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Comparative Effect of Carbimazole, *Citrus sinensis*, Glycine Max and Levothyroxine on Blood Glucose Levels and Body Weight Changes

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Authors' contributions

This work was carried out in collaboration between all authors. Author OUA designed the study, coordinated the research, and wrote the first draft of the manuscript. Author UJA managed the analysis and interpretation of data. Authors OMA and AEJ wrote the protocol and managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: The role of thyroid hormones as important mediator of glucose metabolism and body weight dynamics had long been established. This study was therefore designed to evaluate the effect of standard thyro-active drugs in comparison to crude nutritional extracts, on blood glucose level and body weight changes.
Methodology: Albino wistar rats weighing between 100 - 150g were randomly assigned to seven groups of seven rats each. Group 1 served as control, while groups 2-7 were orally administered Fresh orange juice(FOJ) (1500mg/kg), Fresh soybean(FSB) (0.01mg/kg), levothyroxine (LVT) (0.01mg/kg), carbimazole (Carb) (0.01mg/kg), FSB+LVT

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and FOJ+Carb respectively once daily for twenty eight days. Weekly weight records were taken and the difference calculated as weight changes. The animals were sacrificed and blood samples collected by cardiac puncture. Serum was obtained by standard procedure and used for blood glucose estimation.
Results: Separate treatment with both FOJ and FSB significantly (P<0.05) increased the blood glucose levels and body weight compared to control. Levothyroxine significantly (P<0.05) increased the blood glucose levels but significantly (P<0.05) decreased the body weight compared to the FOJ treated group. Carbimazole significantly (P<0.05) decreased the blood glucose levels compared to the control, FOJ and FSB treated groups. FSB+LVT treatment significantly (P<0.05) decreased the blood glucose levels compared to the LVT group and also significantly (P<0.05) decreased the body weight compared to the control, FOJ and FSB groups. FOJ+Carbimazole combination treatment significantly (P<0.05) decreased the blood glucose levels compared to LVT treatment and also significantly (P<0.05) decreased the body weight compared to the FOJ and FSB groups. FOJ significantly reduced T_4 and T_3 levels compared to control. FOJ, Carb and FOJ+Carb significantly ($p<0.05$) reduced T_4 level compared to control.
Conclusion: The acclaimed antithyroid activity of FOJ and FSB did not appear to directly correlate with that of carbimazole, a standard antithyroid drug, considering their effect on blood glucose level and body weight changes. However their combination treatment with Carb and LVT respectively, showed similar pattern with carbimazole.

Keywords: Carbimazole; Citrus sinensis; Glycine max; Levothyroxine; blood glucose; body weight.

1. INTRODUCTION

Thyroid hormones exert profound effects in the regulation of glucose homeostasis. These effects include modifications of circulating insulin levels and counter-regulatory hormones, intestinal absorption, hepatic production and peripheral tissues (fat and muscle) uptake of glucose [1].

For effective execution of glucose homeostasis, synthesis and release of thyroid hormones must be adequately regulated in the body. Both thyroid disorders and substances that disrupt the synthesis and secretion of thyroid hormone have a major impact on glucose control [2]. When thyroid dysfunction ensues, the glucose homeostatic balance is broken.

Synthetic thyroactive drugs are produced for treatment of thyroid disorders, such as carbimazole, a pro-drug of methimazole and propylthiouracil both used in the treatment of hyperthyroidism.

Another synthetic analogue of thyroxine, levo-thyroxine is used as a hormone replacement therapy for patients with hypothyroidism [3].

The stability of body weight depends on the energy intake and expenditure. When energy intake exceeds output, the excess energy is stored in the body as carbohydrates, protein or fats and this causes a gain in body weight. The converse is also true when energy expenditure exceeds energy intake, body weight will decrease.

It has been appreciated for a very long time that there is a complex relationship between thyroid disease, body weight and metabolism. Thyroid hormone regulates metabolism in both animals and humans. Metabolism is determined by measuring the amount of oxygen used by the body over a specific amount of time. If the measurement is made at rest, it is known as the basal metabolic rate (BMR). Indeed, measurement of the BMR was one of the earliest tests used to assess a patient's thyroid status. Hypothyroid patients were reported to present with low BMR while patients with hyperthyroidism had high BMR [4].

