Melatonin and Vitamin C Attenuate Cassava Diet-Induced Alteration in Thyroid Function

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Abstract

Background

Cyanide is present in cassava and is well known to cause adverse effects on the male reproductive functions. This study evaluated the effect of melatonin and/or vitamin C on body weight, thyroid function and reproductive parameters in male Wistar rats treated with cyanide-enriched cassava-diet (CD), and their possible mechanisms of actions.

Methods

Forty-five (45) animals were divided into 9 groups (n = 5 each) that received the following treatments for 28 days. Groups I-III received normal saline (control), melatonin (15 mg/kg), and vitamin C (100 mg/kg) only. Groups IV-VI received 40% CD, while groups V and VI were additionally treated with melatonin only and melatonin and vitamin C respectively. Groups VII-IX received 80% CD, but groups VIII and IX were additionally treated with melatonin only and melatonin and vitamin C respectively.

Results

Melatonin and/or vitamin C supplement increased body weight in CD-treated rats. The sperm count (but not other semen parameters) was increased by CD and melatonin, while combination of melatonin and vitamin C in CD-treated rats increased all semen parameters. Neither CD alone nor its co-administration with melatonin and /or vitamin C affected plasma luteinising hormone (LH) and testosterone. The 40% CD and 80% CD increased triiodothyronine (T3), but the increase by the former was abolished by melatonin alone while the increase by the latter was neither affected by melatonin alone nor its combination with vitamin C. Moreover, the 40% CD and 80% CD increased thyroxine (T4), but was neither affected by melatonin alone or its combination with vitamin C. The levels of thyroid stimulating hormone (TSH) were not different across all treatment groups. Both 40% CD and 80% CD increased the thiocyanate level, which was ameliorated by melatonin but abolished by combination of melatonin and vitamin C. Both 40% CD and 80% CD decreased the total antioxidant capacity (TAC) level, which was abolished by melatonin.

Conclusion

In conclusion, this study suggests that CD increases weight gain, thyroid hormone and oxidative stress, which were attenuated by anti-oxidants melatonin and vitamin C.

Background

Cassava (Manihot spp) has been a staple crop of the tropics for many years. Owing to the presence of cyanogenic glycosides in cassava, various methods of detoxification have been employed [1, 2]. The traditional method of reducing cassava toxicity in Nigeria is by fermentation. Products from such fermentation include *garri* flour and *fufu* [3]. *Garri* flour derived from cassava is a major staple food for many people in most African and Latin American countries. The soaking of cassava in water, rinsing and baking effectively reduce cassava cyanide content, but improper processing techniques can yield toxic food product [3]. The efficiency of cyanogen removal depends largely on the kinds of unit operations involved in the processing method as well as the initial cyanogen load [4]. The toxicity of cyanogenic glycosides results from the production of hydrogen cyanide, and consequently cyanide poisoning.

Cyanide is a highly toxic compound with both acute and chronic effects [5] stemming from ability to inhibit respiration and the action on some metalloenzymes. The majority of the population is exposed to very low levels of cyanide in the general environment. There are, however, specific subgroups with higher potential for exposure. These include individuals involved in large-scale processing of cassava and those consuming significant quantities of improperly prepared foods containing cyanogen glycosides [6]. The cassava root contains a sufficient amount of cyanogens which require special processing to reduce the danger of toxicity [7]. Cyanide has been shown to be a reproductive toxicant in male dog [8]. Manzano *et al*. [9] examined the effects of sub chronic potassium cyanide in large white pigs and observed histological alterations of the thyroid gland in all the treated animals. Okafor *et al*. [10] also reported increase in the serum level of liver enzymes of rats fed with varied proportion of unprocessed cassava. Okolie and Uansoeje [11] observed the toxic effect of prolonged intake of cassava-borne organic cyanide and inorganic cyanide in some rabbit tissues. There was increase in the level of serum lactate dehydrogenase following cyanide exposure which is an indication of shift in aerobic to anaerobic metabolism, causing lactic acidosis [12].

Melatonin (N-acetyl-5-methoxytryptamine) possesses strong antioxidant activity by which it protects cells, tissues, and organs from the oxidative damage caused by reactive oxygen species (ROS), especially the hydroxyl radical, which attacks deoxyribonucleic acid, proteins, and lipids and causes pathogenesis [13]. The direct effects of melatonin on the male reproductive system and testosterone synthesis from Leydig cells have also been examined in the studies on animals [14]. Because melatonin binding sites have been detected in the reproductive system of different species [15, 16], it also seems reasonable to assume that melatonin exerts its actions through direct interaction with the steroidogenic cells of the reproductive organs [14]. Vitamin C (Ascorbic acid) is an antioxidant that works in aqueous environment of the body. Humans cannot synthesise vitamin C, it must be provided exogenously in the diet and transported intracellular. Ascorbic acid reduces tocopheryl radical formed by the reaction of vitamin E with lipid radicals, protects membranes against oxidation, and prevents lipid peroxidation and affect the regeneration of vitamin E [17].

The exact mechanism by which cyanide exerts a damaging action on tissues is not clear. However, some researchers have proposed that oxidative stress may be implicated in the harmful effects of cyanide

poisoning [18], by increasing ROS and reactive nitrogen species (RNS) [19] and inhibiting antioxidant systems and mitochondrial function. The ameliorative effects of antioxidants on toxicities elicited by many toxicants have also been reported. For instance, Obianime and Robert [20] have observed the ameliorative effect of vitamin C on kidney and testes of cadmium-induced toxicity of male Wistar rats. Vitamin C has also been shown to ameliorate testicular toxicity due to lead exposure in albino rats [21] and associated with improvement in semen quality in humans [22], rabbit [23] and rats [24]. Melatonin and vitamin C have been reported to ameliorate cannabis-induced gonadotoxicity in male rats *in vivo* and *in vitro* [25–27]. The inhibitory effect of melatonin on thyroid growth and function has also been reported [28].

The present study aimed at investigating the effect of melatonin and vitamin C on body weight, thyroid function and gonadal parameters in male rats treated with CD, and their possible mechanisms of actions.

Methodology

Animals

Forty-five (45) adult male Wistar rats (weight range: 180–220 g) were obtained from a trusted commercial breeder. They were housed in wooden cages maintained under standard conditions (12-hr light/dark cycle, 27–30 °C, 50–80% relative humidity), and were acclimatised in the laboratory for two weeks before the commencement of the study. The rats were fed with standard palletised rodent diet (Ace Feeds, Ibadan, Nigeria) and water *ad libitum*. All the animals were well-catered according to the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science and approved by the Ethical Research Committee of the University of Ilorin, Nigeria.

Experimental protocol

Freshly harvested cassava was obtained from International Institute of Tropical Agriculture, Ibadan, Nigeria after proper identification by Mr. Peter Iluebbey (International Trial Manager, Yam-barn Unit) of the Institute, and the specie identification number (TM-91934) was provided by the Institute. The cassava root was peeled to remove the external coat (brownish part) and whitish part was sliced into small pieces. It was later air-dried and pounded into the size of the grower's feed. This enables the cassava to mix properly with grower's feed according to the required proportion. This form of inadequately processed cassava was used throughout the study.

