared with control animals, very similar to other studies where iodine accumulation in other tissues including thyroid, ovary, and testis has been studied [24, 25]. Urinary iodine amounting to nearly 90% of iodine excretion from the body and serving as a marker of iodine intake status [26, 27] increased in iodine-treated animals as well. Thus, in general, the results indicate that iodine in excess taken up in surfeit and well above normal requirements by the excess-iodide-treated animals and the body weight, along with the TH status, are indicators

Table 5: Histomorphometric changes in hepatocytes, pancreatic islets, glomerulus and Bowman's capsule, skeletal and cardiac muscle in excess iodine exposed experimental animals (H and E stained).

Parameters	Control	Excess iodine treated
Long diameter of hepatocytes, mm	16.75 ± 3.81	18.01 ± 2.94[a]
Short diameter of hepatocytes, mm	11.43 ± 2.13	13.2 0 ± 3.01[a]
Long diameter of central vein, mm	46.75 ± 4.56	98.84 ± 7.32[a]
Short diameter of central vein, mm	32.21 ± 3.65	77.56 ± 6.32[a]
β cell density, %	0.97 ± 0.05	0.56 ± 0.013[a]
β cell mass, mg	3.72 ± 0.73	2.08 ± 0.86[a]
Islet cell density, %	1.67 ± 0.67	0.63 ± 0.21[a]
Islet cell mass, mg	4.11 ± 0.88	3.23 ± 0.71[a]
Glomerulus		
Area, μm^2	0.045 ± 0.006	0.064 ± 0.003[a]
Length, mm	0.137 ± 0.021	0.257 ± 0.036[a]
Width, mm	0.127 ± 0.011	0.199 ± 0.018[a]
Bowman's capsule		
Area, mm^2	0.052 ± 0.004	0.078 ± 0.016[a]
Length, mm	0.222 ± 0.032	0.302 ± 0.021[a]
Width, mm	0.182 ± 0.010	0.243 ± 0.034[a]
Bowman's space		
Area, mm^2	0.011 ± 0.002	0.021 ± 0.004[a]
Length, mm	0.031 ± 0.009	0.058 ± 0.006[a]
Width, mm	0.031 ± 0.042	0.087 ± 0.031[a]
Skeletal muscle fibre diameter, μm	57.05 ± 7.65	39 ± 7.29[a]
Number of nuclei/field view	23.00 ± 4.00	11.00 ± 2.00[a]
Cardiac muscle fibre diameter, μm	60.77 ± 8.43	52.34 ± 5.78[s]
Number of nuclei/field view	34.00 ± 7.00	20 ± 3.00[a]

Data are presented as mean ± SD, n = 6. Values bearing superscripts are significantly different by t-test; $p < 0.05$.

of induction of hypothyroidism. Excess iodide is also known to cause hypothyroidism generally, while hyperthyroidism occurs in some preconditioned situations [18].

Exposure to excess iodide induced low thyroidal activity, and under such physiological condition, general parameters of carbohydrate and lipid metabolism were looked into. Blood glucose levels of excess-iodide-treated animals were augmented significantly compared with the control animals; this can be attributed to increased gluconeogenesis and insulin sensitivity due to decline in thyroidal activity [28, 29]. Low TH in the blood promotes the risk of developing type 2 diabetes, especially in people with prediabetic conditions [30, 31]. It is already established that hypothyroidism is a threat to raised blood sugar levels, but the exact mechanism is yet to be clearly defined. Glycogen levels in the liver, heart, kidney, and skeletal muscles were estimated, and it was found that glycogen levels in the excess-iodide-treated group decreased from storage organs of liver and skeletal muscle but increased significantly in the kidney and heart. Greater loss of glycogen from the storage organs is in concurrence with the increased blood glucose levels, as hyperglycaemia is often associated with reduced glycogen synthase as well as increased sugar levels in the peripheral system than in stored form [32, 33]. Earlier, it has also been proposed that in hypothyroid rat skeletal muscle, there is decreased beta-adrenergic responsiveness of the enzymes of glycogen metabolism due to increased activity of phosphoprotein phosphatases and reduced beta-adrenergic receptors and adenylate cyclase activity leading to decreased muscle glycogen [34]. This manifestation of glycogen accumulation in restricted glucose supply via compromised tissue uptake is likely to appear as a paradoxical metabolic stress response. However, previous rodent studies have presented that the cardiac glycogen content increases in parallel with the depletion of liver and skeletal muscle glycogen stores, suggesting that glycogen handling responses are tissue specific [35]. Hyperglycaemic conditions have also been associated with glycogen accumulation in renal tubules, which is in fact considered a morphologic change in diabetic rat kidney [36]. The changes in the essential parameters of carbohydrate metabolism under the influence of excess iodine thus point towards the development of a hyperglycaemic condition, which is likely an effect of a low thyroid function.

