High Dietary Consumption of Iodine induced thyroid cytotoxicity in diabetic intoxicated rats and oxidonitrergic stress in non-diabetic rats

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Abstract

This study aimed to investigate the role of iodine intake on thyroid function in diabetic rats. Twenty four (24) male Wistar rats were grouped into four (n=6): Group 1 (Non-diabetic without iodine), Group 2 (Non-diabetic + iodine), Group 3 (Diabetic without iodine) and Group 4 (Diabetic + iodine). 10mg/kg bw of iodine were mixed with the feeds. Serum triodothyronine (T3), thyroxine (T4), Thyroid Stimulating Hormone (TSH), thyroglobulin and thyroperoxidase antibodies were assessed using ELISA. Serum MDA, SOD and NO levels were assessed with spectrophotometry. In diabetic rats, lower mean serum T4 and TSH concentrations were observed (T4: 13.16±0.55 Vs 11.75±0.21 mg/dL, TSH: 2.62±0.11 Vs 2.28±0.08 IU/mL). Iodine treatment further reduced T4 and increased TSH concentrations (T4: 11.75±0.21 vs 6.75±0.22 mg/dL, TSH: 2.28±0.08 Vs 3.08±0.15 IU/mL). Thyroglobulin and thyroperoxidase antibodies

were absent in all the rats. It was also observed that iodine intake caused an increase in oxidative stress in in both diabetic and non-diabetic treated rats (MDA; 18.4±1.3 Vs 22.2±2.7 µmol/l X 10-5, NO; 14.08±0.38 Vs 13.24±0.07µm/l) and increased SOD levels in diabetic rats (44.44±2.94 Vs 68.94±0.91 mg/ml); this increased could be due to the increased TSH. Consumption of excess iodine suppressed thyroid function in diabetic rats and induced oxidative stress in both diabetic and non-diabetic treated rats

Keywords: *Iodine supplentation, Diabetes, Oxidative stress, Thyroid Function.*

1. Introduction

The two endocrinopathies that affect people most frequently are diabetes and thyroid disease.As both insulin and thyroid hormones are essential in cellular metabolism, an excess or lack of one might cause difficulties with the other's functionality (Mohamed et al., 2017). Thus, it is possible for diabetes and thyroid problems to coexist in individuals. Patients with type 1 diabetes experience hyperthyroid symptoms, while those with type 2 diabetes typically experience hypothyroid symptoms (Mohamed et al., 2017). Thyroid disorder seems to induce oxidative stress in the testis by reducing the levels of testicular enzymatic and non-enzymatic defenses (Mohamed et al., 2017).

Diabetes mellitus is a disease of metabolic dysregulation (Chijiokwu et al., 2022) accompanied by long-term vascular and neurological complications (Rhoades and Tanner, 2003). The prevalence of diabetes for all ages worldwide was estimated at 2.8% in 2000 and 4.4% by 2030 (Wild, Roglic, Green, Sicree, King, 2004). It has long been recognized that thyroid disorder is related with an increased prevalence of poor glucose metabolism (Kabadi and Eisenstein, 1980). Following that, numerous in vivo and in vitro tests were carried out in attempt to identify the fundamental pathophysiologic abnormalities underlying the relationship of hyperglycemia with

thyrotoxicosis (Wajchenberg et al., 1978). In numerous reports on hyperthyroidism, fasting blood sugar levels were found to be either normal or excessive. More so, expanded bodies of evidence have also shown a relationship between thyroid function and blood glucose levels (Ogbonna et al., 2019; Wenhua et al., 2019; Dandan et al., 2021). Notably, Arigi, Fabiyi and Fasanmade (2014) observed that both hypothyroidism and hyperthyroidism caused dysfunction in glucose tolerance and led to increased fasting blood glucose in non-diabetic rats. Macini *et al* (2019) discovered that there was a higher risk of development of type 2 diabetes in individuals consuming high levels of iodine in their diets. Ravindra *et al.* (2011) observed that diabetic serum had a significantly lower ability to bind and transport iodine.