After acclimatisation, the forty-five (45) animals were blindly divided (allocation to groups was done by an invited neutral person who knew nothing about the study) into nine (9) treatment groups (n = 5 each) as follows, Groups I-III received normal saline (control), melatonin (15 mg/kg; Sigma Company Ltd), and vitamin C (100 mg/kg; BioPharma Nig Ltd.) only. Groups IV-VI received 40% CD, while groups V and VI were additionally treated with melatonin only and melatonin and vitamin C respectively. Groups VII-IX received 80% CD [29], but groups VIII and IX were additionally treated with melatonin only and melatonin and vitamin C respectively. Except in the groups that received CDs, other animal groups received standard

animal diet. All animals had unrestricted access to their assigned diet throughout the experimental period, while melatonin and vitamin C were administered daily to the animals in the same order between 10:00 am – 12:00 noon for 28 days.

Animals were euthanised a day after the last treatment under chloroform anaesthesia and blood was collected by cardiac puncture after dissection. The blood was then spun for 10 minutes at 4,000 revolutions per minute and the supernatant plasma from each centrifuged blood was transferred into separate plain bottles and stored at 20ºC before assays of the biochemical parameters. Then, one of the testes was removed and fixed in Bouin's fluid for the histological examination, while the caudal epididymis semen was taken for sperm analysis.

Determination of Biochemical parameters

The TAC (Product Code: BXC0553; Fortress diagnostics Limited, United Kingdom), LH (Catalog No: BXE0651A; Fortress diagnostics Limited, United Kingdom), follicle stimulating hormone, FSH (Catalog No: BXE0631A; Fortress diagnostics Limited, United Kingdom), Testosterone (Catalog No: TE187S; Calbiotech Inc., Spring Valley), TSH (Calbiotech, Spring Valley, CA), T3 (Calbiotech, Spring Valley, CA), and T4 (Calbiotech, Spring Valley, CA) were determined spectrophotometrically (Spectramax Plus; Molecular Devices, Sunnyvale, CA, USA) according to the kit manufacturers' instructions. The serum thiocyanate was determined as previously described [30].

For the assay of T3, 50 µl of the control, specimen, and serum reference were added into the designated well, followed by 100 µl of T3-enzyme conjugate solution and gentle swirling for 20–30 seconds. For the assay of T4, 25 µl of the standards, specimen, and control were added into the designated well, followed by the addition of 50 µl each of the working T4-enzyme conjugates solution and T4-antibody-biotin solution to all wells. For assay of TSH, 50 µl of TSH standards, sample, and control were added into the designated wells and 100 µl of the conjugate reagent was added. In all the assays, the microplate was then incubated for 60 minutes. The wells were emptied of the liquid and were washed three times with wash buffer, followed by blotting on absorbent paper towels. Then, 100 µl of 3,3',5,5'-Tetramethylbenzidine substrate solution was added to all wells and the plates were then covered and incubated for 15 minutes, followed by 50 µl of stop solution and gentle mixing for 15–20 seconds. Within 15 minutes of adding stop solution, the ELISA reading of the absorbance was done at 450 nm. The concentration of T3, T4 or TSH was extrapolated from the standard curve plotted for each of them.

Determination of Cyanogenic Glycoside

The method used for this assay is obtained from the Association of Official Analytic Chemist [31]. Briefly, 4 g of sample was soaked in a mixture containing 40mls of distilled water and 2mls of orth-phosphoric acid. The mixture was totally steroid stopped and left overnight at room temperature to set free all bounded hydrocyanide acid. The resulting sample was transferred into the distillation flask and a drop of paraffin wax is added (anti-foam 1 mg agent) together with broken chips (anti-bump).

The distillation flask was filled to other distillation apparatus and the distilled. About 5 ml of distillate was then collected in the receiving flask that contain 0.1 g of sodium hydroxide pellets. The distillate was then transferred to 50mls volumetric flask and made up to mark with distilled was collected and placed in the conical flask after which 1.6 ml of 5% potassium iodide was added to the filtrate. The resulting mixture was titrated against 0.01. The calculated cyanide content in the cassava used for this study from the method stated above was 3.71 mg/kg.

Estimation of epididymal semen parameters

The caudal epididymis was dissected out from the testis length. Several lacerations were made on the epididymis using sterile scalpel in separate specimen bottles containing normal physiological saline solution at 27 °C. The liberated spermatozoa could then swim out within 3–5 minutes. Caudal epididymal sperm motility and concentration were estimated using the methods of Brooks and Welbster [32].

Epididymal sperm motility was assessed by examining undiluted fluid from the caudal epididymis. A drop of undiluted epididymal fluid was placed on a clean glass slide and examined under the light microscope at a magnification of x400. The mass activity was scored by estimating microscopically the intensity of wave motion.

The right epididymis was minced with anatomic scissors in 5 ml of normal saline, placed in a rocker for 10 min and allowed to incubate at room temperature for 2 min. After incubation, the supernatant was diluted at 1:100 with a solution containing 5 g sodium bicarbonate and 1 ml formalin (35%). The new improved Neuber's counting chamber (haemocytometer) was used in counting the total numbe2r of spermatozoa. About 10 ml of the diluted sperm suspension was transferred to each counting chamber of the haemocytometer and was allowed to stand for 5 min, and thereafter observed under a binocular light microscope.

The proportions of abnormal spermatozoa were examined by random observation of at least 200 spermatozoa on the slide prepared for the estimation of live and dead spermatozoa. The proportion of bent tail and detached head (abnormal sperm cells) were determined, respectively.

Statistical analysis

Data were blindly analysed (by an independent scientist) using the Statistical Package for Social Sciences (SPSS) software version 16 (IBM Corporation, Armonk, NY, USA) and expressed as means ± standard error of the mean (SEM) of the values. One-way analysis of variance (ANOVA) was used to compare the data, followed by post-hoc LSD multiple comparison test to determine the significance at $p < 0.05$. In addition to the comparison of all groups with the control (normal saline) group, the groups that received CD in combination with melatonin and/or vitamin C were additionally compared to the group that received the corresponding dose of CD.

Results

Proximate composition of cassava

The proximate composition of the cassava sample is described in terms of nutrients and their respective percentage of composition. It shows that the cassava sample has dry matter as its highest composition, followed by moisture, crude fibres, crude proteins, total ash, and total fat (Table 1).

Bodyweight in rats given cassava with(out) melatonin and/or vitamin C

When compared to the baseline, there were weekly increases in the weight of the rats in all the groups. Before administration, there was no statistical difference in the weight of the rats across various groups when compared to control. At week 1, the weights of rats that received melatonin (214.4 ± 6.0 g), 40% CD (185.2 ± 22.9 g), 80% CD (174.8 ± 12.4 g), 40% CD + Melatonin (208.4 ± 5.5 g), vitamin C (190.8 ± 14.8 g), 40% CD + Melatonin + Vitamin C (186.0 ± 9.2 g), and 80% CD + Melatonin + Vitamin C (181.6 ± 4.9 g) were higher than that of the rats that received normal saline (140.4 ± 25.3 g). At week 2, the weights of rats that received melatonin (215.0 ± 5.7 g), 40% CD (180.0 ± 20.5 g), 40% CD + Melatonin (188.0 ± 21.4 g), 80% CD + Melatonin (152.0 ± 16.6 g), vitamin C (192.0 ± 11.6 g), 40% CD + Melatonin + Vitamin C (190.0 ± 8.4 g), and 80% CD + Melatonin + Vitamin C (175.0 ± 6.3 g) were higher than that of the group that received normal saline (156.0 ± 26.2 g). At week 3, the weights of rats that received melatonin (212.0 ± 3.7 g), 40% CD (181.0 ± 20.6 g), 40% CD + Melatonin (183.0 ± 22.9 g), 80% CD + Melatonin (141.0 ± 20.02 g), vitamin C (195.0 ± 11.4 g), 40% CD + Melatonin + Vitamin C (187.0 ± 11.6 g), and 80% CD + Melatonin + Vitamin C (171.0 ± 4.9 g) were higher than that of the group that received normal saline (164.0 ± 21.9 g). At week 4, the weights of rats that received melatonin (212.0 ± 5.2 g), 40% CD (186.0 ± 21.4 g), vitamin C (205.0 ± 11.5 g), and 40% CD + Melatonin + Vitamin C (205.0 ± 7.8 g) were higher than the control (159.0 ± 23.3 g) (Fig. 1).