The lipid metabolic parameters including total cholesterol, VLDL, and LDL levels, which are considered "bad" cholesterol, were increased, while HDL level, which is the "good" cholesterol, decreased significantly in excess-iodide-treated animals compared with the control group. Thyroid dysfunction has an unfavourable impact on lipids as well as a number of other cardiovascular risk factors even within the normal range of TSH values; a linear raise in total cholesterol, LDL, and triglycerides and a linear reduction in HDL levels have been observed with increasing TSH [37]. THs are known to induce 3-hydroxy-3-methylglutarylcoenzyme A (HMG-CoA) reductase, the first step in cholesterol biosynthesis. Moreover, T_3 upregulates LDL receptors by effecting the LDL receptor gene activation [38]. This T_3 interceded gene activation is carried out by the direct binding of T_3 to definite TH responsive elements (TREs) [39]. T_3 also controls sterol regulatory element-binding protein-2 (SREBP-2), which in turn regulates LDL receptor's gene expression [40]. Although reduced thyroid activity is associated with diminished activity of HMG-CoA reductase, LDL levels are increased in patients with overt hypothyroidism [41, 42]. This is due to the decreased LDL receptors' activity, resulting in decreased catabolism of LDL and IDL [43, 44]. Influence of THs on HDL metabolism involves augmenting cholesteryl ester transfer protein (CETP) activity, which exchanges cholesteryl esters from HDL to VLDL and triglycerides to the reverse direction

