ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Comparative Effect of Carbimazole, Glycine Max and Citrus Sinensis on Serum Electrolytes and Urea.

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ABSTRACT

This study investigated the comparative effect of carbimazole, glycine max (fresh soybean) and citrus sinensis (fresh orange juice) on serum electrolytes and urea. Twenty eight (28) male albino wistar rats weighing between 100 – 150g were used for the experiment. The rats were randomly divided into 4 groups of 7 rats each. Group 1 served as the control and were given distilled water while groups 2 – 4 were oral gavaged once daily for 28 days with the following; carbimazole 0.01mg/kg, fresh soybean 207.84mg/kg and fresh orange juice 1500mg/kg respectively. The LD_{50} of fresh orange juice and fresh soybean were determined by the method of Lorke's. Blood sample was collected by cardiac puncture and processed to obtain serum for electrolyte and urea analysis. The results showed that Na^+ was significantly ($p<0.05$) reduced in the carbimazole treated group when compared to the control, fresh orange juice and fresh soybean groups. The serum K^+ levels was significantly ($p<0.05$) reduced by carbimazole when compared to the control and fresh orange juice groups. The carbimazole plus fresh soybean combination also significantly ($p<0.05$) reduced serum bicarbonate levels when compared to the fresh orange juice group. It is suggestive from these results that treatment of hyperthyroidism with carbimazole may be associated with derangement in serum electrolyte levels. On the other hand, fresh orange juice and fresh soybean also reported to exert antithyroid activity lack this side effect.

Keywords: carbimazole, glycine max, citrus sinensis, urea, electrolytes

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ISSN: 0975-8585

INTRODUCTION

Electrolytes are substances that are ionized in solution and acquire the capacity to conduct electricity. Electrolyte balance is essential for the normal function of cells and organs in the body. A good number of substances or bio-active molecules are known to affect their values in blood and body fluids.

Common electrolytes that are measured in clinical practice include sodium, potassium, chloride and bicarbonate, creatinine and urea. This measurement is utilized for diagnostic purposes, treatment response and prognosis.

Sodium is the major cation in the ECF and principally regulates its volume thus, Na^+ plays a cardinal role in blood pressure regulation. Sodium transmission in and out of the cells plays a critical role in body functions. Many processes in the body especially in the brain, nervous system and muscles operate through electrical signals generated by the electrolytes. Therefore, extremes in the blood levels could cause the cells to malfunction and may be fatal.

Hypernatremia may result from kidney disease, water deprivation, diarrhea and vomiting. On the other hand, hyponatremia may result from liver and kidney disease, in patients with congestive heart failure, in burns, etc.

Potassium is the major cation of the Intracellular fluid (ICF). Altered levels of potassium in blood could result in arrhythmia which may be fatal at extreme levels.
The major ECF anion is chloride. Hyperchloremia may be seen in diarrhea, kidney diseases and hyperactivity of the parathyroid glands. Bicarbonate ion acts as a buffer in the maintenance of blood pH. Disruptions in the normal bicarbonate level may be due to diseases that interfere with the respiratory functions, kidney disease, altered metabolic conditions. etc.

Blood urea levels rises when a high protein diet is consumed, when there is excessive breakdown or in the presence of gastrointestinal bleeding [1]. The kidneys in their role as regulators of blood urea nitrogen (BUN) levels filter urea in the glomeruli and then reabsorb it in the tubules. This enables maintenance of a normal BUN, which is in the range of 8-25mg/dl (2.9-8.9mmol/L) in humans [1].

The renal tubules are permeable to urea, which means that the longer the tubular fluid remains in the kidneys, the greater is the reabsorption of urea into the blood. Only small amounts of urea are reabsorbed into the blood when the GFR is high but relatively large amount of urea are returned to the blood when GFR is reduced.
Increased metabolic activity due to a rise in thyroid hormones level is known to be associated with elevation in the various electrolytes levels in the body [2].

Reports on the effect of thyroid hormone on urea have been inconclusive or contradictory so far. Croft et al, 1996 [3] experimenting on hyperthyroid rats reported that

there were no changes in urea biosynthesis. On the other hand, moderate hyperthyroidism had been reported to decrease liver amino acid-nitrogen conversion [4].