Certain herbal preparations and other synthetic thyroactive drugs are known to influence the synthesis and metabolic fate of thyroid hormones. Some of the primary axes of influence appears to be through alterations in peripheral conversion, hepatic antioxidant enzyme systems and other avenues of thyroid hormone metabolism. The effect of soy products on thyroid hormone function and metabolism in humans is still being researched; however, animal evidence is suggestive of an impact on aspects of peripheral conversion. In animal experiments soy protein elevated plasma T_4 concentrations [5]. However, some naturally occurring flavonoids found in orange fruits (*Citrus sinensis*) and other synthetic derivatives, have the potential to disrupt thyroid hormone metabolism. Synthetic flavonoid derivatives have been shown to decrease serum T_4 concentrations and inhibit both the conversion of T_4 to T_3 [6]. Naturally occurring flavonoids appear to have a similar inhibitory effect.

Although several health benefits have been associated with consumption of both soy beans and orange juice, there is also a need to consider their safety, particularly in relation to thyroid function. Previous reports on the effect of *Citrus sinensis* and glycine max on thyroid hormone has been inconsistent. Some studies claim increase thyroid hormone level with FOJ treatment and decrease thyroid hormone level with FSB treatment, other studies have reported the reverse respectively.

On the basis of the above observations, the need for further studies became imperative. Considering the profound effect of thyroid hormones on blood glucose metabolism and body weight dynamics, this study therefore aims to provide an indirect means to assess the effects of these nutritional extracts on the above physiologic indices, compared to standard thyro-active drugs and their combination treatment.

2. MATERIALS AND METHODS

2.1 Materials

Fresh soy bean seeds were bought from Akpan Andem Market, while the sweet orange fruit was purchased from Itam market both in Uyo. Their identities were confirmed by a botanist in the Department of Botany, Faculty of Sciences, University of Uyo, Uyo, Nigeria. The voucher specimens were kept in same department with the following numbers UUS 5671 and UUS 3769 for *Citrus sinensis* and *Glycine max* respectively. The beans were pulverized into powder (70g) using electric blender, and sieved. The pulverised powder was macerated in 400ml of distilled water and incubated for 12 hrs at room temperature.

The final filtrate was dried in a hot plate to obtain a brown gummy paste which was weighed with a mettler P163 electronic weighing balance. The stock solution of the extract was prepared by dissolving 1gm of the extract in 10ml of water to give a concentration of 100mg/ml. The stock solution was labeled appropriately and stored in a refrigerator until it was needed for administration.

The stock concentration of sweet orange juice was determined by taking 1ml of orange juice into 10ml beaker of a known weight, then concentrated to dryness using hot plate. The weight difference between empty beaker and the concentrated content were recorded. This was repeated 3 times and the average was recorded as 90g/ml.

The median lethal dose (LD_{50}) of both soybean and fresh orange juice extract was determined by modified method of Lorke (1983) [7].

2.2 Animal Preparation and Experimental Design

A total of 49 adult male albino rats weighing between 100 - 150g obtained from the disease free stock of the animal house, Physiology Department, College of Medical Sciences University of Calabar, Calabar, Nigeria, were used for the study. The animals were kept in conducive and well ventilated rooms. Each rat in a study group was individually housed in a stainless steel cage with wooden bottom grid and a wire screen top. They were fed with pelletized guinea feed grower mash and allowed food and water *ad libitum.* Good hygiene was maintained by constant cleaning and removal of faeces and spilled feed from cages daily. They were handled according to the recommendation of the local and national ethic committees.

The rats were randomly assigned into seven (7) groups of seven (7) rats in each group and were designated and treated as follows:

Group 1: Control given only clean water
Group 2: Was given fresh orange juice [1500mg/kg]
Group 3: Was administered fresh soybean [207.84 mg/kg of stock concentration]
Group 4: Was administered Levothyroxine [0.14 µg/kg]
Group 5: Was administered carbimazole [0.01mg/kg]
Group 6: Was given fresh soybean and levothyroxine [207.84mg/kg + 0.14µg/kg respectively]
Group 7: Was given fresh orange juice and carbimazole [1500mg/kg + 0.01 mg/kg]
The treatment was once daily for 28 days.