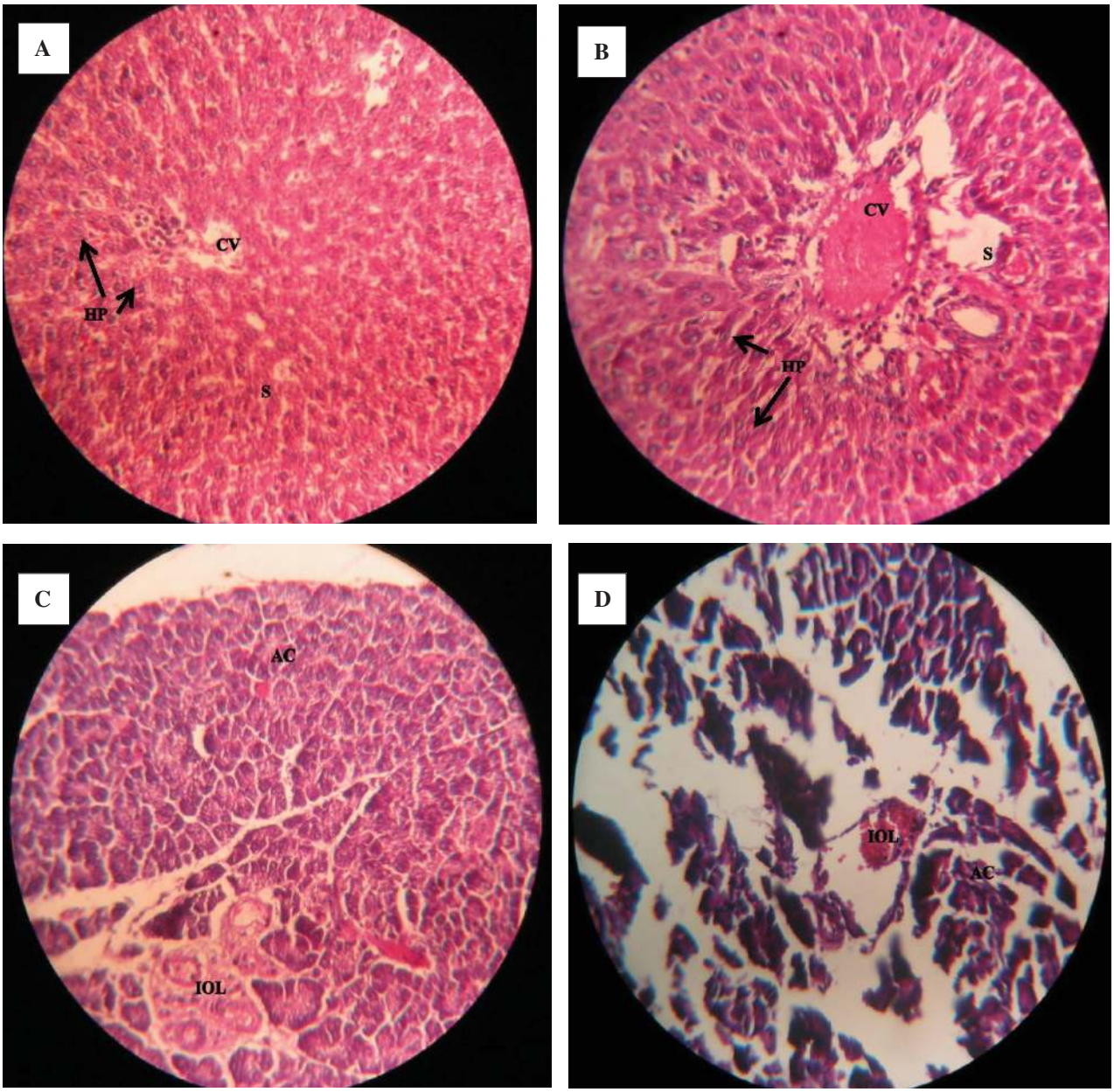

Figure 4: Photomicrographs of H and E stained (A) control liver and (B) excess-iodine (3.5 mg KI/100 g bw for 60 days)-treated liver at 20× magnification. Both photomicrographs indicate the presence of central vein (CV), hepatocytes (HP), and sinusoids (S). Photomicrographs of H and E stained control pancreas are shown in (C), while excess-iodine-treated (3.5 mg KI/100 g bw for 60 days) pancreas at 20× magnification is shown in (D). Both show presence of pancreatic acini (AC) and islets of Langerhans (IOL).

[45]. Subclinical hypothyroidism has also been associated with increased triglyceride and decreased HDL levels [46]. In fact, previous studies have proposed that dyslipidemic patients should undergo a biochemical screening for thyroidal abnormalities [47].

Histological sections of the excess-iodine-treated pancreas demonstrated total disruption and no recognizable islets, which likely corresponds to the developed hyperglycaemic state due to compromised insulin secretion and anomalous glucagon secretion resulting from changes in pancreatic islet cell function and/or mass as corroborated by both histology and histomorphometry [48]. Disruptive changes in the treated liver comprising degenerated hepatocytes, associated decrease in hepatocytes and central vein diameters, and prominent vacuolations could be the primary cause for the loss of stored glycogen from this organ. Similar degenerative changes in the skeletal muscle showing decrease in diameter with the dissolution of muscle fibre and loss of nucleus are the answer to the prominent change of glycogen loss from the organ as well.

