

# Comparative effect of *Citrus sinensis* and carbimazole on serum $T_4$ , $T_3$ and TSH levels

Okon Uduak Akpan, Elemi John Ani<sup>1</sup>, Emmanuel Columba Inyang Etoh<sup>2</sup>, Adienbo Ologbago Macstephen<sup>3</sup>

Departments of Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Uyo, Uyo, Akwa Ibom, <sup>3</sup>University of Port Harcourt, Port Harcourt, Rivers State, <sup>1</sup>College of Medical Sciences, University of Calabar, Calabar, Cross River, <sup>2</sup>Department of Obstetrics and Gynaecology, University of Uyo Teaching Hospital, Uyo, Akwa Ibom, Nigeria

## ABSTRACT

**Background:** There are previous independent reports on the anti-thyroid property of *Citrus sinensis*. This isoflavones and phenolic acid-rich natural agent is widely consumed as dietary supplement, thus the need to investigate its comparative effect with a standard anti-thyroid drug on  $T_4$ ,  $T_3$  and thyroid stimulating hormone (TSH) levels. **Objective:** To compare the effect of *Citrus sinensis* and carbimazole (CARB) on blood levels of thyroid hormones ( $T_4$  and  $T_3$ ) and TSH. **Materials and Methods:** Male wistar albino rats weighing 100–150 g were employed in this research. The rats were randomly assigned to four groups of seven rats per group. Group I served as control and were administered distilled water while groups II–IV were administered with 1500 mg/kg of *Citrus sinensis* (fresh orange juice; FOJ), 0.1 µg/g of levothyroxine (LVT) and 0.01 mg/g of CARB, respectively, per oral once daily for 28 days. The animals were sacrificed under chloroform anaesthesia and blood sample collected by cardiac puncture and processed by standard method to obtain serum. TSH,  $T_4$  and  $T_3$  were assayed with the serum using ARIA II automated radioimmunoassay instrument. **Results:** The results showed that TSH level was significantly ( $P < 0.05$ ) decreased in LVT treated group compared with the FOJ group.  $T_4$  was significantly ( $P < 0.05$ ) decreased in the FOJ and CARB groups compared with the control and LVT groups. LVT significantly increased  $T_4$  when compared with FOJ group.  $T_3$  was significantly ( $P < 0.05$ ) decreased in the CARB group compared with the control. **Conclusion:** These findings suggest that FOJ alters thyroid hormones metabolism to reduce their serum levels with a compensatory elevations of TSH level in a direction similar to CARB.

**Key words:** *Citrus sinensis*, carbimazole, thyroid hormones, thyroid stimulating hormone

### Address for correspondence:

Dr. Okon Uduak Akpan, Department of Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Uyo, Uyo, Akwa Ibom, Nigeria.  
E-mail: chairmo2013@gmail.com

## INTRODUCTION

Several standard drugs and natural products are known to affect the synthesis, activity and pharmaco-dynamics of thyroid hormones. Thyroid hormones are key regulators of metabolism and are known to have pleiotropic effects in many different organs. The thyroid gland synthesises and releases thyroxine ( $T_4$ ) and tri-iodothyronine ( $T_3$ ), which represent the only iodine containing hormones in vertebrates.  $T_4$  is the main product of thyroid secretion and local deiodination in peripheral tissues produces  $T_3$ , the biologically active thyroid hormone.  $T_3$  is several times more active than  $T_4$ , which is largely a prohormone,<sup>1</sup>

however, the duration of action is more for  $T_4$  than  $T_3$ . More than 99% of the circulating  $T_4$  and  $T_3$  are protein bound, mainly to  $T_4$ -binding globulin and to a lesser extent to transthyretin and albumin.<sup>1</sup> Thyroid hormones can rapidly be released from these proteins, this process facilitating their entry into cells but  $T_3$  has less affinity for plasma proteins and combine loosely with them so that it is released quickly, while  $T_4$  has more affinity and strongly binds with plasma proteins so that it is released slowly.<sup>2</sup>

