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Citrus Sinensis Separately Increases Serum Plasma Proteins while its Combination Treatment with Carbimazole Reduces-the Thyroid Hormones Connection

Okon Uduak Akpan^{1,*}, Ofem Ofem Effiong³, Olubobokun Titilope Helen¹, Akpan Henry Daniel²

¹Dept of Physiology, University of Uyo, Uyo, Akwa Ibom State, Nigeria

²Dept of Biochemistry, University of Uyo, Uyo, Akwa Ibom State, Nigeria

³Dept of Physiology, University of Calabar, Cross Rivers State, Nigeria

Abstract Plasma proteins play a vital role in the bioavailability and by implication, the activity of thyroid hormones. This study therefore became expedient in order to investigate the effect of Citrus sinensis on serum plasma proteins in comparison with standard thyro-active drugs. Albino wistar rats were randomly divided into 5 groups of 7 rats each. Group I served as control, group II received 1500mg/kg of fresh orange juice (FOJ), group III received 0.1ug/g of Levothyroxine (LVT), group IV received 0.01mg/kg of Carbimazole (CARB) and group V received FOJ (1500mg/kg)+ CARB (0.01mg/g) once daily per oral for 28 days. The animals were sacrificed after a night fast and blood samples obtained by cardiac puncture and processed by standard procedure to obtain the serum which was used for plasma protein analysis. The results showed that FOJ and LVT significantly ($p < 0.05$) increased total protein, albumin and globulin levels while FOJ+CARB significantly ($p < 0.05$) decreased them. It appears that the separate effect of Citrus sinensis and LVT on plasma protein is unrelated to their effect on thyroid hormone levels. The combination treatment appears to synergistically potentiate their antithyroid effect irrespective of the singular effect of Citrus sinensis on plasma proteins. Thus, there may be need for caution on the excessive consumption of Citrus sinensis during treatment with Carbimazole

Keywords Citrus Sinensis, Carbimazole, Levothyroxine, Thyro-Active Drugs, Plasma Proteins

1. Background

There is a widespread consumption of Citrus sinensis (Fresh Orange Juice) either as fruit juice or beverages and its effect on thyroid hormone levels have been reported. Serum proteins are very important component of blood plasma. The total serum protein is about 7.0-9.5 g/dl which makes up about 7% of total blood volume[1]. Serum proteins play numerous roles in the continuity of life; they act as transport system for hormones, vitamins, minerals, lipids, drugs and also help to balance the osmotic pressure of blood and tissue[1]. Serum proteins also help in regulating the cellular activities of the cell, functioning of the immune system and as a source of energy for tissues when the level of carbohydrates has been depleted in the body[2]. Serum proteins component include albumin, globulin, fibrinogen and lipoprotein[3].

The transferrin family of plasma proteins is involved in the regulation of iron, which is essential for the proper

oxygenation of blood (4). Although albumin, immunoglobulins and clotting factors are always present in healthy blood plasma and make up the bulk of plasma protein content, the presence of other proteins can be important indicators of illness[5].

Albumin is synthesized mainly in the liver. It contributes to the generation of blood oncotic pressure and is also involved in tissue growth and healing. Serum albumin is the most abundant protein in human blood plasma[6]. It constitutes about half of the blood serum protein. Albumin transports hormones, fatty acids, and other compounds, buffers pH among other functions. Globulins are divided into alpha-1, alpha-2, beta, and gamma globulins[7]. Some globulins are synthesized in the liver, while others are produced by the immune system[8]. Certain globulins bind with hemoglobin. Other globulins transport metals, such as iron in the blood and help to fight infection[8]. A test for total serum protein reports separate values for total protein, albumin, and globulin[7].

Levothyroxine is a synthetic thyroid hormone used for the treatment of hypothyroidism[9]. Levothyroxine increases serum protein level, it exerts its effect through the control of DNA transcription and protein synthesis[10]. Levothyroxine diffuses into the cell nucleus and binds to thyroid hormone

* Corresponding author:

chairmo2013@gmail.com (Okon Uduak Akpan)

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receptor protein on the DNA to form hormone nuclear receptor complex which activates gene transcription and synthesis of protein[9]. Thyroxine binding globulin (TBG), thyroxine binding pre-albumin (TBPA) and thyroxine binding albumin (TBA) are the major transport proteins for thyroid Hormones (T_4 and T_3)[11]. Their serum concentrations are known to inversely affect the synthesis and activity of thyroid hormone.