2.3 Blood Sample Preparation

At the end of the experiment, rats were anesthetized with chloroform. About 1 - 1.5ml of blood was collected directly from the heart through cardiac puncture of the anaesthetized dissected rats using 2 ml syringe and a 23 gauge needle. The collected blood samples were stored in heparinised vials for serum separation.

2.4 Assay of Blood Glucose

Glucose was analysed from serum sample using Accu-Check Advantage II, Roche Diagnostic GmbH, Mannheim, Germany. Values obtained using the glucometer have been shown to correlate excellently with those from the use of standard biochemical methods [8,9].

2.5 Determination of Body Weight Changes

Rats' body weight were measured at the start of the experimental period, 1st, 2nd, 3rd and 4th weeks, using a digital balance. These weights were determined at the same time during the morning and values in grams were recorded in the study log book.

2.6 Tri-iodothyronine (T_3) Assay

Tri-iodothyronine assay was done using DS-EIA-THYROID-T_3 TOTAL RT kit, with lower detection limit at 0.2 ng/ml. The sensitivity was calculated by determining the variability of the 0 ng/ml calibrator and using the 2 SD (95% certainty) statistics.

2.7 Thyroxine (T_4) Assay

Thyroxine assay was done using DS-EIA-Thyroid-T4 Total RT kit, with lower detection limit at 5.0 nmol/l. The sensitivity was calculated by determining the variability of the 0 nmol/l calibrator and using the 2 SD (95% certainty) statistics.

2.8 Statistical Analysis

The obtained data are presented as means ± standard error of mean (SEM). The difference between the groups was calculated using analysis of variance (ANOVA) followed by multiple mean comparisons. Mean differences between treatments groups were tested using least significant difference (LSD) at $p<0.05$ level of significance.

3. RESULTS

3.1 Comparison of Blood Glucose in the Different Experimental Groups

The mean ± SEM blood glucose concentrations were 5.46 ± 0.15, 5.93 ± 0.12, 6.10 ± 0.13, 6.33 ± 0.33, 5.21±0.21, 5.22±0.40, and 5.34 ± 0.23 mmol/L for control, FOJ, FSB, LVT, Carb, FSB+LVT, FOJ+Carb groups respectively. From the results obtained, FOJ and FSB significantly ($P<0.05$) increased the blood glucose levels when compared to the control group. LVT also significantly ($p<0.05$) increased the blood glucose levels when compared to the control group. Carbimazole significantly ($P<0.05$) decreased the blood glucose levels compared to the control, FOJ and FSB treated groups. FSB + LVT treatment significantly ($P<0.05$) decreased the blood glucose levels compared to the LVT group. FOJ + Carbimazole combination treatment significantly ($P<0.05$) decreased the blood glucose levels compared to LVT treatment. (Fig. 1).