Feed intake in rats given cassava with(out) melatonin and/or vitamin C

Before administration, there was no statistical difference in the feed intake of the rats across various groups when compared to control. At week 1, the feed intake was higher in rats that received 40% CD (35.0 ± 1.6 g) and 80% CD (35.0 ± 1.6 g), lower in rats that received Melatonin (20.0 ± 1.6 g), 80% CD + Melatonin (20.0 ± 2.2 g) and 80% CD + Melatonin + Vitamin C (20.0 ± 1.6 g) but unchanged in rats that received 40% CD + Melatonin (30.0 ± 1.6 g), Vitamin C (30.0 ± 2.2 g) and 40% CD + Melatonin + Vitamin C (30.0 ± 1.6 g) when compared to control (30.0 ± 2.7 g). At week 2, the feed intake was higher in rats that received melatonin (35.0 ± 1.4 g), 80% CD + Mel (25.0 ± 1.6 g), 40% CD + Melatonin + Vitamin C (40.0 ± 2.7 g) and 80% CD + Mel + Vit C (27.0 ± 2.0 g), lower in rats that received 40% CD + Melatonin (15.0 ± 1.6 g) but unchanged in rats that received 40% CD (20.0 ± 1.6 g), 80% CD (23.0 ± 2.0 g) and Vitamin C (20.0 ± 1.4 g) when compared to control (20.0 ± 1.6 g). At week 3, the feed intakes were lower in all groups (except 40% CD) when compared to control. At week 4, the feed intakes were higher in rats that received 40% CD (25.0 ± 3.5 g), 80% CD (30.0 ± 2.7 g), 80% CD + Melatonin (25.0 ± 1.6 g), vitamin C (25.0 ± 1.6 g)

but lower in rats that received melatonin (15.0 ± 1.6 g), 40% CD + Melatonin + Vitamin C (11.0 ± 1.0 g), and 80% CD + Meatonin + Vitamin C (15.0 ± 1.6 g) when compared to control (20.0 ± 1.5 g) (Fig. 2).

Semen parameters in rats given CD with(out) melatonin and/or vitamin C

Vitamin C, but not melatonin, increased the semen parameters (except sperm viability) in rats when compared to control. The 80% CD, but not 40% CD, increased the sperm count when compared to control. The sperm count, but not other semen parameters, was increased by melatonin in 40% CD-treated rats. Combination of melatonin and vitamin C in CD-treated rats increased the semen parameters when compared to control and CD only (Table 2).

Gonadotropins and testosterone levels in rats given CD with(out) melatonin and/or vitamin C

The insignificant decreases in plasma FSH caused by both doses of CD were potentiated by melatonin co-administration to a noticeable level. However, neither CD alone nor its co-administration with melatonin and /or vitamin C caused any noticeable effect on the plasma LH and testosterone when compared to the control (Table 3).

Thyroid hormones and TSH levels in rats given CD with(out) melatonin and/or vitamin C

Melatonin (0.76 ± .08 ng/ml) or vitamin C (0.61 ± 0.11 ng/ml) caused no significant change in the plasma T3 when compared to control (0.68 ± 0.1 ng/ml). The 40% CD (1.11 ± 0.12 ng/ml) and 80% CD (1.01 ± 0.13 ng/ml) increased T3, but the increase by the former was abolished by melatonin alone (0.69 ± 0.13 ng/ml) while the increase by the latter was neither affected by melatonin alone (0.95 ± 0.12 ng/ml) or its combination with vitamin C (0.91 ± 0.12 ng/ml) (Fig. 3).

Melatonin (0.98 ± 0.4 µg/ml) or vitamin C (1.21 ± 0.5 µg/ml) did not affect T4 when compared to control (0.63 ± 0.1 µg/ml). The 40% CD (1.21 ± 0.11 µg/ml) and 80% CD (0.99 ± 0.09 µg/ml) increased T4, which was neither affected by melatonin alone (1.15 ± 0.13 µg/ml; 1.00 ± 0.13 µg/ml respectively) or its combination with vitamin C (1.35 ± 0.12 µg/ml; 1.15 ± 0.10 µg/ml respectively) (Fig. 4).

The levels of TSH were not different across all treatment groups (Fig. 5).

Thiocyanate and Total Antioxidant Capacity (TAC) in rats given CD with(out) melatonin and/or vitamin C

Vitamin C (4.95 ± 0.12 µg/ml), but not melatonin (4.16 ± 0.99), increased the thiocyanate level when compared to control (4.19 ± 0.37 µg/ml). Both 40% CD (7.94 ± 0.61 µg/ml) and 80% CD (7.36 ± 0.63 µg/ml) increased the thiocyanate level, which was ameliorated by melatonin (5.29 ± 0.31 µg/ml;

5.88 ± 0.91 µg/ml) but abolished by combination of melatonin and vitamin C (4.29 ± 0.59 µg/ml; 4.72 ± 0.36 µg/ml) (Fig. 6).

Melatonin (1.30 ± 0.35 µg/ml) or vitamin C (1.96 ± 0.08 µg/ml) did not affect TAC when compared to control (1.90 ± 0.09 µg/ml). Both 40% CD (1.69 ± 0.03 µg/ml) and 80% CD (1.64 ± 0.07 µg/ml) decreased the TAC level, which was abolished by melatonin (1.85 ± 0.06 µg/ml; 2.04 ± 0.14 µg/ml) (Fig. 7).

Table 1. Proximate composition of the cassava

Nutrient	Composition (%)
Dry matter	87.50
Moisture Content	12.50
Crude fibres	5.20
Crude Protein	3.60
Total Ash	2.18
Crude Fats	0.55

Table 2: Effect of melatonin and/or vitamin C on semen parameters of rats treated with CD.

roups	Sperm count (10^6/ml)	Sperm motility (%)	Sperm morphology (%)	Sperm Viability (%)
ontrol	49.13±1.05	82.74±1.09	88.03±1.24	90.94±1.87
elatonin	52.60± 1.13	82.12±0.28	86.76±0.80	90.72±0.94
tamin C	63.73±1.96*	87.34±0.15*	91.84±1.42*,	90.61±2.73
)% CD	46.87±0.70	81.88±1.28	85.29±0.05	82.76±0.64
)% CD + melatonin	55.87±1.62*	83.81±0.97	86.82±1.73	90.34±2.15
)% CD + Melatonin Vitamin C	56.93±1.09*#	86.61±1.75*#,	90.04±1.74#	92.51±1.40#
)% CD	53.33±0.48*	82.65±0.81	85.47±0.86	88.53±1.07
)% CD + Melatonin	54.53+1.79*	82.40±0.34	86.58±0.92	89.92±0.90
)% CD + Melatonin Vitamin C	64.47±2.33*#	87.82±0.12*	92.53±0.58*#	95.84±0.56*#

*p<0.05 when compared to control; CD denotes Cyanide-enriched cassava-diet

Table 3: Effect of melatonin and/or vitamin C on gonadotropins and testosterone of rats treated with CD.