Carbimazole is an anti thyroid drug used in the treatment of hyperthyroidism. It decreases the secretion of thyroid hormone thereby slowing down the metabolic activity of the body[12]. Carbimazole decreases albumin and globulin synthesis[14].

Citrus sinensis plays a role in the regulation of thyroid function which suggests its potential to ameliorate hyperthyroidism[15]. Citrus sinensis extract contains phytochemicals with antiperoxidative activity[15]. Citrus sinensis extract increases total protein in the body. Its active component that is responsible for the elevation of serum proteins is d- limonene[16].

Since plasma proteins plays important role in the synthesis, transportation and activity of thyroid hormones, an investigation of its plasma levels during separate and combination treatment with known thyromimetic (Levothyroxine) and an anti thyroid agent (Carbimazole and Citrus sinensis), may offer some clue to elucidate if their mechanism of action on thyroid hormone are related to their effect on plasma proteins.

2. Materials and Methods

2.1. Collection and Identification of Plant Materials

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 -4°C .

The stock concentration of the sweet orange extract was determined by taking 2ml of the sweet orange extract and then concentrated to dryness using a hot plate and an evaporating dish. The difference 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 90mg/ml.

The median lethal dose was estimated by the method of Lorke's (1983) (17). There was no mortality at the highest dose of 5000mg/kg, therefore 30% of 5000mg/kg was used i.e. 1500mg/kg.

2.2. Animals Preparation, Treatment and Sample Collection

35 male albino wistar rats weighing 100-150g 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 department

of the 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 conditioners of daylight, night darkness and normal room temperature, with wood shavings as their beddings and were allowed to acclimatize 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 palletized Guinea feed. The experimental procedures involving the animals and their care were in line with the approved guidelines by the local research and ethical committee.

The rats were randomly assigned into five groups of seven[7] rats per group. Rats in group I served as control and were administered distilled water while groups II, III, IV and V were administered with 1500mg/kg of Citrus sinensis or Fresh Orange Juice (FOJ), 0.1 $\mu\text{g/g}$ Levothyroxine (100 μg) and 0.01mg/g of Carbimazole 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.

After administration for 28 days, the animals were sacrificed using chloroform anesthesia and blood sample was obtained by cardiac puncture into sample bottles for analysis. The blood in the sample bottle without anticoagulant was spin for serum. 12 sample bottles were spin at a time for 5mins at 1200rpm.

2.3. Plasma Proteins Assay

Plasma proteins were assayed using reagents from Randox, UK. Total serum protein was assayed by adding 20 μl of serum sample to 1ml of biuret reagent, incubated at room temp for 10 mins, read at 540nm and the concentration was calculated. For albumin, 10 μl of sample was added to 3ml of bromocresol green reagent, incubated at room temp for 5 mins and read at 630nm. Value for Globulin was obtained from the difference between total protein and albumin.

2.4. Statistical Analysis

Data gotten from the study were subjected to descriptive statistics and the results presented as Means \pm 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).

3. Results

Figure 1 shows the results were presented as Mean \pm SEM. The Mean \pm SEM values for Total protein were 6.37 \pm 0.21, 7.77 \pm 0.68, 7.47 \pm 0.51, 7.21 \pm 0.15 and 5.79 \pm 0.30 for Control, FOJ, LVT, CARB and FOJ+CARB groups respectively. There was a significant increase in the total

protein level in the FOJ and LVT groups when compared to the control at $p < 0.05$ level of significance. A significant decrease was also observed in the FOJ+CARB group when compared to the FOJ group. No significant change was observed in the CARB group.

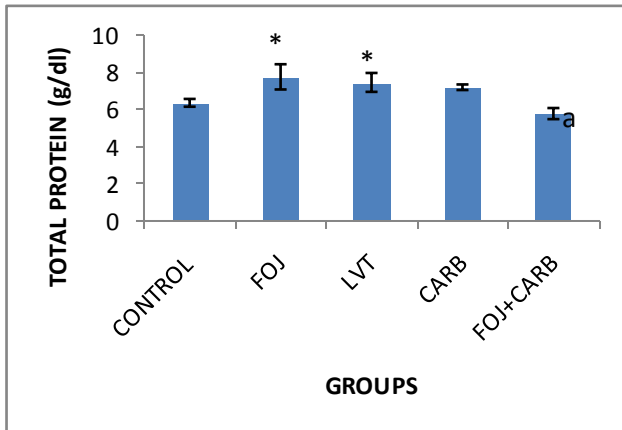


Figure 1. Comparison of total protein in the different experimental groups. *= $p < 0.05$ vs control; a= $p < 0.05$ vs FOJ