The production of thyroid hormones is controlled by thyroid stimulating hormone (TSH) synthesised by the anterior pituitary gland in response to TSH-releasing hormone (TRH), which is secreted by the hypothalamus. Unbound or free  $T_4$  and  $T_3$  ( $fT_3$  and  $fT_4$ , respectively) exert a negative feedback on the synthesis and release of TSH and TRH in order to maintain circulating thyroid hormone levels within the normal range. The actions of thyroid hormones are initiated by their interaction with thyroid hormone receptors (TRs), which belong to a large superfamily of steroid hormone receptors, other members of which include the sex steroid receptors, vitamin

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10.4103/0300-1652.132049

D receptors and retinoic acid receptors.<sup>3</sup> The functional TRs thus serve to promote or inhibit the transcription of thyroid hormone responsive genes.<sup>4</sup>

*Citrus sinensis* is a rich source of isoflavonoids and phenolic acids. Much attention has been paid to the beneficial antioxidant effect of this natural phenolic acids.<sup>5,6</sup> This has led to their proposed use as anti-carcinogens<sup>7</sup> and cardioprotective agents,<sup>8</sup> these and other reasons has prompted a dramatic increase in their consumption as dietary supplements.

The phytochemicals like isoflavones found in *Citrus sinensis* has anti-peroxidative activity.<sup>9</sup> Studies have shown that *Citrus sinensis* extract significantly decreases the level of serum thyroxine ( $T_4$ ) in rats.<sup>10</sup> The anti-thyroidal role of *Citrus sinensis* might be mediated through the inhibition of thyroid peroxidase (TPO)<sup>11</sup> the key enzyme in thyroid hormone biosynthesis, as it contains the phenolic compound naringin, which inhibits the activity of TPO.<sup>11-13</sup> Due to its anti-peroxidative activity, *Citrus sinensis* extract has anti-thyroidal properties, which suggest its potential to ameliorate hyperthyroidism.<sup>14</sup>

Levothyroxine (LVT) is a synthetic thyroid hormone used for the treatment of hypothyroidism.<sup>15</sup> LVT, when administered during hypothyroid state, up-regulates TSH receptors, which result in increased  $T_4$ ,  $T_3$  secretion.<sup>15</sup> LVT increases serum protein level, it exerts its effect through the control of DNA transcription and protein synthesis.<sup>16</sup>

Carbimazole (CARB) is an anti-thyroidal drug, which interferes with the synthesis of thyroid hormones and as a result reduces the level of thyroid hormones.<sup>17</sup> Animal studies carried out on pregnant mice, rats and rabbit showed that treatment with CARB, resulted in hypothyroidism in their offspring.<sup>18,19</sup>

CARB is a commonly used standard drug for the treatment of hyperthyroidism and fresh orange juice (FOJ) is gradually gaining prominence as a natural anti-thyroid agent. It is important that their efficacy be investigated in the same experimental setting and environment.

## MATERIALS AND METHODS

### Materials

Fresh orange fruit was obtained from Akpan Andem market in Uyo, Akwa Ibom State Nigeria. The orange fruit was peeled and cut into two parts to make it easier to squeeze. After squeezing, the juice was filtered using filter paper, which was placed inside a funnel and the filtrate was preserved in the refrigerator at a temperature of about  $-4^{\circ}\text{C}$ .

The stock concentration of the sweet orange extract was determined by taking 2 ml of the sweet orange extract and then concentrated to dryness using a hot plate and an evaporating dish. The differences in the weight of the

evaporating dish when empty and after evaporation was determined. This was repeated three times and the mean value was recorded as 90 mg/ml.

The median lethal dose was estimated by the method of Lorke.<sup>20</sup> There was no mortality at the highest dose of 5000 mg/kg, therefore 30% of 5000 mg/kg was used, that is, 1500 mg/kg body weight.

### Animal preparation, experimental groupings and treatment

Twenty-eight male albino wistar rats weighing 100-150 g were obtained from the Animal House Unit, of the Department of Physiology, University of Calabar and were housed in a cross-ventilated room in the animal house unit of the department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, Uyo. The animals were kept in conventionally and environmentally adapted wooden cages with wire netting under uniform husbandry condition of daylight, night darkness and normal room temperature, with wood shavings as their beddings and were allowed to acclimatise for 2 weeks before the commencement of the research. The animals were kept in dry and hygienic condition with access to feed and water *ad libitum*. Before and during the research, the animals were fed with palletised Guinea feed.