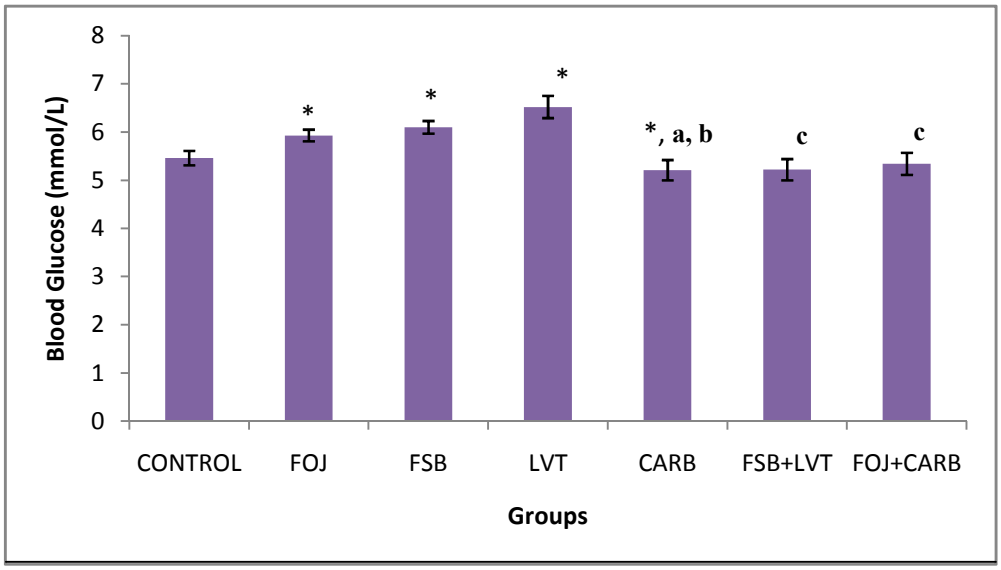

Fig. 1. Comparison of blood glucose in the different experimental groups

Values are mean ± SEM, n = 7.
**= p<0.05 vs Control; a= p<0.05 vs FOJ;*
b= p<0.05 vs FSB; c = p<0.05 vs LVT

3.2 Comparison of Body Weight Changes in the Different Experimental Groups

The mean ± SEM body weight changes were 69.71 ± 4.00, 90.71± 6.67, 82.85 ± 5.28, 72.71 ± 3.11, 62.00 ± 2.59, 51.57 ± 4.13, and 67.00 ± 7.73g for control, FOJ, FSB, LVT, Carb, FSB + LVT, FOJ + Carb groups respectively. From our results, FOJ and FSB significantly ($p<0.05$) increased the body weight of the experimental rats. The weight gain was consistently progressive from the first week of treatment to the last week of treatment. Levothyroxine treatment significantly ($p<0.05$) decreased the body weight when compared to the FOJ treated group. The weight loss was recorded from the second week of treatment. The highest percentage of weight loss was recorded in the last measurement (4^{th} week). Carbimazole significantly decreased body weight when compared with FOJ and FSB treated groups. FSB + LVT treatment also significantly ($p<0.05$) decreased the body weight compared to the control, FOJ, FSB and LVT groups. FOJ + Carbimazole combination treatment significantly ($p<0.05$) decreased the body weight compared to the FOJ and FSB groups. The effect of all the combination treatments on body weight changes were progressive with the duration of treatment. (Fig. 2).

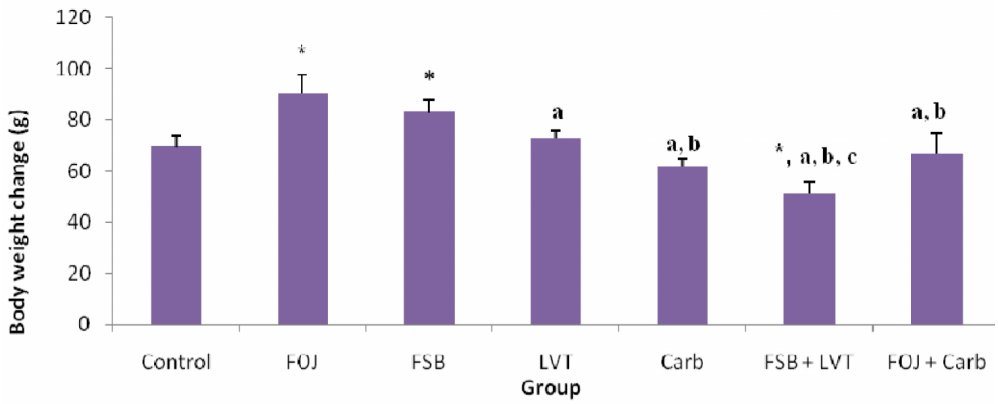

Fig. 2. Comparison of body weight change in the different experimental groups Values *are mean + SEM, n = 7.*
**p<0.05 vs control; a = p<0.05 vs FOJ;*
b = p<0.05 vs FSB; c = p<0.05 vs LVT

3.3 Comparison of Triiodothyronine (T_3) Concentration in the Different Experimental Groups

The mean ± SEM T_3 values were 34.86 ± 2.64, 26.71 ± 2.90, 30.00 ± 2.54, 29.29 ± 2.68, 33.29 ± 1.67, 35.57 ± 1.77, and 38.57 ± 1.82ng/dl for control, FOJ, FSB, LVT, carb, FSB+LVT and FOJ+carb group respectively. FOJ significantly (p<0.05) reduced T_3 when compared with the control group. FSB+LVT significantly (p<0.05) increased T_3 level compared to FOJ. FOJ+Carb significantly (p<0.05) increased T_3 level compared to FSB and LVT. (Fig. 3).