Thyroid hormones [tri-iodo-thyronine (T3) and thyroxine (T4)] are known to regulate a variety of biochemical processes throughout the body that are required for appropriate growth, metabolism, and brain activity. Iodine is an essential component of T3 and T4, and it has unique effects on the thyroid gland and the immune system. Iodine is a non-metallic element belonging to the halogen family in Group VIIA of the periodic table (Cooper, 2007). It is a dark purple, crystalline and lustrous solid at room temperature (Cooper, 2007). Iodine is necessary to living organisms in which it is actively concentrated in the thyroid gland for the synthesis of thyroid hormones; triiodothyronine (T_3) and thyroxine (T_4) (Haldimann, Bochud, Burnier, Paccaud, & Dudler, 2015). The recommended dietary intake (RDI) of iodine for adults is 150µg/day. It could be obtained by consuming foods such as seaweed, milk and dairy products, iodised table salt and seafood (DOH-UK, 1995). According to Wolf and Chaikoff (1948), consumption of high amounts of iodine inhibits three steps in the synthesis of thyroid hormones; iodide trapping, thyroglobulin iodination (wolf-Chaikoff effect) and thyroid hormone release from the thyroid gland which can lead to hypothyroidism. Excessive iodine consumption can also induce

hyperthyroidism and this is known as the Jod-Basedow effect. Excess thyroid hormones cause "thyroid diabetes" (Hartoft-Nielsen et al., 2009), whereas hyperthyroidism causes glucose intolerance in animals and humans (Hartoft-Nielsen et al., 2009). Diabetes mellitus has been proven to coexist with a range of thyroid disorders. The thyroid gland regulates carbohydrate metabolism at the levels of pancreatic islets and glucose-using target tissues, raising crucial therapeutic and diagnostic challenges. There is however limited information regarding the effect of high iodine intake on the thyroid gland of diabetic rats. In context of the above, this study was carried out to investigate the effect of high iodine intake on thyroid function in diabetic rats and its underlying mechanism.

2. Materials and Methods

2.1.Animal Handling

Adult male Wistar rats weighing 150 to 200 g (7-9 weeks old) used for this study were obtained from the Central Animal house, Faculty of Basic Medical Sciences, University of Ibadan, Nigeria and were kept in plastic cages under normal standard condition of about 25 ± 2 °C in 12:12 h day and night cycle. The animals were left to acclimatize for at least 14 days with unrestricted access to water and standard rat chow before the commencement of the experiments. The study protocols used in handling the animals were in line with the establishments by National institutes of Health (NIH) Guideline for the Care and Use of Laboratory Animals (Publication No. 85-23, revised). Six animals per group was used in this study based on the principle of the three Rs (3Rs: Replacement, Reduction and Refinement) by Oyovwi et al. (2021).

2.2.Ethical Approval

The University of Ibadan's Ethics Committee for Animal Care and Use (ACUREC), reference number UI-ACUREC/19/0029, approved the use of animals in this study. The Animal Care and Use Ethics Committee (ACUREC) guarantees that all adverse events are promptly reported to ACUREC and those institutional policies and laws are followed.

2.3. Determination of iodine and caloric content of feeds

The standard rat feed were analyzed to determine the following parameters;

a. *Determination of Caloric content (using a bomb calorimeter):*

The apparatus used was the Gallenkamp Ballistic Bomb Calorimeter. Reagent used for calibration was Benzoic acid. Determination: 0.25g of each sample depending on the bulkiness was weighed into the steel capsule. A 10cm cotton thread was attached to the thermocouple to touch the capsule. The bomb was closed and charged in with oxygen up to 30atm. The bomb was fixed up by depression to ignite switch to burn the sample in an excess of oxygen. The maximum temperature rise in the bomb was measured by thermocouple and galvanometer system.

b. *Determination of iodine content*

The method of A.O.A.C (1984) was used to determine the content of iodine. 5g of sample was dissolved in approximately100ml water. The pH was adjusted to 2.8 using 0.6% HCl. 30mg potassium iodide powder (KI) was added to convert all iodate present to elemental iodine. The liberated iodine was titrated with 0.005N freshly prepared $Na_2S_2O_3$ (sodium thiosulphate solution) using 1% starch solution as the end point or equivalence point indicator. The titre obtained at this point was used to calculate iodine concentration in sample in mg/kg.

2.4.Induction of Diabetes Mellitus

Diabetes was induced in subgroups C and D after 6 weeks with one dose of intraperitoneal administration of Alloxan monohydrate at a dose of 150mg per kg rat body weight (Sikarwar and Patil 2010; Akinola, Gabriel, Suleiman & Olorunsogbon, 2012; Maiffo *et al.*, 2019) after an overnight fast. The Alloxan was diluted in normal saline and administered within a few minutes. One hour after, the rats were given feed *ad libitum* and 5% dextrose.After 7 days, the fasting blood glucose of the rats was assessed with the use of Accu-check glucometre and strip. The rats with fasting blood glucose levels higher than 200mg/dl were considered diabetic.