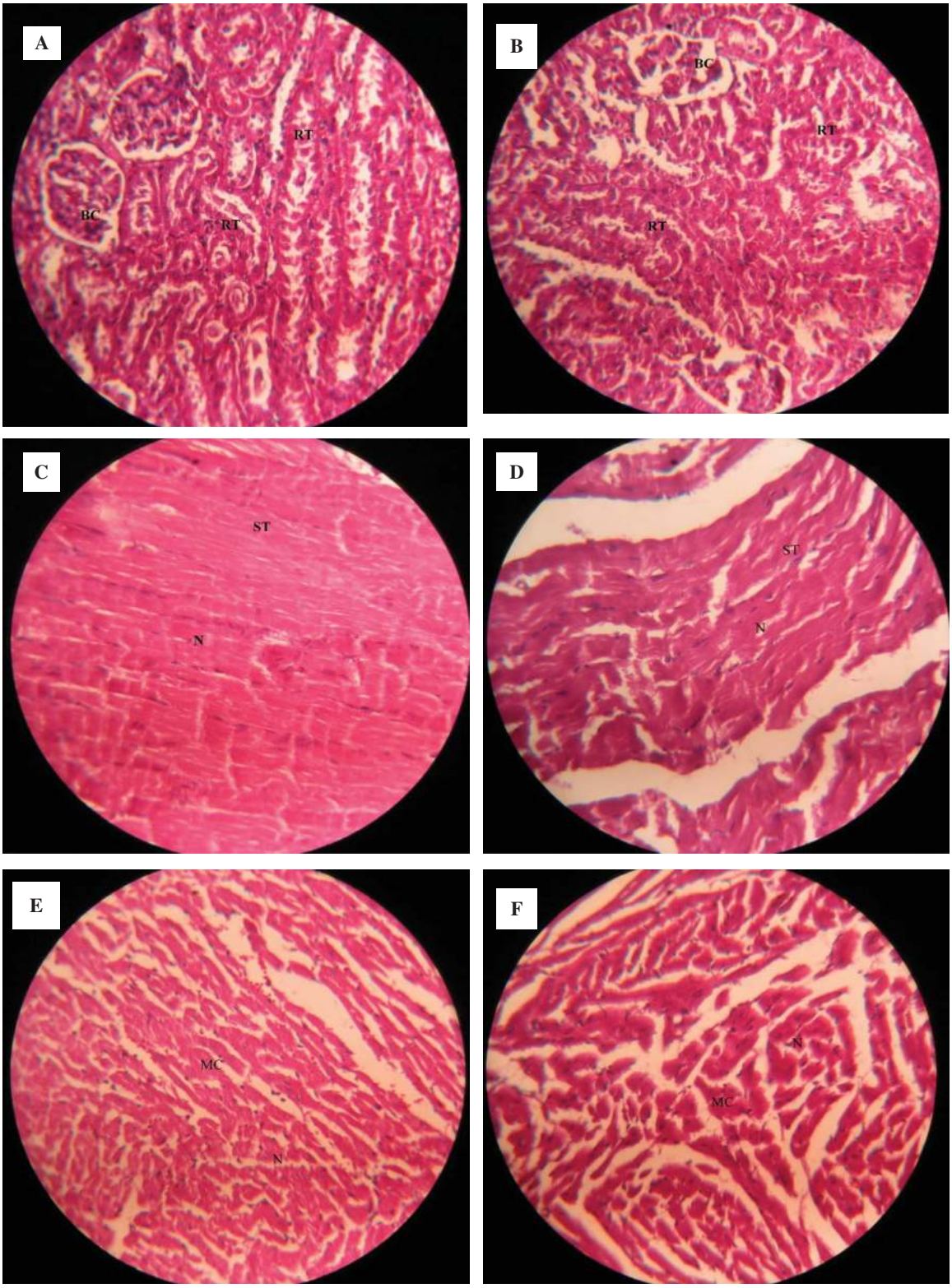

Figure 5: Photomicrographs of H and E stained (A) control kidney and (B) excess-iodine (3.5 mg KI/100 g bw for 60 days)-treated kidney at 20× magnification. Photomicrographs are labelled with the presence of renal tubules (RT) and Bowman's capsule (BC). Photomicrographs of H and E stained (C) control skeletal muscle and (D) excess-iodine (3.5 mg KI/100 g bw for 60 days)-treated skeletal muscle at 20× magnification. Shown in the picture are nuclei (N) and striations (ST). Photomicrographs of H and E stained control heart are represented in (E), while excess-iodine-treated (3.5 mg KI/100 g bw for 60 days) heart at 20× magnification is shown in (F). Figures represent the presence of nuclei (N) and muscle fibre/myocytes (MC).

Moreover, excess iodine administration has been associated with the production of reactive oxygen species (ROS), iodide itself being an exceedingly reactive molecule with abilities to react with all major biomolecules and building bases of proteins, lipids, and nucleic acids by forming intermediary iodo compounds, which can initiate severe peroxidative and ultimately apoptotic processes leading to such noticed disruptive changes in the metabolic organs [49]. In the present study, an increase in oxidant levels and a decrease in antioxidant levels are indicative of such impaired oxidant status, followed by the development of oxidative stress. Iodine itself, as mentioned, is a potent oxidant and, on administration in excess, gets concentrated in tissues, as has been reflected in results. This may further develop oxygen-derived free radicals forming molecular iodine reacting with iodiniumcation. Excess iodine thus may cause enhanced generation of free radicals resulting in overall deterioration.

Increase in blood cholesterol levels inducing a hypercholesteromic state likely impinges on other cardiovascular risk factors and gives rise to a fatty heart, as seen from the histologic view of the excess-iodide-treated cardiac muscles. Low thyroid function is associated with impaired microvascular function and in patients with hypothyroidism, a true enhanced incidence of hypertension for increased peripheral vascular resistance has also been found [50], the evidence for which is visible in the augmented hyalinization and cardiac muscles mutilation in treated animals. Nephropathic degenerative changes along with