Glycine max among its other important constituents is the most abundant source of isoflavones in nature [5]. Isofalvones are said to inhibit thyroid peroxidase thereby reducing thyroid hormone levels which in turn decreases body metabolism and electrolyte levels [6]. A decrease in serum urea level due to the antithyroid function of glycine max (soybean) has also been reported [7].

Citrus sinensis contains a variety of phytochemicals. Hesperetin and Naringenin are flavonoids found in citrus fruits [8]. Naringenin is found to have a bio-active effect on human as anti-oxidant (free radical scavenger), anti inflammatory and immune system modulator. It has been shown to reduce oxidant injury to DNA in in-vitro studies [9].

Fresh orange juice has an anti-peroxidase activity which suggests its potential to ameliorate hyperthyroidism [10]. Since fresh orange juice decrease thyroxine (T_4) level [11], decrease in thyroid hormone could lead to decrease in serum electrolyte levels. There appears to be a scarcity of reports on the relationship between thyroid hormone level and urea, while the report on a slight decrease in urea level by fresh orange juice is yet to be conclusively proven.

Carbimazole is used in the treatment of hyperthyroidism. It is a pro drug and it is converted to the active form, methimazole in the body. The latter prevent the thyroid peroxidase enzyme from coupling and iodinating the tyrosine residues on thyroglobulin, hence inhibiting the production of T_4 and T_3. The antithyroid action of carbimazole results in decrease level of thyroid hormone and consequential reduction in metabolic activity with lowered serum electrolyte [12].

Carbimazole was previously reported to cause little or no change in urea biosynthesis in hyperthyroid rats [4].

On the bases of the antithyroid activity shared in common between Carbimazole, glycine max (soybean) and Citrus sinensis (fresh orange juice), the necessity to investigate their effect comparatively on serum electrolyte and urea became very obvious. This was the major objective of this study.

MATERIALS AND METHODS

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 about -4^0c.

The stock concentration of the sweet orange (FOJ) extract was determined by taking 2ml of the sweet orange juice 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 fresh soybean seeds were also bought from Akpan Andem market, Uyo. The beans were rinsed with water to remove sand and then allowed to drip off water and were then grounded and pulverized into powder using electric blender. After that, 400ml of distilled water was added to the pulverized soybean to become macerated, the soybean was sieved and put into a conical flask which gave weight of 70g, the mouth of the flask was sealed using masking tape and foil paper. The preparation was left for 24 hours and then the mixture was filtered using a satin mesh material and final filtrate was gotten using whatman's filter paper size 1. The final filtrate was dried in a hot plate to obtain a brown gummy paste. A mettler P163 electronic weighing balance was used to weigh the gummy paste before stock solution was prepared. The stock solution of the extract was prepared by dissolving 1gm of extract in 10ml of water to give a concentration of 100mg/ml. the stock solution was labeled appropriately and refrigerated at 4^0c until required for use.

Animals Preparation, Treatment and Sample collection

28 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 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 and were strictly adhered to.
The rats were randomly assigned into four groups of seven (7) rats per group. Rats in group 1 served as control and were administered distilled water while groups II, III and IV were administered with 0.01mg/g of carbimazole, 207.84mg/kg of fresh soy bean (FSB) and 1500mg/kg of citrus sinensis or Fresh Orange Juice (FOJ) respectively for 28 days according to their body weight.

The median lethal dose was estimated by the method of Lorke's (1983) [13]. Administration of the aqueous extract was done orally by means of calibrated syringe with attached rubber cannula. The serum electrolyte (Na^+, K^+, Cl^-, and $HC0_3^-$) concentration were analysed using an ion selective electrode (ISE) Baur Biolyte-5 manufactured by Baur Biomedical Electronic, Germany and Urea by Betholet method using Randox Kit, UK.

ISSN: 0975-8585

STATISTICAL ANALYSIS

Data gotten 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≤0.05. Data analysis was done using the statistical software package SPSS for windows version 17.0 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

The results were presented as Mean±SEM. The Mean±SEM for Na^+ were 142.14±1.03, 136.86±1.52, 142.14±2.34 and 143.29±0.84 for Control, CARB, FSB and FOJ groups respectively. Only Carbimazole significantly reduced Na^+ level when compared with control, FSB and FOJ as shown in figure 1.