2.5.Distribution and treatment of animals

Twenty four (24) rats were randomly divided into four groups of six rats each. These rats received the various gavage treatments for 14 days as follows: Group 1 (normal control) received 10 mL/kg bw of distilled water; Group 2 (diabetic rats) received 10 mL/kg bw of distilled water; Group 3 (non-diabetic rats) received 2.5 mg/kg bw of the iodine; Groups 4 (diabetic rats) received the iodine at the dose of 10 mg/kg bw. The doses and routes of distilled water (Miaffo et al., 2019) and iodine (Kotyzováa, Eybla, Mihaljevičb & Glattre, 2005) were selected based on previous dose-response effect and preliminary investigation. However, normal saline (10 mL/kg, p.o.) were administered as vehicle to naïve rats in different groups which served as normal control.Notably, in groups 2 and 4, rats were fed with feeds mixed with iodine at concentration of 10mg/kg *ad libitum* throughout the experiment. The requirement was determined by comparing the weight, iodine content and dry matter content of thyroid glands from rats supplemented with various levels of iodine. All treatment was done orally between 8.00am – 9.00am once daily for the period of eight (8) weeks.

2.6.Collection of blood samples and preparation

Blood was collected into plain bottles from the orbital vein with the use of plain capillary tubes. The blood was centrifuged at 3,000 revolutions per minute (rpm) for 20 minutes after which the supernatant was separated with the use of a micropipette into separate plain bottles and frozen at -20°C until the thyroid hormone assays were performed using ELISA strip reader

2.7.*Circulatory concentration of thyroid hormones analysis*

The levels of free T3 (fT3), free T4 (fT4), thyroid stimulating hormone (TSH), Thyroid peroxidase (TPO) and thyroglobulin (TG) antibodies were determined in serum samples using their respective ELISA kits (diagnostic systems laboratories INC.) supplied from (Monobind Inc., USA) according to the manufacturer's recommended protocol.

2.8.Determination of oxidative biomarkers

2.8.1. Determination of Lipid Peroxidation (Malondialdehyde-MDA)

Lipid peroxidation was determined by measuring the thiobarbituric acid reactive substances (TBARS) produced during lipid peroxidation. This was carried out by the method of Varshney and Kale (1990). An aliquot of 0.4ml of the sample was mixed with 1.6ml of Tris-KCl buffer to which 0.5ml of 30% TCA was added. Then 0.5ml of 0.75% TBA was added and placed in a water bath for 45minutes at 80^{o}C. This was then cooled in ice and centrifuged at 3000rpm for 15minutes. The clear supernatant was collected and absorbance measured against a reference blank of distilled water at 532nm. The MDA level was calculated according to the method of Adam-Vizi and Seregi (1982). Lipid peroxidation in units/mg protein or gram tissue was computed with a molar extinction coefficient of $1.56X10^{5}$ M^{-1} Cm^{-1}.

2.8.2. Determination of Superoxide Dismutase (SOD) Activity

The level of SOD activity was determined by the method of Misra and Fridovich (1972). 1ml of sample was diluted in 9ml of distilled water to make a 1 in 10 dilution. An aliquot of 0.2ml of the diluted sample was added to 2.5ml of 0.05M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer and the reaction started by the addition of 0.3ml of freshly prepared 0.3mM adrenaline to the mixture which was quickly mixed by inversion. The reference cuvette contained 2.5ml buffer, 0.3ml of substrate (adrenaline) and 0.2ml of water. The increase in absorbance at 480nm was monitored every 30 seconds for 150 seconds.

2.9.Determination of Total Nitrite (NO)

Nitrite determination was done using the method described by Ignarro, Buga, Wood, Byrns and Chaudhuri, 1987). The assay relies on a diazotization reaction that was originally described by Griess in 1879. The procedure is based on the chemical reaction which uses sulfanilamide and naphthylethylenediaminedihydrochlorate (NED) and under acidic condition. Sulfanilamide and NED compete for nitrite in the Griess reaction.

*2.10. Statistical Analysis***.**

The results were expressed as mean ± S.E.M ((Standard Error of Mean)). Significance of mean values of different parameters between the groups was analysed using one-way analysis of variance (ANOVA). Multiple comparisons were performed using the Bonferroni post hoc analysis. All analyses were performed using Graph Pad Prism 8 and differences were considered statistically significant at probability level less than 0.05 for all tests

3. Result

3.1.*Mean Iodine and Caloric Content of Normal Rat Feed*

Table 1 shows the mean iodine and caloric content of normal rat chow. The iodine content of the feed, measured in mg/kg, is about 5.68 mg/kg, while the caloric content has been shown to be about 3.97 kcal/g of rat feed. However, the iodine content (5,680.01.) in the rat chow was significantly ($p<0.05$) higher than the calorie content (3.97 ± 0.001).