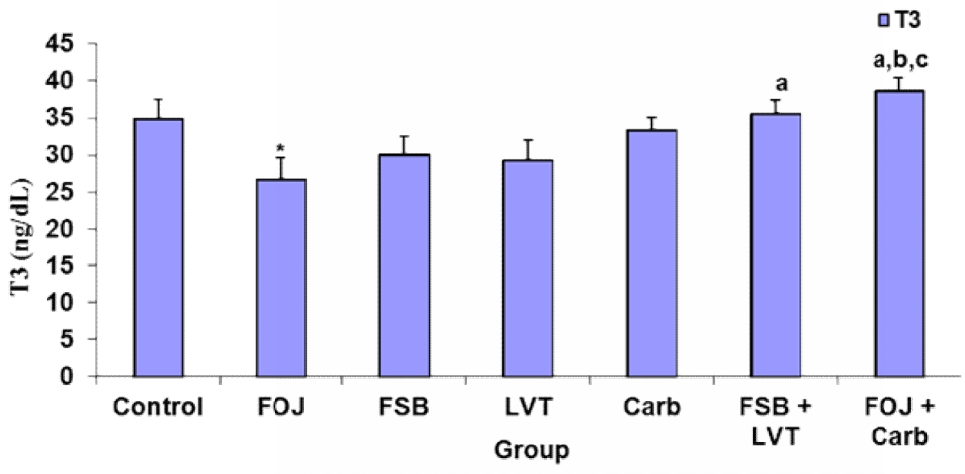

Fig. 3. Comparison of T_3 concentration in the different experimental groups
Values are mean ± SEM. n = 7.
**p<0.05 vs Control; a = p<0.05 vs FOJ;*
b = p<0.05 vs FSB; c = p<0.05 vs LVT.

3.4 Comparison of Thyroxine (T_4) Concentration in the Different Experimental Groups

The mean ± SEM T_4 values were 6.84 ± 0.49, 5.27 ± 0.41, 6.41 ± 0.76, 7.14 ± 0.57, 4.54 ± 0.12, 6.33 ± 0.59, and 5.19 ± 0.37 µg/dl for control, FOJ, FSB, LVT, carb, FSB+LVT and FOJ+carb group respectively**.**. FOJ, Carb and FOJ+carb significantly (p<0.05) reduced T_4 level when compared with the control. LVT significantly increased (p<0.05) T_4 level when compared with FOJ, Carb and FOJ+Carb. Carb significantly (p<0.05) reduced T_4 level when compared with LVT. (Fig. 4).

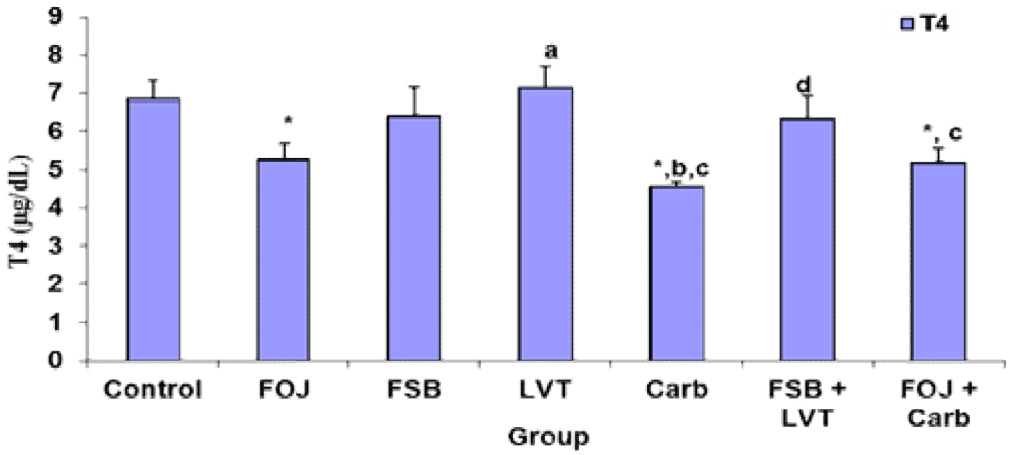

Fig. 4. Comparison of T_4 concentrations in the different experimental groups

Values are mean ± SEM, n = 7.