The rats were randomly assigned into four groups of seven rats per group. Rats in group 1 served as control and were administered distilled water while groups II, III and IV were administered with 1500 mg/kg of *Citrus sinensis* (FOJ), 0.1  $\mu\text{g/g}$  LVT and 0.01 mg/g of CARB, respectively, for 28 days according to their body weight. Administration of the aqueous extract was done orally by means of calibrated syringe with attached rubber cannula. The experimental procedures involving the animals and their care were in line with the approved guidelines by the local research and ethical committee.

### Sample collection

The animals were sacrificed using chloroform anaesthesia and blood sample collected by cardiac puncture and processed by standard method to obtain serum for hormonal assay.

### Hormonal assay

Thyroid stimulating hormone assay

This was done using thyrotropin kit (ELISA kit from Fortress Diagnostic Ltd, UK).

Thyroxine ( $T_4$ ) assay

This 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. The substances, shown in Table 1, were tested for cross reactivity of the assay.

### Tri-iodothyronine (T<sub>3</sub>) assay

This was done using DS-EIA-THYROID-T<sub>3</sub> 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. The substances, shown in Table 2, were tested for cross reactivity of the assay.

### Statistical analysis

Data from the study were subjected to descriptive statistics and the results presented as Means ± Standard Error of Mean. Differences between means were separated by one-way analysis of variance (ANOVA), followed by *post-hoc* multiple comparisons, with the least significant threshold employed at  $P \leq 0.05$ . Data analysis was done using the statistical software package SPSS for Windows version 17.0 (SPSS Inc., Chicago, IL, USA).

## RESULTS

### TSH

The Mean ± SEM values for TSH were  $1.50 \pm 0.05$ ,  $1.68 \pm 0.06$ ,  $1.38 \pm 0.04$  and  $1.61 \pm 0.05$  for Control, FOJ, LVT and CARB groups, respectively. FOJ and CARB significantly increased ( $P < 0.05$ ) TSH levels when compared with the control. While LVT significantly reduced ( $P < 0.05$ ) TSH when compared with FOJ [Figure 1].

### Thyroid hormone (T<sub>4</sub>)

The Mean ± SEM values for T<sub>4</sub> were  $6.84 \pm 0.49$ ,  $5.27 \pm 0.41$ ,  $7.14 \pm 0.57$  and  $4.54 \pm 0.12$  for Control, FOJ, LVT and CARB groups, respectively. FOJ and CARB significantly ( $P < 0.05$ ) reduced T<sub>4</sub> level when compared with the control, LVT significantly increased ( $P < 0.05$ ) T<sub>4</sub> level when compared with FOJ. CARB significantly ( $P < 0.05$ ) reduced T<sub>4</sub> level when compared with LVT [Figure 2].

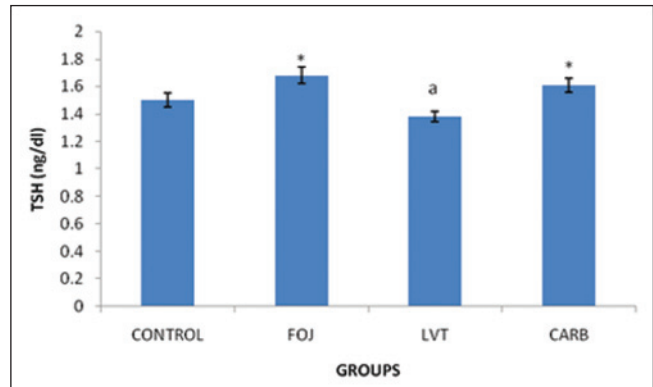
### Tri-iodothyronine (T<sub>3</sub>)