3.2.Effect of high consumption of iodine supplement on thyroid hormonal function in diabetic rats

The mean T4 levels of rats with and without diabetes were not significantly different. Additionally, diabetic rats' serum T4 concentrations were shown to be lower. As seen in Fig. 1, a one-way ANOVA and post hoc analysis revealed that excessive iodine supplementation considerably (p less than 0.05) decreased T4 levels and increased TSH levels in rats as compared to diabetes. In contrast to the control group, neither the non diabetic nor the iodine-supplemented groups alone showed any appreciable alterations in T3 (Fig. 1c).

3.3.Effect of high consumption of iodine supplement on Antibody Index for Thyroperoxidase (TPO)in diabetic rats

As shown in Table. 2, excess iodine supplementation shows negative level for of thyroperoxidase antibody among diabetic and diabetic treated with iodine supplementation groups when compared to the control group. This is an indication that iodine intake did not cause the development of thyroperoxidase antibody.

3.4.Effect of high consumption of iodine supplement on Antibody Index for Thyroglobulin (TG)in diabetic rats

As shown in table. 3, Excessive iodine supplementation shows a negative value for the antibody index for thyroglobulin in diabetics and diabetics treated with iodine supplementation groups compared to the control group. This is an indication that iodine intake did not lead to the formation of thyroglobulin antibodies.

3.5.Effect of high consumption of iodine supplement on oxidative status in diabetic rats

Rats given iodine alone showed a considerable rise in MDA levels compared to controls (Fig. 2a). Although there were no discernible differences between the rats given iodine alone and the control group in terms of their SOD levels (Fig. 2b). A one-way ANOVA and post hoc test revealed that excessive iodine supplementation in diabetic rats increased MDA and SOD significantly (p less than 0.05) in comparison to diabetic rats. In contrast to the control group, neither the diabetes nor the iodine-supplemented groups showed any appreciable alterations in SOD (Fig. 2b).

3.6.Effect of high consumption of iodine supplement on nitric oxide in diabetic rats

In diabetic rats, excessive iodine supplementation increased NO levels significantly (p 0.05) compared to diabetic rats, as demonstrated in Figure 3 from a one-way ANOVA and post hoc test. However, neither the diabetic nor the iodine-supplemented groups showed any discernible differences in NO levels from the control group.

3.7.Effect of high consumption of iodine supplement on body weights of rats

Figure 4 depicts the results of high iodine supplementation. There was a group of diabetic rats, as shown in Figure 4. Additionally, the body weight of diabetic rats alone increased significantly more than that of the iodine-treated group alone as well as the control group.

4. Discussion

The amount of iodine found in the analysis of typical rat food was based on data from Halverson, Zepplin and Hart (1949) The standard rat food had an appropriate amount of iodine, according to the rodents' estimated daily iodine needs. Therefore, too much of the extra iodine (10 mg/kg feed) was given. It is well known that hypothyroidism makes people gain weight. When compared to diabetic rats not given supplementary iodine (group III), group IV's diabetic rats gained more weight, which may be attributable to the lower serum -T4 concentrations in that group. Diabetes affects the pituitary-thyroid axis, increasing the occurrence of thyroid abnormalities. Diabetes appears to alter hypothalamic thyrotropin-releasing hormone (TRH) secretion and pituitary thyrotropin (TSH) release. Excess thyroid hormones has been investigated to cause "thyroid diabetes" (Hartoft-Nielsen et al., 2009), whereas hyperthyroidism causes glucose intolerance in animals and humans (Hartoft-Nielsen et al., 2009). Diabetes mellitus has been proven to coexist with a range of thyroid disorders in human, as indicated in our animal study. In accordance with this, Wolff and Chaikoff (1948) reported that an iodine injection in rats almost completely inhibited organification (iodide oxidation) in the thyroid gland, which lasted for about ten days, and was then followed by an escape phenomenon known as adaptation and Restoration of normal organization of iodine and normal peroxidative function of the thyroid. In free-living populations, Katagiri, R., Yuan, X., Kobayashi, S., and Sasaki, S. (2017) observation that salts with poor control or continuous exposure to excessive iodine from water are risk factors for hypothyroidism.