GROUPS	FSH (mIU/ml)	LH (mIU/ml)	TESTOSTERONE (ng/mol)
Control	8.50± 1.25	5.25± 0.52	2.00±0.20
Melatonin	7.38±0.59	5.25± 0.14	1.75 ± 0.25
Vitamin C	6.63±0.94	5.38± 0.24	1.88 ±0.13
40% CD	6.13 ± 1.07	5.75 ± 0.14	1.88± 0.59
40% CD + Melatonin	5.88±0.69*	5.88±0.43	2.00±0.29
40% CD + Melatonin + Vitamin.C	6.38±0.75	5.25±0.52	1.25±0.14
80% CD	6.25±0.95	5.00±0.71	1.75±0.25
80% CD + Melatonin	4.88±0.13*	5.75±0.32	2.00 ± 0.54
80% CD + Melatonin + Vitamin.C	6.63±0.18	5.75± 0.25	1.38±0.13

*$p<0.05$ when compared to control; CD denotes Cyanide-enriched cassava-diet

IS - interstitial space, ST - seminiferous tubule, SC - Sertoli cells, LC - Leydig cells, GC - germ cells, SP/C - sperm cells). (A) Control group having normal testicular tissue with a well-differentiated germ cell (B) Melatonin-treated rats that show hypertrophic tissue with loss of cellularity (C) Rats that received 40% CD, showing spermatocyte and testicular degeneration with shrunken seminiferous tubules (D) Rats that received 80% CD, showing poorly differentiated hyperplastic tissue with no recognizable cell features (E) Rats that received melatonin in addition to 40% CD, showing hypertrophic testicular tissue with loss of spermatocytes (F) Rats that received melatonin in addition to 80% CD, showing hypertrophic testicular tissue with loss of spermatocytes and widening of the interstitial space (G) Rats that received vitamin C

only, showing normal testicular tissue with mild infiltration (H) Rats that received melatonin and vitamin C in addition to 40% CD, showing normal but hypertrophic tissue (I) Rats that received melatonin and vitamin C in addition to 80% CD, showing normal testicular tissue.

Discussion

Reduction in weight gain in sheep [33], hen [34], broiler chicks [35] and rabbits [36] treated with cassava or cyanide have been reported in a concentration-dependent manner. Avais *et al*. [36] also reported an insignificant effect of cyanide on feed intake in cyanide-treated rats. However, the high nutritional value of cassava has been reported, having 112 calories per 100 gram compared to sweet potatoes and beets having 76 calories per 100 grams and 44 calories per 100 grams respectively [37]. Its ability to provide high calories has made it an important crop for developing countries. Consumption of high calories diet has been associated with weight gain and obesity. For instance, dietary energy density was associated with higher body mass index, waist circumference, elevated fasting insulin, metabolic syndrome in U.S. adults [38]. Normal-weight persons have also been shown to have diets with a lower energy density than obese persons [39]. The increase in weight gain by CD in the present study could thus be partly associated to its high calories as previously reported. While the discrepancy in our observation of high weight gain and the reduction in weight gain reported by others [33, 35, 36] cannot be convincingly justified at the moment, we speculate that the dosage of the cassava administered, species of animals, the geographical effect on different cassava samples and processing methods could be culpable.

Consumption of foods with low energy density (kcal/g) has been said to reduce energy intake and has been recommended for weight management. For instance, men and women with low-energy-dense diet had a lower energy intake (approximately 425 and 275 kcal/d less) than did those with a high-energy-dense diet, even though they consumed more food (approximately 400 and 300 g/d more, respectively). Moreover, persons with high fruits and vegetable intake had the lowest energy density values and the lowest obesity prevalence [39]. In this study, we investigated if supplementation of CD with anti-oxidants having negligible energy density will increase feed consumption and also reduce the bodyweight of rats as noted in humans by Ledikwe *et al*. [39] and Mendoza *et al*. [38]. We observed an increase in body weights of rats that received melatonin and vitamin C supplements in addition to CD throughout the experimental period. We also found that the feed intake was higher in rats that received cassava but lower or unchanged in rats that received melatonin and/or vitamin C with(out) cassava at weeks 1 and 4. Contrarily, the feed intake was higher in rats that received melatonin and vitamin C supplements in addition to cassava at week 2, and also higher in all groups (except in those that received 40% CD) at week 3. Thus, our study suggests that the effect of energy density on weight gain and feed consumption are time-dependent.

Is the cassava-induced increase in body weight related to alteration in thyroid functions? It has been reported that cyanide causes a reduction in the growth rate of hens by inhibiting intra-thyroidal stimulating hormone and thereby causing a reduction in thyroxine level which is necessary for growth [34].

In a Mozambique rural population affected by spastic paraparesis, the anti-thyroid effect of thiocyanate from cassava-derived cyanide exposure manifested as decrease in serum T4 but increase in serum T3, T3/T4 ratio and TSH [40]. Thiocyanate also inhibited sodium-iodide symporter, thus reducing the transport of iodine from circulation into thyroid follicular cells which will impair thyroid hormone synthesis [41]. In weaned mice, thiocyanate decreased thyroid T3, T4 and iodine contents but increased plasma TSH with corresponding hyperthrophy of the thyroid gland, all of which followed recovery after thiocyanate withdrawal [42]. Fresh cassava root-induced elevation of serum thiocyanate was accompanied with no change in thyroid gland size, and thus no goiter [43]. However, our own study disagrees with these previous studies and showed hyperthyroid effect of CD, as we noticed increase in the plasma T3 and T4 without any corresponding change in the TSH. Our study partly agrees with that of Adeniyi *et al*. [44] that also noticed cyanide-induced hyperthyroidism, but slightly disagrees with their study as our own hyperthyroidism is independent on thyrotropin like theirs. Adeniyi *et al*. [44] treated male Wistar rats with hexacyanoferrate III solution for 56 days and reported significant increase in the levels of thyroid hormones (T3 and T4) but reduction in TSH, while the thyroid gland showed marked epithelial hyperplasia with cellular degeneration and scanty cytoplasm. We therefore speculate that the increase in weight gain elicited by CD in our study is related to hyperthyroidism-induced increase in feed intake in the rats.

Does cassava and its cyanide cause oxidative stress? Serum thiocyanate is a stable metabolite and a useful biomarker of cyanide exposure [40], whose implications in oxidative stress have been well-documented [30]. For instance, prolonged sub-lethal cyanide administration caused a decline in superoxide dismutase activity in red blood cells and catalase activities in some tissues of cyanide-toxified rats [45–47]. Cyanide intoxication has been linked to increasing lipid peroxidation leading to the production of malondialdehyde (MDA) which is a pro-oxidant that causes oxidative stress [46, 48]. Hariharakrishnan *et al*. [49] also reported that various concentrations of cyanide caused cytotoxicity in Rhesus monkey kidney epithelial cells, which was accompanied by elevation of MDA, reactive oxygen species (ROS), reactive nitrogen species (RNS), and diminished cellular antioxidant status (reduced glutathione, glutathione peroxidase, superoxide dismutase and catalase). Increase in serum aminotransferases (aspartate and alanine aminotransferase) have also been observed following cyanide exposure indicating damage to the cell membrane of the liver [50]. In the present study, we observed that both doses of CD increased thiocyanate and reduced the total antioxidant capacity (TAC). These observations suggest that CD has pro-oxidant effect and are in agreement with previous studies cited in this paragraph.