histomorphometric alterations are also evident from the microphotograph of excess-iodide-treated rats, and such changes, including severe expansive changes with swelling and fat deposition, can be attributed to the interplay between thyroid and the kidney in each other's functions. Thyroid dysfunction affects renal physiology and development, whereas kidney disease could result in thyroid dysfunction as well [51]. Renal blood flow, glomerular filtration rate, as well as the concentrating ability of the formed urine reportedly decreases in hypothyroidism, which has been attributed to pathologic changes in the glomerular structure in hypothyroidism, such as glomerular basement membrane thickening and mesangial matrix expansion [52], as have been observed in this study. The role of ROS in causing tissue damage even in cardiac and renal tissues is also imperative to the cause of metabolic disruption [49]. Most of the degenerative changes as observed, including disruptive changes in the liver, kidney, heart, pancreas due to generation of oxidative stress, were found consistent with the present available literature.

In brief, excess iodide in the given dose and duration after its uptake followed by the development of biochemical hypothyroidism results in excess iodine induced impaired oxidative state, causing a widespread disruption, which has been reflected in carbohydrate and lipid metabolism, encouraging further molecular look out for such changes and possible interventions for monitoring exposures commonly occurring in iodine-replete environment where uncontrolled iodized salt is in practice under universal salt iodization programme.

Conclusions

It may thus be concluded that excess iodide, when administered at relatively high doses, prompts unfavourable alterations in the carbohydrate and lipid metabolic processes, as evidenced from biochemical investigations and histomorphometric studies of associated organs, which result in the generation of ROS and lead to biochemical hypothyroidism. Excess iodine induced a hyperglycaemic and hypercholesterolemic state and in turn causes the development of cardiovascular risks, renal degeneration, and skeleton muscular disruption; oxidative stress and perturbation of the thyroid metabolic axis or excess iodide itself or both act as a pro-oxidant to start degenerative changes.

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