Given that the experimental period was longer than ten days and that Wolf and Chaikoff (1948) observed the escape phenomenon over a longer period of time, the results in non-diabetic rats are compatible with their findings. Additionally, the lack of thyroid autoimmune antibodies such as

the thyroid peroxidase antibody (TPOAb) and the thyroglobulin antibody (TgAb) suggests that high iodine intake had no appreciable impact on thyroid function in non-diabetic rats fed standard rat food. According to Ravindra et al. (2011) findings, diabetic serum has lower iodine uptake. They claim that elevated blood sugar levels may be the root of the lower iodine intake since they can modify the structure of biomolecules through glycation, which reduces the iodine binding sites. Since less iodine is available for the production of thyroid hormones, this may be the cause of the markedly lower mean serum content of T4 seen in diabetic rats fed standard rat food. However, it is unclear what caused the mean serum TSH to be considerably lower.

Lower T4 levels and higher TSH levels compared to diabetic rats not swallowing supplementary iodine were indications that iodine consumption at a dose of 10 mg/kg food had the ability to further depress thyroid function in diabetic rats. The mean serum T3 levels, however, did not differ significantly from one another. TSH is released more frequently by the anterior pituitary gland when thyroid hormone levels are lower. (Hall, 2008). The higher serum TSH values seen in this study are therefore predicted to be caused by reduced blood T4 levels. Although thyroid peroxide antibodies (TPOAb) and thyroglobulin antibodies (TGAb) were noticed, they have already been characterized by Lindberg, Ericsson, Ljung and Ivarsson (1997) and Otken *et al.* (2006). In this investigation, they were not seen in patients with type 1 diabetes.

MDA levels have been observed to rise in both hypo- and hyperthyroidism (Dumitriu, Bartoc, Ursu, Purice, Ionescu, 1988; Cheserek, Wu, Ntazinda, Shi, Shen, & Le, 2015; Mancini *et al*., 2016, Chakrabarti, Ghosoh, Banerjee, Mukherjee & Chowdhury, 2016). Notably, the considerably elevated serum MDA levels and serum NO levels found with iodine intake show that excessive iodine consumption in rats caused oxidative stress via a mechanism that did not impact thyroid function. According to Verma et al. (1991), injection of TSH led to a marked

decrease of SOD in the adrenal gland. The serum SOD concentrations likely rose as a result of the adrenal glands depletion of SOD. Given that their mean TSH levels were noticeably raised, this may be the cause of the elevated blood SOD levels seen in diabetic rats fed iodine and standard rat food. The creation of ROS, which can result in thyroid gland oxidative damage and put a diabetic patient at risk for thyroid disease, is one of the factors contributing to the thyroid cytotoxicity seen in diabetic rats (Mohamed et al., 2017). According to the findings of Mohamed et al. (2017), DM cases are more prone to experience problems when an abnormality is present. According to the current study, increased iodine intake promotes thyroid cytotoxicity and changes the antioxidant defense system, leading to an increase in oxidative stress in both normal and diabetically poisoned rats.

This study supports Messarah, Saoudi, Boumendjel, Boulakoud and Feki (2010) findings that the antioxidant system was altered in hypo-/hyperthyroidism-induced rats through an increase in the activities of catalase, glutathione peroxidase, and superoxide dismutase, as well as a decrease in glutathione (GSH) concentration. In addition, extra iodine dramatically raised MDA and antioxidants in rats with normal and hypothyroid thyroid function, according to Hussein, Abbas, Wakil, Elsamanoudy and El Aziz (2012). The high iodine intake seen in this study is particularly notable for the increased accumulation of oxido-nitrogen stress indicators like MDA and NO as well as the alteration in antioxidant function. To avoid oxido-nitrogen stress-induced thyroid cytotoxicity and enable more effective therapeutic intervention, it is crucial to moderate iodine intake in the management of the diabetic condition.

5. Conclusions

The regular rat food's iodine content was adequate to provide the daily dose. thyroid function was reduced by diabetes mellitus. The thyroid function of diabetic rats was further inhibited by excessive iodine consumption, and both diabetic and non-diabetic rats had an increase in oxidative stress. Iodine needs for diabetics are higher than for non-diabetics, although excessive iodine consumption should be avoided as it can harm thyroid function.

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Institutional Review Board Statement: The University of Ibadan's Ethics Committee for Animal Care and Use (ACUREC), reference number UI-ACUREC/19/0029, approved the use of animals in this study. The Animal Care and Use Ethics Committee (ACUREC) guarantees that all adverse events are promptly reported to ACUREC and those institutional policies and laws

are followed. The research could not be changed without first receiving ACUREC's clearance. All study participants gave their informed consent after receiving all necessary information.