Is there a link between the cassava-induced hyperthyroidism and oxidative stress? The involvement of ROS and oxidative stress in the development of hyperthyroidism and autoimmune diseases like Graves' disease has been well documented. For instance, hyperthyroidism increases oxygen consumption, dysfunction in the mitochondrial respiratory chain, elevated intracellular adenosine triphosphate consumption and increased ROS production [51, 52]. Genesis of Graves' disease and its orbitopathy [53], in

addition to hyperthyroidism-induced damage such as thyrotoxic myopathy and cardiomyopathy [54] have been strongly linked to oxidative stress. Untreated hyperthyroidism also reportedly increased oxidative stress parameters, while restoration of euthyroidism with antithyroid drug reversed the biochemical abnormalities associated with oxidative stress [55]. In fact, animal and human studies suggest that increased ROS directly contribute to some clinical manifestations of the disease. Our simultaneous observation of hyperthyroidism and oxidative stress in cassava-treated rats show that there is a link between these two conditions as reported by others.

Can the oxidative stress be ameliorated by melatonin and/or vitamin C? Treatment of 24 hyperthyroid patients with propylthiouracil for 5 days combined with vitamin C for 1 month potentiated the antioxidant defense system and oxidative stress in them [56]. Antioxidants treatment also improved clinical picture of hyperthyroid patients and led to earlier attainment of euthyroid state [57, 58]. Asayama *et al*. [59] also reported that vitamin E protects against thyroxine-induced lipid peroxidation in muscles. Similarly, we also observed in this study that melatonin and/or vitamin C ameliorates cassava-induced oxidative stress and hyperthyroidism in rats.

Exposure of dogs to cassava-borne cyanide diet for 14 weeks has been reported to elicit testicular degenerative changes and liver lesion [8]. In our present study, the sperm count (but not other semen parameters) was increased by 80% CD, but not 40% CD, an effect that was augmented by melatonin. Combination of melatonin and vitamin C in cassava-treated rats increased the semen parameters when compared to control and CD only. Our data also showed that CD did not significantly affect testosterone and gonadotropins, neither did melatonin and/or vitamin C caused any change when administered alone or combined with cassava treatment. Though most of the semen parameters did not show significant effect in cassava-treated animals, the degeneration of the seminiferous tubules as evident from the testicular histology suggests gonadotoxicity in these animals. It also suggests that the gonadotoxic effect of cassava in male rats are independent on the hormones but might be attributed to either the direct or indirect effect of cyanide and/or its metabolite (thiocyanate) on the seminiferous tubules via induction of oxidative stress.

Conclusion

In conclusion, this study suggests that CD increases weight gain, thyroid hormone and oxidative stress, which were attenuated by anti-oxidants melatonin and vitamin C.

Abbreviations

CD: Cyanide-enriched cassava-diet; LH: Luteinising hormone; T3: Triiodothyronine; T4: Thyroxine; TSH: Thyroid stimulating hormone; TAC: Total antioxidant capacity; ROS: Reactive oxygen species; RNS: Reactive nitrogen species; FSH: Follicle stimulating hormone; SCN: Thiocyanate; Mel: Melatonin; Vit C: Vitamin C; MDA: Malondialdehyde.

Declarations

Ethics Approval and consent to participate

All the animals were well-catered according to the criteria outlined in the ‘Guide for the Care and Use of Laboratory Animals’ prepared by the National Academy of Science and approved by the Ethical Research Committee of the University of Ilorin, Nigeria.

The consent to participate in the study is not applicable.

Consent for publication

Not applicable

Competing interest

The authors declare that they have no competing interest

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Authors’ contributions

IAA and LAO conceived and supervised study. IAA designed the study. OOM and CMI carried out the study and re-analysed the data. IAA and OOM interpreted the data and drafted the manuscript. All authors read and approved the final manuscript to be published.

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None

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Figures

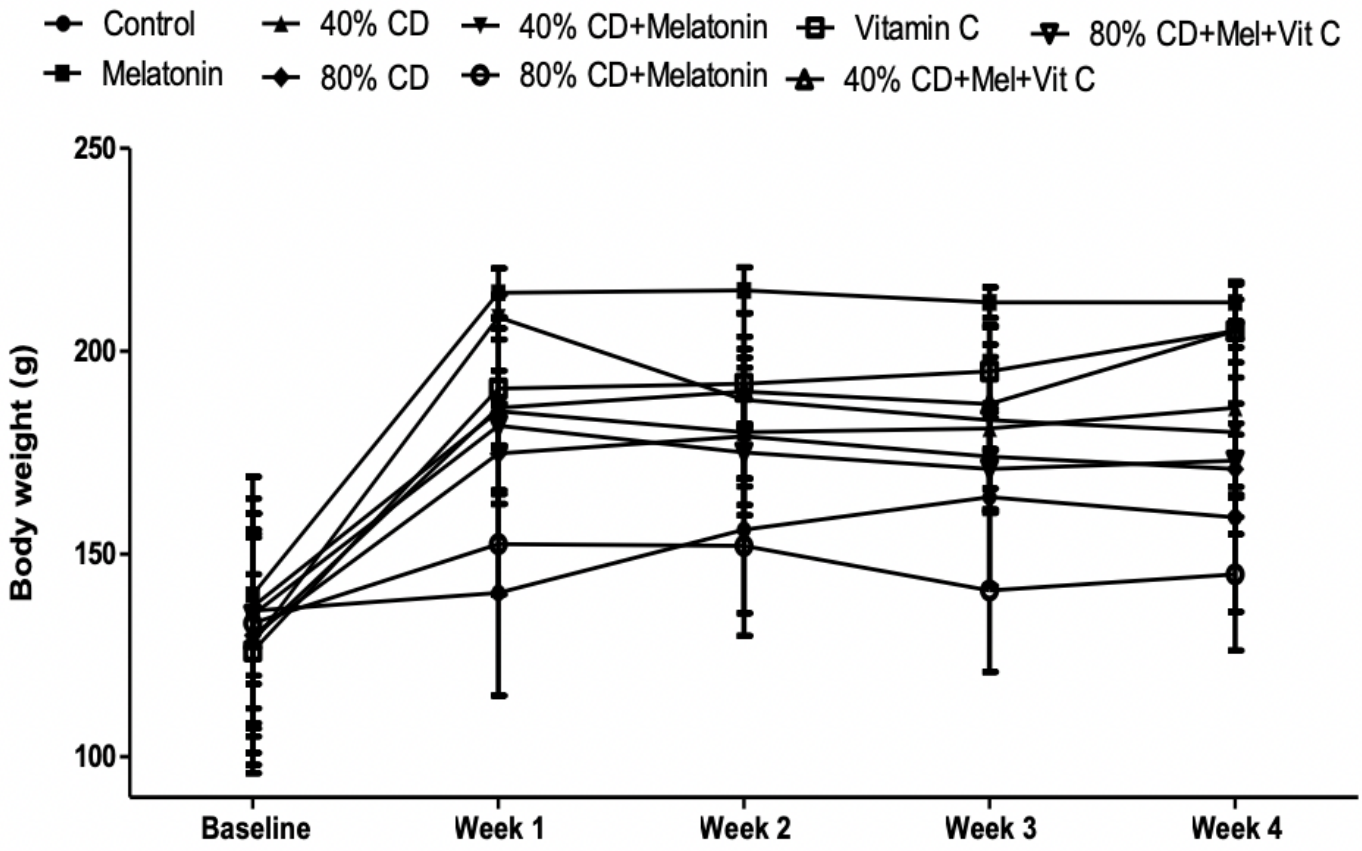

Figure 1

Effect of melatonin and/or vitamin C on bodyweight of rats treated with Cassava-diet. CD denotes cyanide-enriched cassava-diet; Mel denotes melatonin; Vit C denotes vitamin C