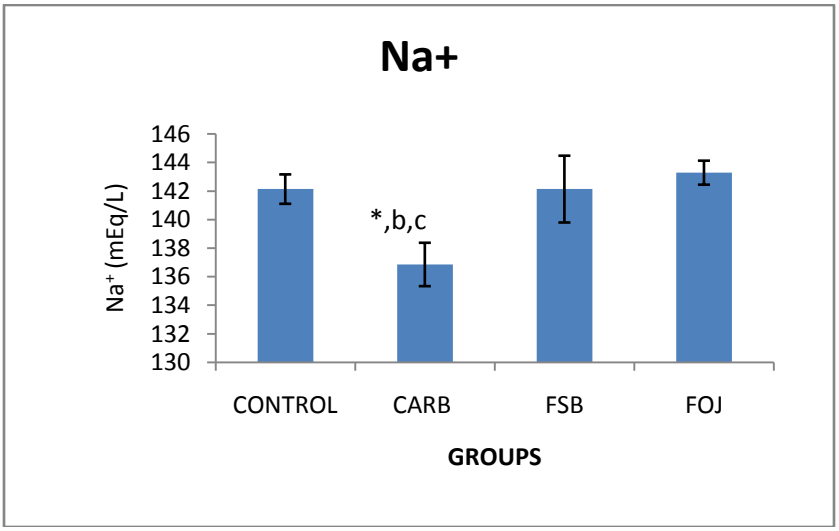

Figure 1: Comparison of Na^+ levels in the different experimental groups*=p<0.05 vs control; b= p<0.05 vs FSB, C= p<0.05 vs FOJ

ISSN: 0975-8585

The Mean±SEM for K^+ were 5.34±0.27, 4.29±0.47, 5.41±0.47 and 5.27±0.26 for Control, CARB, FSB and FOJ groups respectively. Carbimazole significantly reduced K^+ levels when compared to control and FOJ as shown in figure 2.

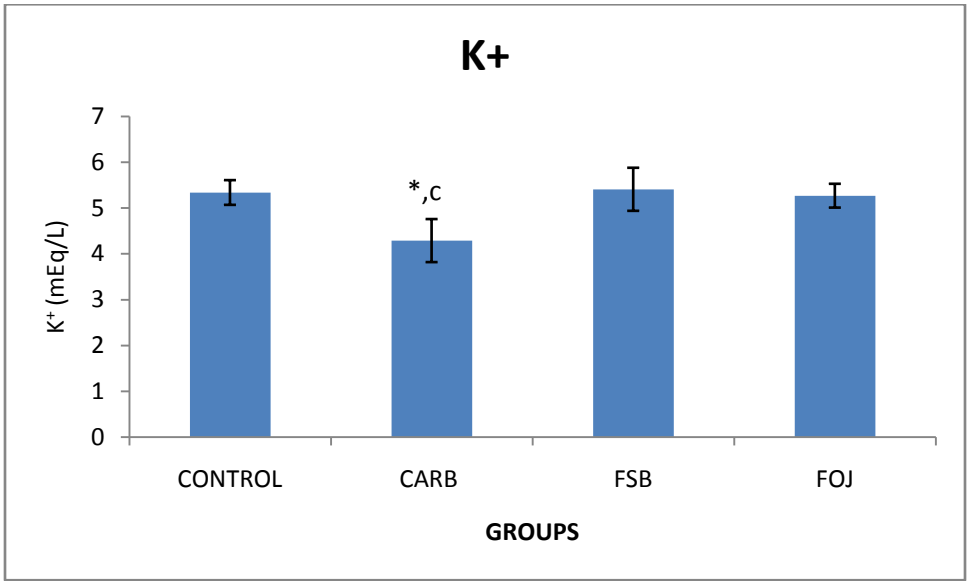

Figure 2: Comparison of K^+ levels in the different experimental groups *=p<0.05 vs control; C= p<0.05 vs FOJ

The Mean±SEM for Cl^- were 98.29±0.82, 99.71±0.89, 103.14±2.41 and 105.27±0.99 for Control, CARB, FSB and FOJ groups respectively. None of the extract caused a significant change in Cl^- levels as shown in figure 3.