**p<0.05 vs Control; a = p<0.05 vs FOJ; b = p<0.05 vs FSB; c = p<0.05 vs LVT; d = p<0.05 vs Carb; e = p<0.05 vs FSB+LVT*

4. DISCUSSION

There exist an inextricable relationship between body weight and blood glucose levels based on metabolic indices, investigative results and clinical evidence. Also, the influence of thyroid hormones on glucose metabolism and body weight dynamics are well established. These assertions therefore underscored our compelling interest to study the effect of natural agents (*Citrus sinensis* and glycine max) and standard drugs (Carbimazole and levothyroxine) all of which are known to affect the biosynthesis, pharmacodynamics and metabolism of thyroid hormones.

The results obtained from this study showed that FOJ (*Citrus sinensis*) and FSB (glycine max) increased the blood glucose levels and the body weight of the experimental rats. The increased body weight in the FOJ and FSB treated groups could be related to the double dimensional impact of both nutritional and phytochemical constituents of both agents and their adverse consequential effect on thyroid function. Vitamin C, a proven antioxidant in addition to phenols, flavone glycosides, polymethoxylated flavones and hydroxycinamate are active phytoconstituents of FOJ. [10,11]. FOJ is rich in protein especially storage globulins 11 - S- glycinin and 75 β-conglycinin [12], large amount of polyunsaturated fatty acids (PUFAs) [13] and carbohydrates mostly oligosaccharides [14]. Other phytochemical

constituents of FOJ and FSB include: isoflavone, glycetin, glycosides, sterols [15], phospholipids and saponins found to have several biological activities [16], and ferritin, a multimeric iron storage protein [17].

It is evident from these results that nutritional and antioxidant substance could have promoted anabolic metabolic pathways with concomitant increase in body weight. Most of the major phytochemical constituents of both FOJ and FSB has been reported to exhibit anti-peroxidase activity with consequential reduction in thyroid hormone levels, which will in turn lead to decreased BMR and weight gain. It should be noted that the later mechanism may contribute less significantly to body weight increase since hypothyroidism usually develops over a long period of time.

The increase in blood glucose levels in the FOJ and FSB experimental group of animals may be partially related to their antithyroidal activities. Decreased glucose disposal has been proven in hypothyroid patients by different methods including clamp studies [18,19]. Moreover, hypothyroidism results in decreased liver glucose outputs, thereby compensating for insulin resistance present in peripheral tissues and accounting for the diminished insulin requirement for glycaemic control in hypothyroid diabetic individuals [20]. Regarding beta cell function, normal or reduced basal plasma insulin levels have been described in hypothyroidism. These findings to the extent of the limitations earlier noted are quite consistent with the idea of attenuated endogenous glucose production in the hypothyroid state [21].

The decrease in body weight and elevation of blood glucose levels in the levothyroxine treated group is in line with known metabolic mechanisms. It has been previously reported that thyrotoxic individuals show an increased glucose turn over with increased gastrointestinal glucose absorption, post absorptive hyperglycemia and elevated hepatic glucose output, along with elevation of post - prandial insulin, proinsulin, free fatty acid concentrations and peripheral glucose transport and utilization [20].

From our results, levothyroxine treatment appears to have created, understandably, elevation of thyroid hormone levels thus mimicking a hyperthyroid state. In this state, there is likely massive arrival of glucose to the peripheral cells that overwhelms the Kreb's cycle resulting in an increased metabolism of glucose through the non-oxidative pathway. Lactate produced in large quantity in the cells returns to the liver and participates in the Cori cycle where four ATP molecules are wasted for each glucose molecule created [22].