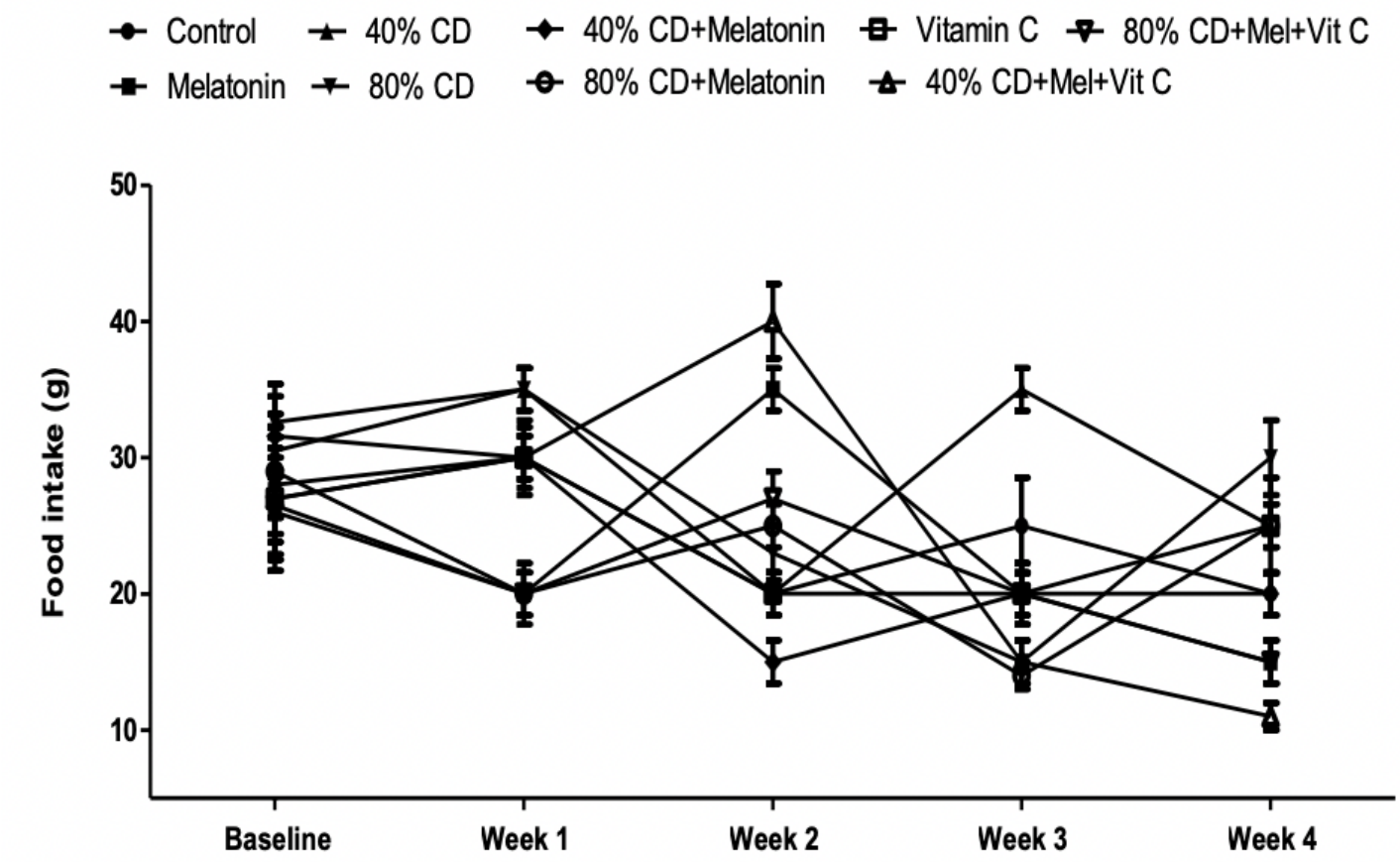

Figure 2

Effect of melatonin and/or vitamin C on feed intake of rats treated with Cassava-diet. CD denotes cyanide-enriched cassava-diet; Mel denotes melatonin; Vit C denotes vitamin C

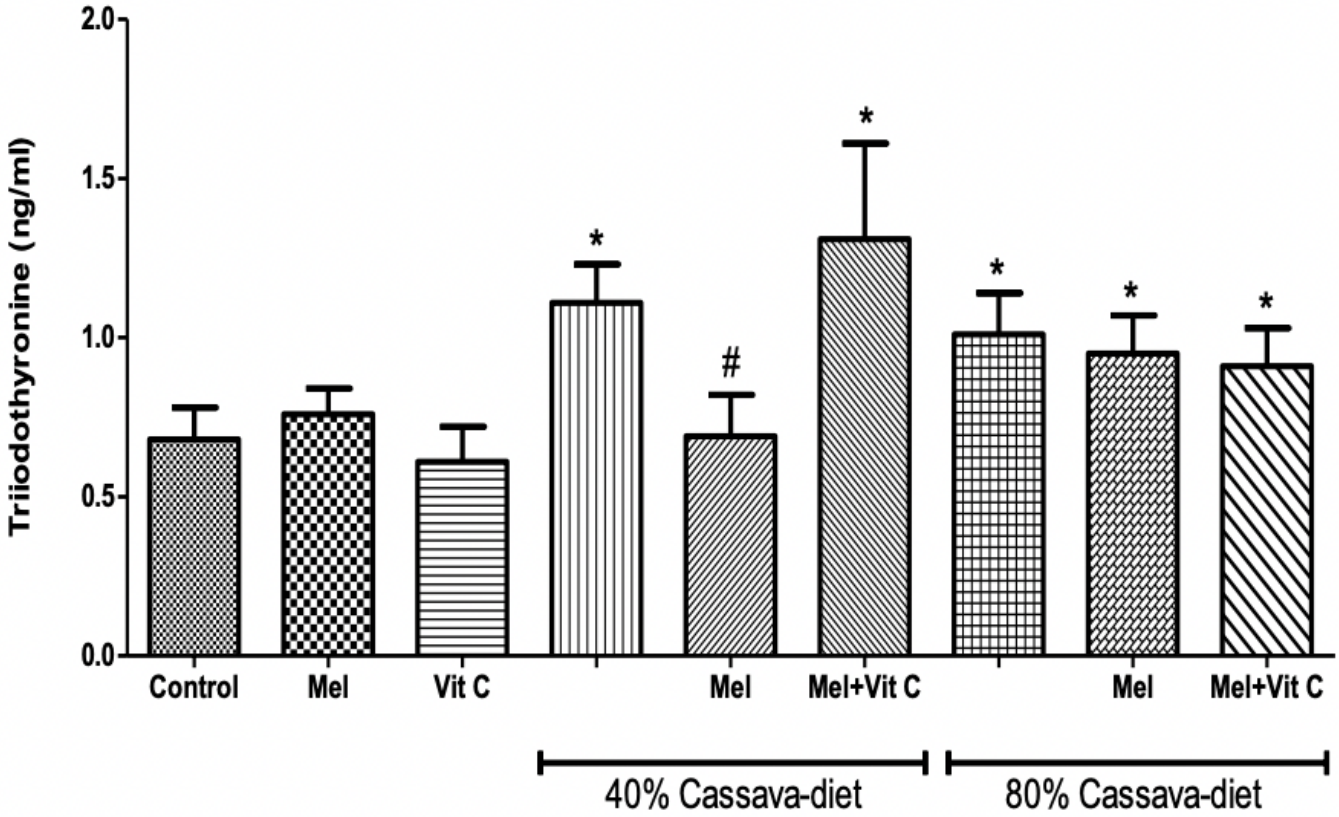

Figure 3

Effect of melatonin and/or vitamin C on triiodothyronine of rats treated with Cassava-diet. *p<0.05 when compared to control; #p<0.05 when compared to 40% Cassava-diet; Mel denotes melatonin; Vit C denotes vitamin C