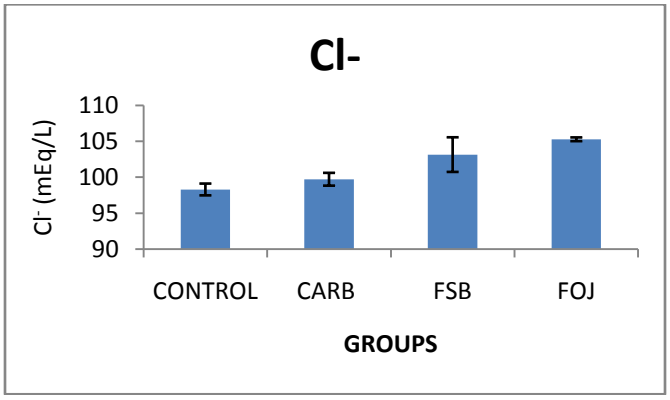

Figure 3: Comparison of Cl^- levels in the different experimental groups

ISSN: 0975-8585

The Mean±SEM for $HC0_3^-$ were 24.43±0.43, 21.86±0.59, 21.00±0.38 and 24.29±0.42 for Control, CARB, FSB and FOJ groups respectively. Carbimazole and FSB significantly reduced $HC0_3^-$ levels when compared to control and FOJ as shown in figure 4

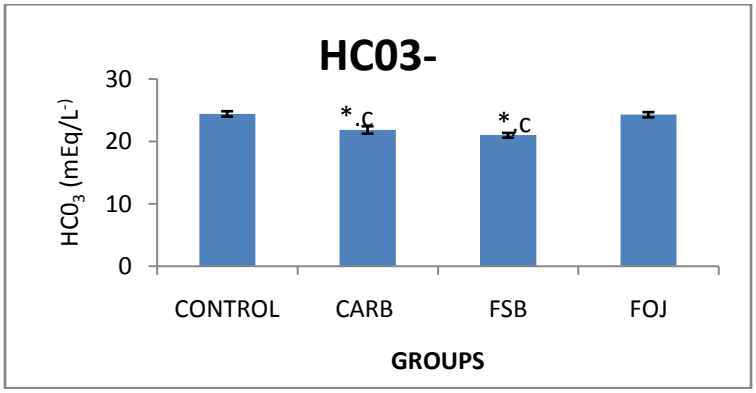

Figure 4: Comparison of $HC0_3$- levels in the different experimental groups *=p<0.05 vs control; C= p<0.05 vs FOJ

The Mean±SEM for Urea were 5.23±0.26, 4.77±0.26, 4.86±0.34 and 5.73±0.27 for Control, CARB, FSB and FOJ groups respectively. Carbimazole and FSB significantly reduced $HC0_3^-$ levels when compared to FOJ as shown in figure 5.

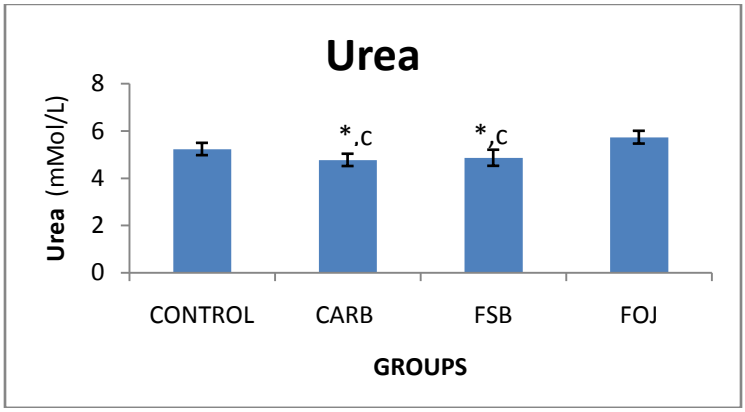

Figure 5: Comparison of Urea levels in the different experimental groups *=p<0.05 vs control; C= p<0.05 vs FOJ

This study presented a comparative analysis of the blood levels of electrolytes and urea in albino wistar rats treated with carbimazole, fresh soy bean, and fresh orange juice. From the results, carbimazole was found to significantly reduce the blood levels of all the electrolytes and urea with the exception of chloride ions while on the other hand fresh orange juice had no significance effect on any of the electrolytes and urea. Treatment with fresh soy bean (glycine

max) significantly lowered the blood levels of bicarbonate ions and urea with no effect on the other electrolytes measured (i.e. sodium, potassium and chloride).