Although glucose uptake in peripheral tissues has been described as either normal or increased [22], reduced insulin stimulated peripheral glucose utilization has also been demonstrated in hyperthyroidism [20,23]. The notion that insulin stimulation of glucose uptake in thyrotoxic tissues may be impaired can be interpreted in the context of lower glucose extraction from serum in proportion to increased blood flow [24].

Our results have shown that Soybean levothyroxine (FSB+LVT) combination significantly decreased body weight when compared to single treatment with either FSB or LVT. This finding is rather curious, especially with previous reports that dietary genistein contained in soybean decreases body weight [25]. The relations among dietary isoflavones, weight loss and fat content have not been explored adequately; however, invivo studies support the role of isoflavones in the treatment of obesity. Isoflavone-rich diets have been reported to improve lipid metabolism and antidiabetic effects in obese rats [26]. In recent years, substantial data from epidemiologic surveys and nutrition intervention studies have

suggested the beneficial effect of soy isoflavone genistein on obesity in humans. It was shown that BMI was inversely related to 24-h urinary isoflavone excretion, the biomarker of soy intake, in a worldwide epidemiologic study [27]. However, the mechanisms by which isoflavones in soy exert their beneficial effect on body fat and obesity are unclear. Due to its structural similarity to endogenous estrogens, genistein can act as a weak estrogen and bind to the ER in various tissues, including adipose tissue. Naaz et al., 2003 [25] showed that genistein did not decrease adipose tissue in ovariectomized ERα knockout mice, demonstrating that genistein's antilipogenic effect requires ERα and regulation of estrogen-dependent processes. Experimental evidence suggests that genistein may also exert effects via non-ER–mediated mechanisms. Genistein modulates cell-signaling mechanisms and nuclear related cell proliferation and differentiation mechanisms by inhibiting protein tyrosine kinase, protein kinase and topoisomerase II [28]. Interestingly, in our study, soybean levothyroxine combination (FSB+LVT) treatment resulted in weight loss. The probable hypothesis to elucidate this occurrence could be that the combination treatment synergistically stimulated catabolic pathways with consequential reduction in weight.

The molecular mechanism whereby thyrotoxicosis causes weight loss is not settled, but thyroid hormone is thought to increase sodium-potassium adenosine triphosphatase (ATPase) activity in many tissues, suggesting that the diminished efficiency of ingested calories is due to futile cycle of adenosine triphosphate (ATP) synthesis and breakdown with energy lost as heat.

The potency of FOJ treatment to increase body weight was further observed in the result of the FOJ+Carb treatment. The weight reduction in the carbimazole treated group was significantly increased in the combination treatment. This points to the efficacy of FOJ to subjugate the effect of carbimazole in the combination treatment. If in a long term treatment the antithyroid effect of both FOJ and carbimazole treatment could come to play is a subject for further research. From this result, it is clearly evident that the antithyroid factor did not play a dominant role.

The combination treatment of both FSB+LVT and FOJ+Carb resulted in significant reduction in blood glucose levels when compared to LVT group. The pattern of activity is indicative of the hypoglycemic property of FSB and Carb, especially. There was no difference in the level of blood glucose reduction for Carb and FOJ+Carb treatment, while FSB+LVT treatment significantly brought down the blood glucose levels. Thus, FSB appears to have sufficiently blocked blood glucose elevation by LVT while Carb appears to have adequately blocked elevation of blood glucose by FOJ. Whether this occurrence in the case of FSB is related to its antithyroid activity is likely, but for FOJ the mechanism is not clear and may call for further studies. It should be noted however, that other metabolic variables not considered in this study, could contribute to the above observations.

5. CONCLUSION

The effect of both FOJ and FSB were more or less similar to that of the thyroid hormone analogue, Levothyroxine, on blood glucose levels and body weight changes. However, the combination treatment of FOJ with Carb and FSB with LVT showed activities correlating to that of carbimazole. Thus the acclaimed antithyroid property of FOJ and FSB could not be confirmed from their action on blood glucose level and body weight changes except with their combination treatment with standard thyro-active drugs levothyroxine and carbimazole.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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