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Figure

List of Figures

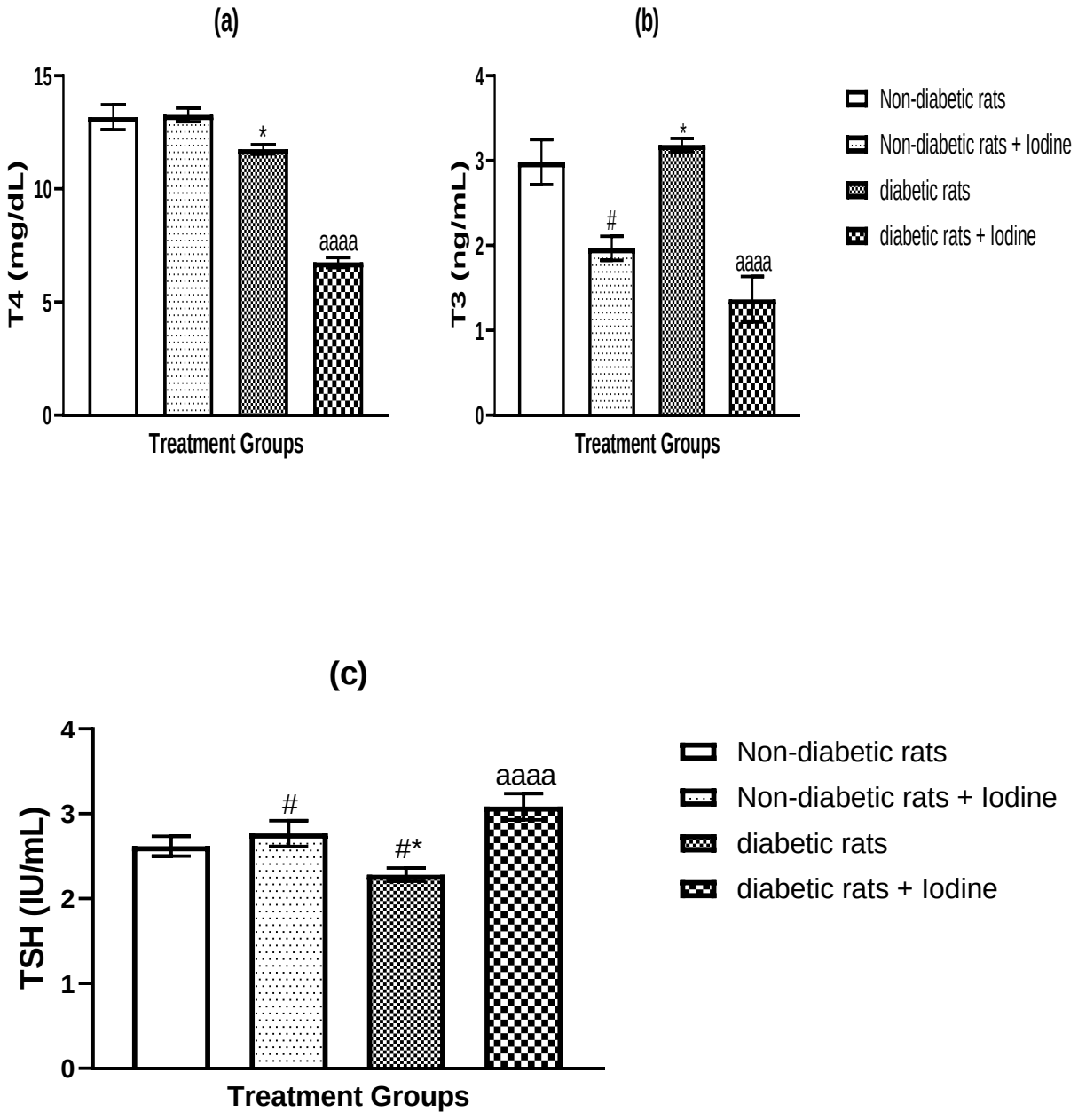

Fig. 1a-c.