The Mean ± SEM values for T<sub>3</sub> were  $34.86 \pm 2.64$ ,  $26.71 \pm 2.90$ ,  $29.29 \pm 2.68$  and  $28.21 \pm 1.67$  for Control, FOJ, LVT and CARB groups, respectively. FOJ and CARB significantly

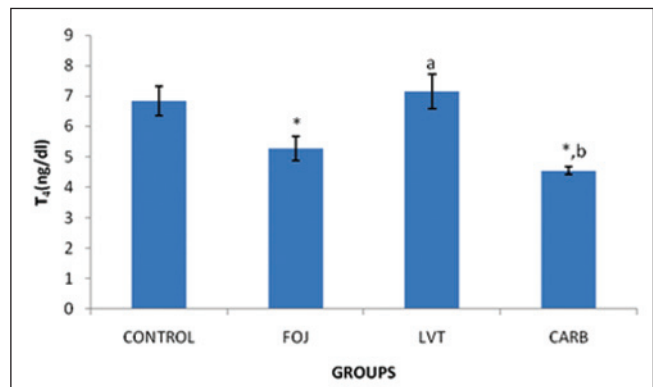
( $P < 0.05$ ) reduced T<sub>3</sub> when compared with the control group [Figure 3].

## DISCUSSION

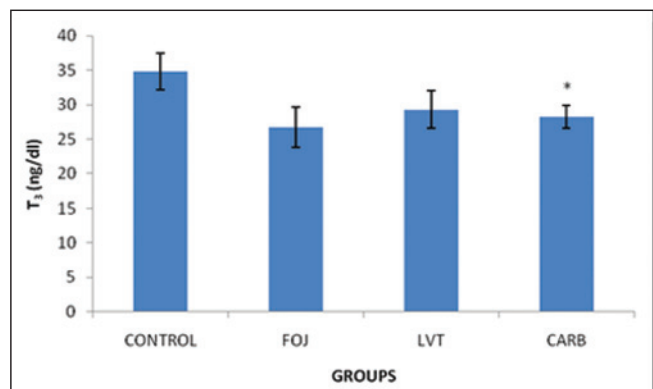
Thyroid hormone synthesis and secretion is regulated by the hypothalamic-pituitary-thyroid axis. Thyrotropin-releasing hormone (TRH) released from the hypothalamus



**Figure 1:** Comparison of TSH levels in the different experimental groups =  $P < 0.05$  vs control; a =  $P < 0.05$  vs FOJ



**Figure 2:** Comparison of T<sub>4</sub> levels in the different experimental groups =  $P < 0.05$  vs control; a =  $P < 0.05$  vs FOJ; b =  $P < 0.05$  vs LVT



**Figure 3:** Comparison of T<sub>3</sub> levels in the different experimental groups Values are Mean ± SEM,  $n = 7$ . \* =  $P < 0.05$  vs control

**Table 1: Thyroxine (T<sub>4</sub>) Specificity**

Substance	Cross-reactivity (%)
L-Thyroxine	100
Tri-iodothyronine	1.46
Di-iodothyronine	0.001
Tetraiodoacetate	2.53

**Table 2: Tri-iodothyronine (T<sub>3</sub>) Specificity**

Substance	Cross-reactivity (%)
L-Thyroxine	0.0001
Tri-iodothyronine	100
Di-iodothyronine	0.0001
Tetraiodoacetate	0.0001

stimulates secretion of thyroid-stimulating hormone (TSH) from the anterior pituitary. TSH, in turn, is the physiologic stimulus for the synthesis and secretion of thyroid hormones, L-thyroxine ( $T_4$ ) and L-tri-iodothyronine ( $T_3$ ), by the thyroid gland. Circulating serum  $T_4$  and  $T_3$  levels exert a feedback effect on both TRH and TSH secretion. When serum  $T_4$  and  $T_3$  levels increase, TRH and TSH secretion decrease. When thyroid hormone levels decrease, TRH and TSH secretion increase.