This result indicates that treatment of hyperthyroidism with carbimazole could lead to hyponatraemia, hypokalemia, acid-base disorders and probably impaired hepatic deamination. The mechanism by which hypothyroidism induces hyponatrema is not completely understood. The principal abnormality in fluid intake regulation appears to be an inability to maximally suppress antidiuretic hormone (ADH) [14]. The glomerular filtration is also decreased in hypothyroidism. This can directly diminish free water excretion by diminishing water delivery to the diluting segments [14]. Decreased delivery may be particularly important in those cases in which hyponatraemia develop despite appropriate suppression of ADH release [15]. The pathophysiology of this condition is that regardless of the mechanism the net effect of the impairment in water excretion is the retention of ingested water and a reduction in the plasma sodium concentration. The implication of this theory is that alteration of sodium balance could lead to alteration of the osmotic pressure of body fluid. This is due to the fact that abnormal concentration of sodium in serum can affect the osmotic pressure of the body fluid which is related to blood pressure [16].

The relationship between sodium and hypertension is based on the capacity of the mineral to “attract” water. Blood pressure which depends in part on blood volume increases as retained water increases. The reverse is the case when the serum sodium level decreases. In this way, serum levels of sodium affect the blood pressure. However, it is important to note that the causes of primary hypertension are multifactorial and not limited to sodium mechanism. We can therefore summit that carbimazole, and glycine max could be effective in the management of hyperthyroidism induced hypertension. Alteration in potassium concentration can occur by two major mechanisms: either from redistribution between extracellular and intracellular compartments or an increase potassium loss through the gastrointestinal tract or the kidney. Numerous agents can cause a shift of potassium from the extracellular compartments into cells but the total body fluid potassium remains usually normal. Hypokalemia may sometimes appear apparently minor but this should not be underestimated. In this present report, the actual mechanism underlying the decrease potassium ion concentration is not very clear apart from its correlation with the depressed metabolic activity due to the anti-thyroid action of carbimazole, though this was not applicable for citrus sinensis and glycine max, probably because of their less potent antithyroid effect.

The bicarbonate buffer system is the most important amongst blood buffers when blood pH is considered [17]. The reduction in the serum bicarbonate ion levels implies that the blood pH was lowered. This reduction could be linked to either respiratory excretion via hyperventilation or increased renal excretion of bicarbonate [18]. There was no hyperventilation observed in the experimental rats in this study. Therefore, the renal route of excretion was the likely mechanism of loss for the carbimazole and glycine max treated groups. The implication of this observation is that administration of carbimazole or glycine max could trigger any of the several pathophysiological mechanisms involved in metabolic acidosis. Chloride ion appears to be an exception amongst all the electrolytes investigated in this study,

as all the agents administered in the various groups had no effect on chloride ion. These findings call for further research.

Serum urea concentration analysis is important to assess the functional status of the kidney and the liver. A high level of serum urea indicates optimal filtering capacity of the kidney while a low serum urea level can be a sign of impaired liver function. Again, from our results it appears that carbimazole and glycine max possess some nephro-toxic potential. Thus their use in patient with renal or hepatic impairment should be with extra caution.

This results however raises the question if really the action of Carbimazole on the sodium electrolytes is related or not, to its anti thyroid activity in view of the observation that FOJ also known to possess antithyroid properties had no significant effect on serum electrolyte and urea. Nevertheless, it is also possible that this could be attributed to the degree of potency of these agents to inhibit thyroid hormone synthesis and pharmacodynamics. We can also deduce that FOJ in particular could be useful future treatment for hyperthyroid In patients with concormittant, renal and or hepatic impairment due to its apparent neutral effect on the serum electrolyte and urea dynamics.

CONCLUSION

It is suggestive from this study that carbimazole, a standard anti-thyroid drug commonly used in the treatment of hyperthyroidism may also cause electrolyte derangement in the course of such treatment. While FOJ though yet to be developed and standardized as an antithyroid agent appears to lack these side effects. A concerted effort on the development of these natural agents for use as standard anti-thyroid drug is strongly advocated.

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