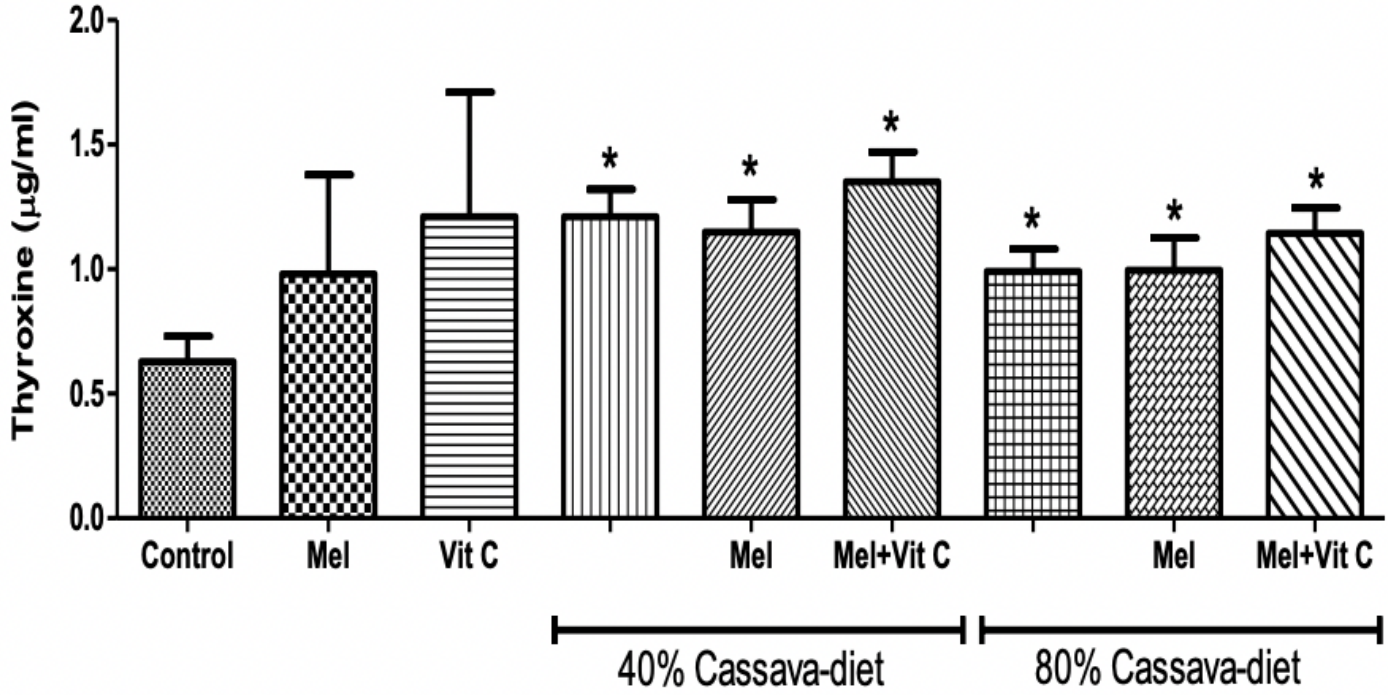

Figure 4

Effect of melatonin and/or vitamin C on thyroxine of rats treated with Cassava-diet. *p<0.05 when compared to control; Mel denotes melatonin; Vit C denotes vitamin C.

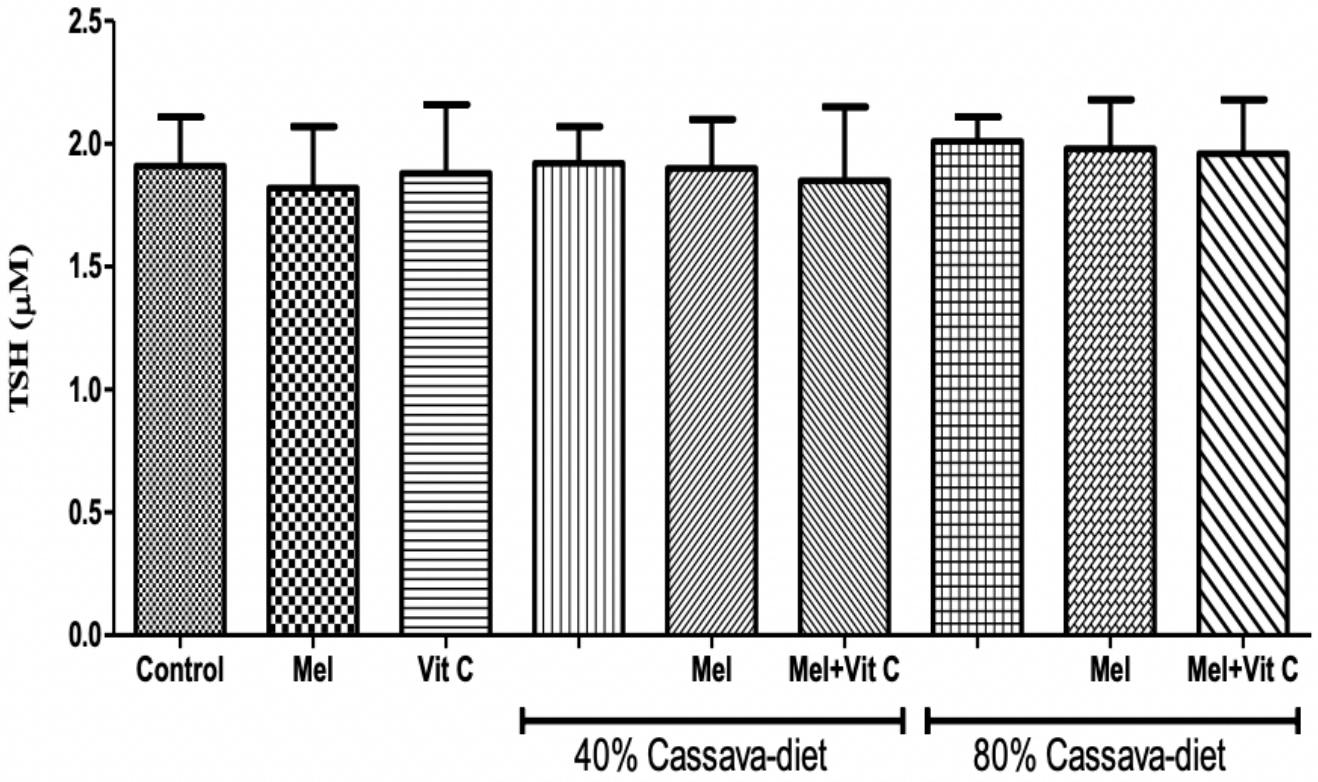

Figure 5

Effect of melatonin and/or vitamin C on thyroid-stimulating hormone of rats treated with Cassava-diet. TSH denotes thyroid-stimulating hormone; Mel denotes melatonin; Vit C denotes vitamin C.