In this study, it was observed that FOJ and CARB significantly increased TSH level while it significantly decreased  $T_4$  and  $T_3$  levels. Due to its anti-peroxidative activity, *Citrus sinensis* extract has anti-thyroidal properties, which suggest its potential to ameliorate hyperthyroidism.<sup>14</sup>

Nicolosi *et al.*,<sup>10</sup> reported that *Citrus sinensis* extract administered in rats significantly decreased the level of serum thyroxine ( $T_4$ ). Peels from *Citrus sinensis* has also been found to inhibit the thyroid, where reduction in both the thyroid hormones was observed, in response to both the juice and the peel extract.<sup>10</sup> It was suggested that *Citrus sinensis* might be inhibiting thyroid hormones not only at glandular level, but also at the level of peripheral conversion of  $T_4$  to  $T_3$ .<sup>10</sup>

In the study, the goitrogenic effect of FOJ was illustrated by the significant decrease in  $T_3$  level and compensated negative feedback elevation of serum TSH level in the experimental rats.

LVT significantly reduced TSH when compared with the FOJ group while increasing  $T_4$  level but it did not significantly increase  $T_3$  level. LVT is identical to that produced naturally in the human thyroid gland. It acts like the endogenous thyroid hormone thyroxine,  $T_4$ , (a tetra-iodinated tyrosine derivative). In the liver and kidney,  $T_4$  is converted to  $T_3$ , an active metabolite. Thyroxine ( $T_4$ ) is the form of thyroid hormone that is solely secreted by the thyroid gland while about 80% of  $T_3$ , which is the biologically active thyroid hormone in circulation, is formed by the de-iodination of  $T_4$ .  $T_3$  produced by the thyroid gland is also in small quantity, it has a rapid onset of action and metabolises quickly, thereby accelerating its elimination rate when compared with  $T_4$ . The above peculiarities may account for the selective elevation of  $T_4$ , with no significant elevation of  $T_3$ .

CARB is a thionamide. It is converted to methimazole, which inhibit the enzyme TPO, thus blocking the conversion of iodide to iodine and inhibiting iodide organification. Methimazole also inhibits the coupling of iodotyrosines, monoiodotyrosine/diiodotyrosine (MIT/DIT) to DIT. These actions will result in reduced production of thyroid hormones. Animal studies carried out on pregnant mice, rats and rabbit showed that treatment with CARB resulted in hypothyroidism in their offspring.<sup>18,19</sup> In this study,

CARB decreased  $T_4$  and  $T_3$  level when compared with the control group while it significantly increased TSH levels. In a previous study by Abraham *et al.*,<sup>17</sup> it was observed that CARB has an anti-thyroidal property, which inhibit TPO enzyme activities. It might also reduce  $T_4$  level by increasing the level of iodine in circulation, which will inhibit the thyroid gland activity (Wolf Chaicoffs effect) by decreasing iodide organification (inhibition of TPO) and release of  $T_3$  and  $T_4$ . A study by Wise *et al.*,<sup>21</sup> on the mode of action of CARB in Graves' disease, observed that CARB does not act solely by inhibiting intrathyroid hormone-genesis, but more definitively by affecting thyroid hyperstimulation at a pre-biosynthetic level. It is also possible that CARB and FOJ might also inhibit thyroid hormone by inhibiting the hypothalamic-pituitary-thyroid axis.

Thus, there appears to be a direct correlation between the action of CARB and FOJ (*Citrus sinensis*) on the inhibition of thyroid hormone metabolism. However, despite the high dose of FOJ administered, CARB appears to have a more potent effect in reducing both  $T_4$  and  $T_3$  levels at a lower dose. We can therefore suggest that consumption of high quantity of FOJ may result in the reduction of serum  $T_4$ ,  $T_3$  levels and if taken during treatment of hyperthyroidism with other anti-thyroid drugs may potentiate their effect. It is possible that with further research, FOJ may be developed into a veritable adjuvant treatment for hyperthyroidism.

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**How to cite this article:** Uduak OU, Ani EJ, Etoh EI, Macstephen AO. Comparative effect of *Citrus sinensis* and carbimazole on serum T<sub>4</sub>, T<sub>3</sub> and TSH levels. *Niger Med J* 2014;55:230-4.

**Source of Support:** Nil, **Conflict of Interest:** None declared.

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