Figure 2 shows the Mean \pm SEM values for serum albumin levels were 3.98 ± 0.13 , 4.18 ± 0.29 , 4.09 ± 0.19 , 4.01 ± 0.12 and 3.31 ± 0.23 for Control, FOJ, LVT, CARB and FOJ+CARB groups respectively. FOJ significantly ($p < 0.05$) increased serum albumin level when compared with the control. A significant ($p < 0.05$) decrease was observed in the FOJ+CARB group when compared with the FOJ, LVT and CARB group.

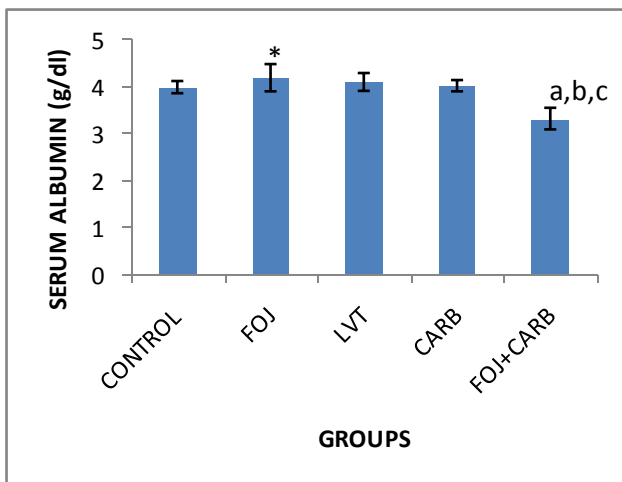


Figure 2. Comparison of albumin in the different experimental groups. *= $p < 0.05$ vs control; a= $p < 0.05$ vs FOJ; b= $p < 0.05$ vs LVT; c= $p < 0.05$ vs CARB

Figure 3 shows the Mean \pm SEM values for Globulin levels were 2.39 ± 0.18 , 3.54 ± 0.51 , 3.38 ± 0.46 , 3.21 ± 0.25 and 2.33 ± 0.17 for Control, FOJ, LVT, CARB and FOJ+CARB groups respectively.

There was a significant ($p < 0.05$) increase in serum globulin level in the FOJ and LVT group when compared to the control group. FOJ+CARB significantly ($p < 0.05$) decreased serum globulin level when compared with the FOJ

group.

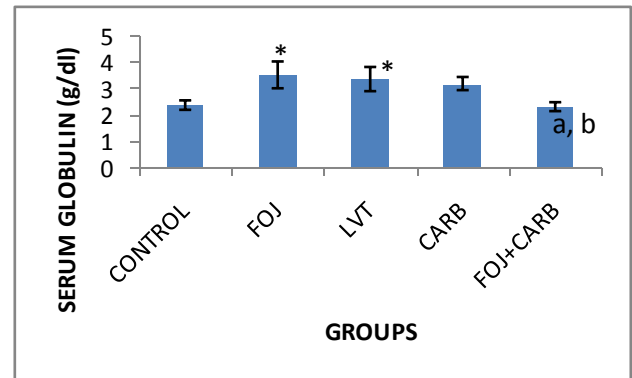


Figure 3. Comparison of Globulin in the different experimental groups. *= $p < 0.05$ vs control; a= $p < 0.05$ vs FOJ; b= $p < 0.05$ vs LVT

Fresh orange juice increased the total protein, albumin and globulin level significantly ($p < 0.05$) when compared to the control.

Levothyroxine increased the total protein and globulin level significantly ($p < 0.05$) when compared to the control.

Carbimazole Citrus sinensis combination significantly ($p < 0.05$) reduced total protein, albumin and globulin level when compared to the FOJ.

4. Discussion

The significant increase in the TP level in the FOJ group could be attributed to an active component, d-limonene, which is responsible for the elevation of serum total protein (TP) as reported by Kerneva *et al.*, 1987[16]. Citrus sinensis contains isoflavones which has antiperoxidative activity[18], this could in turn result in direct stimulation of protein receptors synthetic pathways.