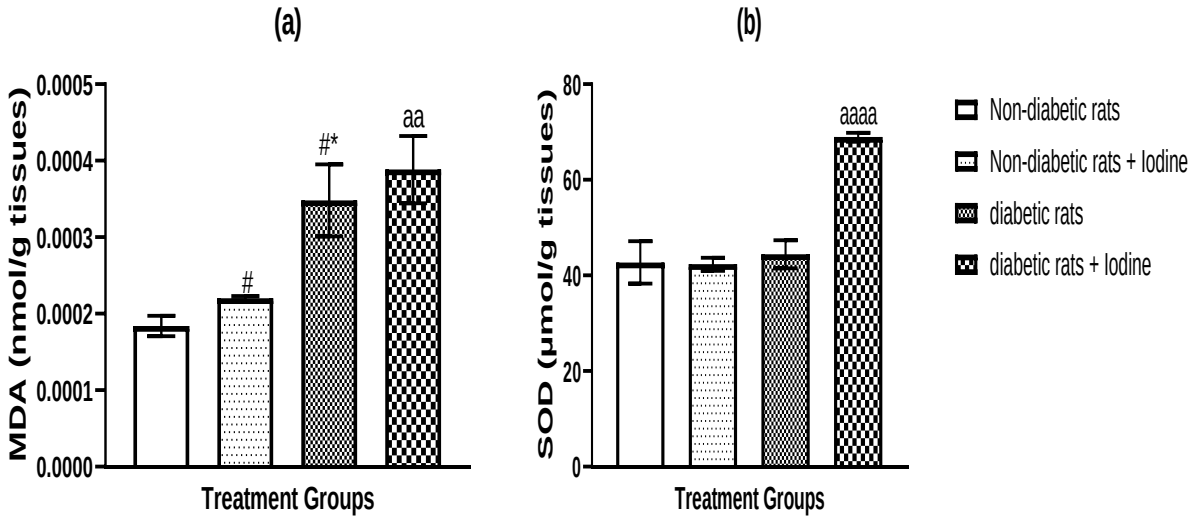
(a)
(b)
MDA (nmol/g tissues)
0.0005
0.0004
0.0003
0.0002
0.0001
0.0000
#
#*
aa
Treatment Groups
SOD (µmol/g tissues)
80
60
40
20
0
aaaa
Treatment Groups
Non-diabetic rats
Non-diabetic rats + Iodine
diabetic rats
diabetic rats + Iodine

Fig. 2a-c.

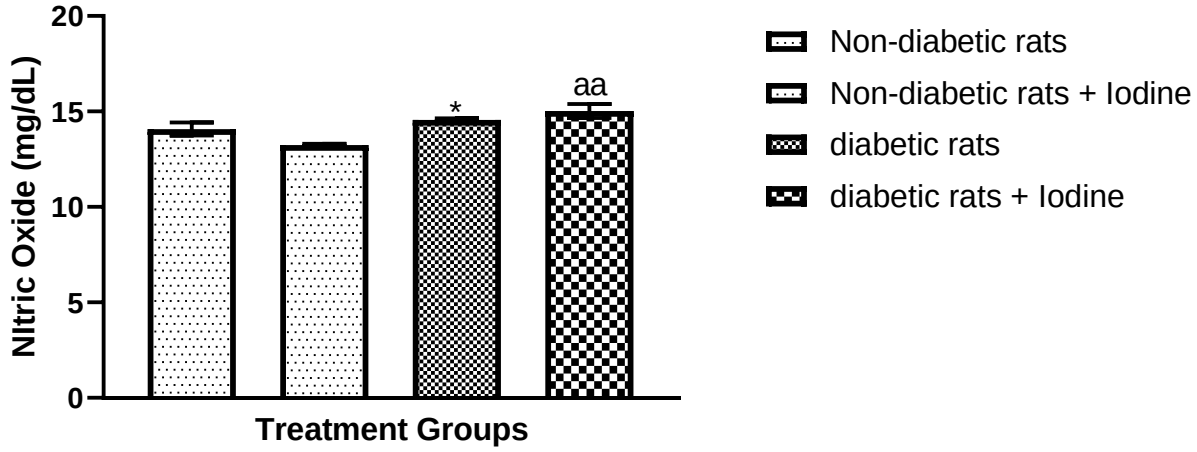
Nitric Oxide (mg/dL)
20
15
10
5
0
*
aa
Treatment Groups
Non-diabetic rats
Non-diabetic rats + Iodine
diabetic rats
diabetic rats + Iodine

Fig. 3.