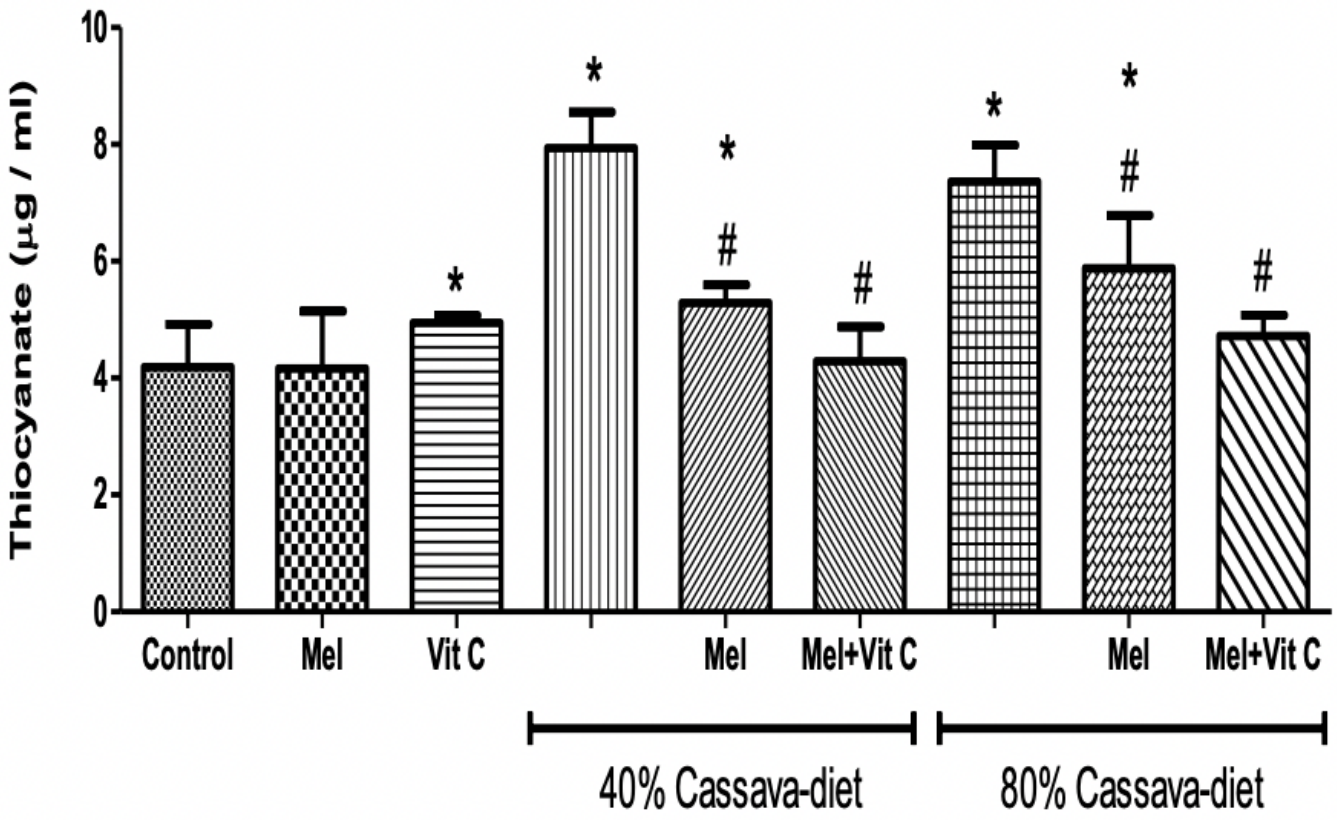

Figure 6

Effect of melatonin and/or vitamin C on thiocyanate of rats treated with cassava-diet. *p<0.05 when compared to control; #p<0.05 when compared to the corresponding dose of Cassava-diet; Mel denotes melatonin; Vit C denotes vitamin C.

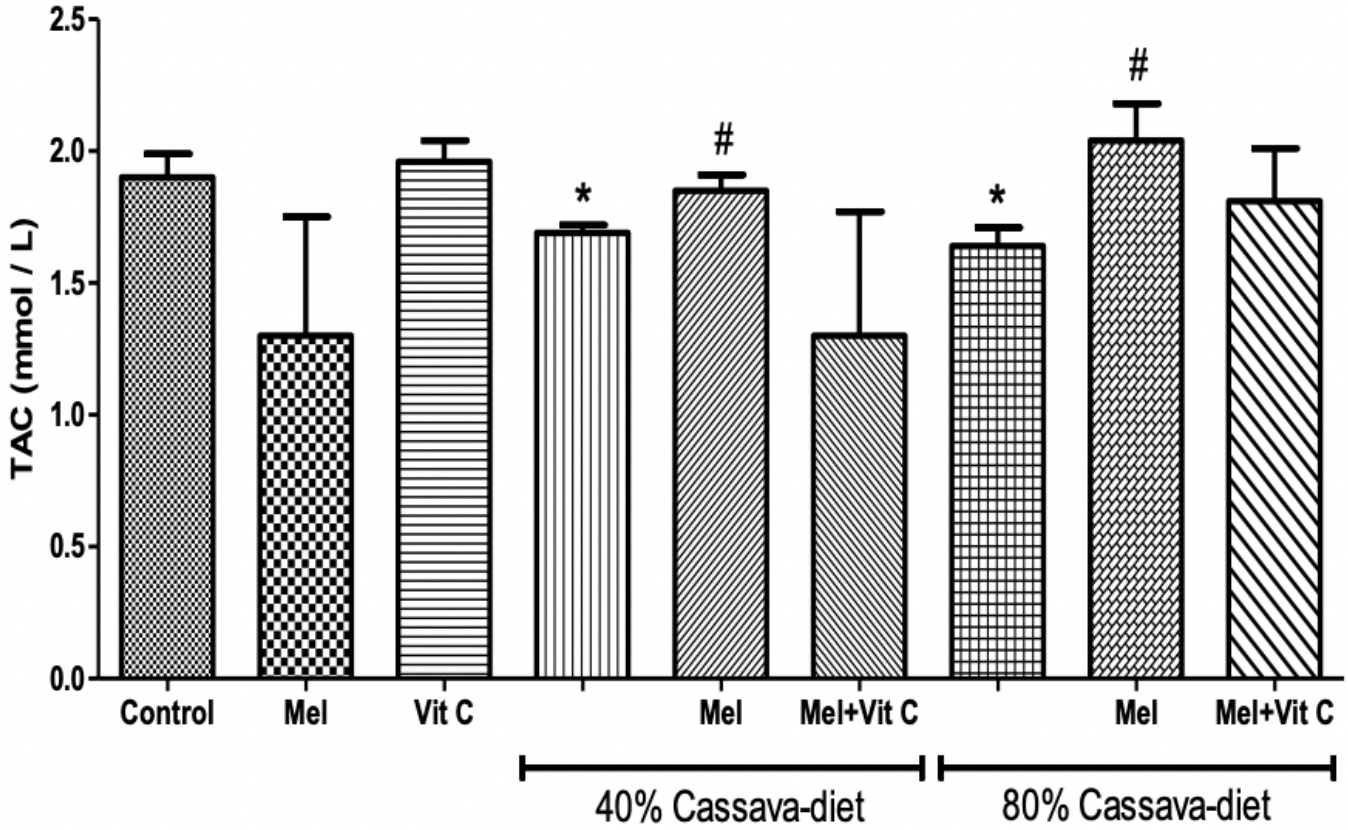

Figure 7

Effect of melatonin and/or vitamin C on total antioxidant capacity of rats treated with cassava-diet. *p<0.05 when compared to control; #p<0.05 when compared to the corresponding dose of Cassava-diet; Mel denotes melatonin; Vit C denotes vitamin C.

Figure 8

Effect of melatonin and/or vitamin C on testicular histology of rats treated with cassava-diet (CD).

Supplementary Files

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