Levothyroxine acts like the endogenous thyroid hormone, thyroxine. In the liver and kidney, T_4 is converted to T_3 , the active metabolite. In order to increase solubility, thyroid hormones attach to thyroid hormone binding proteins, thyroxin-binding globulin, and thyroxin-binding prealbumin (transthyretin). Transport and binding to thyroid hormone receptors in the cytoplasm and nucleus then takes place which leads to increase production of proteins including serum total proteins[9]. Levothyroxine also binds to plasma proteins (thyroxine binding globulin, thyroxine binding prealbumin and albumin) by 99%. This action of Levothyroxine could be responsible for the increase level in serum total protein observed in this study.

Carbimazole is a carbithoxy derivative of methimazole. Its antithyroid action is due to its conversion to methimazole after absorption. It is used in the treatment of hyperthyroidism e.g. in thyrotoxicosis. It decreases the uptake and concentration of inorganic iodine by the thyroid; it also reduces the formation of di-iodotyrosine and thyroxine[11]. Once converted to its active form, it prevents the thyroid peroxidase enzyme from coupling and iodinating the tyrosine residues on thyroglobulin, hence reducing the

production of the thyroid hormones T_4 and T_3 , thereby reducing the production of proteins including serum proteins (19). In this study, carbimazole on its own did not have a significant effect on serum proteins level, this supports the study by Siersbaek-Nielsen et al, 1978[20], who worked on the extrathyroidal effects of propylthiouracil and carbimazole on serum T_4 , T_3 , reverse T_3 and TRH-induced TSH-release in man, they observed no significant changes in serum T_4 , basal serum TSH or response to TRH.

The Carbimazole Citrus sinensis combination treatment however reduces serum total proteins in this study when compared with the group that was administered with Citrus sinensis only.

Studies have shown that Citrus sinensis extract administered in rats significantly decreases the level of thyroid hormone[6]. Increased blood level of thyroid hormone is known to increase serum plasma protein levels[9]. It therefore follows that the combined synergistic antithyroid effect of both Citrus sinensis and Carbimazole could be responsible for the reduction of the serum total proteins in this group. It is also evident that the antithyroid effect of Carbimazole might have been less potent to cause a significant effect; if this could be due to the dosage administered in this study could also be considered. Citrus sinensis has been reported to increase serum total protein in previous studies[16] in line with our current findings. Even though Citrus sinensis was found to cause the elevation in total plasma protein, it is likely that this action may be mediated by some other unknown mechanism or that may selectively override its anti-thyroid effect. Since Carbimazole on its own could not cause a significant reduction, this therefore strongly suggests a possible synergistic antithyroid interaction between Carbimazole and Citrus sinensis.

The significant increase in the albumin level in the FOJ group could be linked to d-limonene.

The paradoxical effect of Citrus sinensis on plasma proteins when administered alone and in combination with Carbimazole is rather curious! It is possible that some active Constituents of FOJ may activate some enzymes in the synthetic pathway of serum albumin while some other constituents on the other hand may potentiate the action of Carbimazole, there may be need to further investigate this hypothesis.

The significant increase in serum globulin in the Citrus sinensis group may also be attributed to the presence of d-limonene, an active component in Citrus sinensis. This supports the study by Lehman et al, 1989[21] where they evaluated the association between d-limonene and alpha 2μ -globulin, their result showed that there was excessive accumulation of alpha 2μ -globulin in kidneys of male rats exposed to d-limonene.

There was a significant increase in the level of serum globulin in the Levothyroxine group. Levothyroxine is a synthetic form of thyroxine (T_4) which increase serum globulin level as stated by Lauberg, 1984[10], where he

reported that levothyroxine mimic T_4 by controlling DNA transcription to produce increased serum globulin level.

A significant decrease in serum globulin levels was also observed in the group administered with the Carbimazole Citrus sinensis combination. This was in line with the result for total protein and albumin. Similar findings were reported by Peavy et al, 1981[13].

5. Conclusions

It therefore appears that the separate effect of Citrus sinensis and Levothyroxine on plasma protein is unrelated to their effect on thyroid hormone levels. Levothyroxine and Citrus sinensis were previously reported to increase and decrease thyroid hormone levels respectively. The effect of Carbimazole Citrus sinensis combination treatment is in consonant with their antithyroid activity. It is likely that the combination treatment could synergistically potentiate this effect irrespective of the singular effect of Citrus sinensis on plasma proteins. Thus, there may be need for caution on the excessive consumption of Citrus sinensis during treatment with Carbimazole.

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