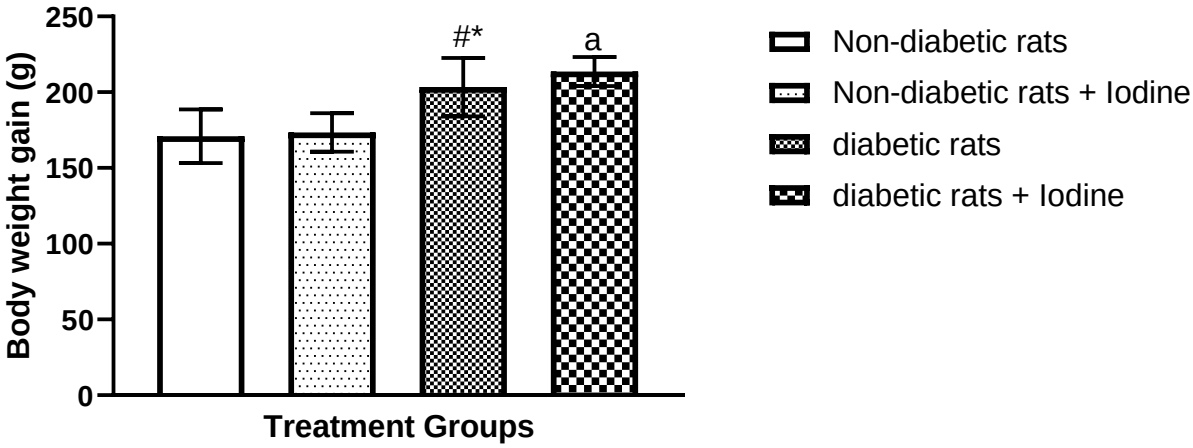
250
200
150
100
50
0
Body weight gain (g)
#*
a
Treatment Groups
Non-diabetic rats
Non-diabetic rats + Iodine
diabetic rats
diabetic rats + Iodine

Fig. 4.

List of table

Table 1

Sample	Iodine (mg/kg)	Gross Energy (Kcal/g)
Normal Rat feed	5.68 ± 0.01*	3.97 ± 0.001

Table 2:

Groups	1	2	3	4
	0.1	0.1	0.1	0.1

Table 3

Groups	1	2	3	4
	0.1	0.1	0.1	0.1

Dear Chief Editor,

Sir,

RESPONSE TO EDITORIAL/REVIEWERS' COMMENTS

I want to thank you for your critical review of our article. We have made the necessary corrections and below are our point by point responses to your comments to the authors.

Reviewing: 1

Comment (Abstract): 1. Please re-write abstract. It is better to make sentences more compact or concise. 2. The abstract in cover page was not similar with the main text. 3. Line 29-30 and line 33-34, what this result had compared with that sample?

Response: That has been taken care of. The abstract in the main text has been worked on to be in similarity to that of the cover page. Line 29-30 and line 33-34 as indicated may be due to the increased TSH; this finding corroborated the findingsof Vernia et al., 1991

Comment (Introduction): .1. The authors should narrow down to your particular focus. Please, state the objectives of the work and provide an adequate background, avoiding a detailed literature and mention rational of this study or research gap eg. oxidative thyroid or thyroid related with diabetic was not mentioned. 2. Please more introduce the literature or information about thyroid or thyroid related with diabetic disease in human. It leads to importance study in rat model. 3. Please check line 54, page 4, this sentence was incorrect? 4.4% in 2030?

Response: Adequate background has been provided with focus on thyroid related diabetic as well as its oxidative implication. The objective has been stated at the end of the introduction. The sentence in line 54 has be corrected.

Reviewer Comment (Materials and Methods): The author should add EC for animal study. 2. Why the author determines soybean and cassava? (line 78, page 5). 3. Regarding introduction, why the author determines oxidative biomarker following iodine treatment? 4. Why the author analysis the data by using Newman-Keuls'test?

Response: That has been taken care of and corrected accordingly. The EC so indicated was at the end of the manuscript before the references but has be included in the materials and methods. The soya bean and the cassava so determined were for further study which was not part of this

paper. Hence, it has been deleted. The oxidative biomarkers were determined to establish the possible underlying mechanism of iodine relative to thyroid related diabetics. The Newman-Keuls'test was actually used in our further study while trying to compared unequal sample size. What we actually used here is the Bonferroni post hoc test for multiple comparism since our sample size was small. Hence, it has been corrected to the statistical tool used

Reviewer Comment (Results and Discussion): 1. Please check significant $p< 0.05$ in line 169, 189, and 194. 2. Please re-write and clarify the table2. 3. Please discuss this study with others relevance study especially in diabetic disease in human.

Response: That has been taken care of and corrected accordingly as compared to other human and animal studies

The Editor,

we wish to commend you for your prompt review of our article and hoped that our answers to your comments are convincing enough.

We will be very grateful if our article can be published in your revered journal as soon as possible.

Thank you for your anticipated cooperation