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The Effect of Acute Co-administration of Fansidar® with Vitamin C on Some Serum Electrolytes and Body Weight Changes in Male and Female Wistar Albino Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AAA and RUU designed the study, wrote the protocol and wrote the first draft of the manuscript. Author AAA also performed the statistical analysis. Author JEO managed the analyses of the study. All Authors managed the literature searches and approved the final manuscript.

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ABSTRACT

Aims: To investigate the effect of fansidar® and vitamin C co-administration on serum electrolyte and body weight indices of Wistar albino rats.
Place and Duration of Study: Department of Medical Biochemistry, Cross River University of Technology, Okuku Campus, between August 2013 and June 2014.
Methodology: Sixty (60) Wistar albino rats were divided into three groups I, II, III (n=20; 10 male, 10 female) weighing between 180-200 g. Group I was designated as the control and received distilled water, groups II and III were treated with 14.29 mg/kg body weight of fansidar® and 14.29 mg/kg body weight each of fansidar® + vitamin C respectively for 14 days. The animals were

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then sacrificed and blood collected for serum electrolyte analysis.
Results: The results obtained showed that the serum [Na^+] was significantly (P<0.05) increased in group II females only compared with control. There was a significant (P<0.05) increase in the serum [K^+] of groups II and III males and group III females compared with control. There was also a significant (P<0.05) elevation of [CO_2] in group II rats (irrespective of sex) compared with the control. The [Cl^-] of only the males in groups II and III was significantly (P<0.05) reduced compared with the control. The results of body weight indices investigated showed a significant (P<0.05) decrease in mean weight increase (MWI) and growth rate (GR) of all the test groups compared with the control, however, group III compared with group II was significantly (P<0.05) increased.
Conclusion: The alterations in serum electrolyte on fansidar® administration were pronounced in males than females except in the sodium electrolyte levels. The co-administration of vitamin C may reverse the adverse alterations in serum electrolyte and body weight changes caused by fansidar® administration.

Keywords: Fansidar®; vitamin C; serum electrolyte; body weight; Wistar rat.

1. INTRODUCTION

Malaria has been recognized since the Greek and Roman civilizations over 2,000 years ago, with different patterns of fever described by the early Greeks [1]. Each year, approximately 500 million people are infected with malaria worldwide, of those infected, roughly 2 million die of the disease [2].

Malaria is caused by six plasmodium species; *falciparum, vivax, ovale curtisi, ovale wallikeri, malariae* and *knowlesi* [1]. At any one time, an estimated 300 million people are said to be infected with at least one of these plasmodium species [3]. Around the world, the malaria situation is serious and getting worse. It threatens the lives of 40% of the world's population particularly pregnant women and children under five who suffer from malaria-infected morbidity and mortality [4,5].Together with pneumonia, diarrhoea, measles and malnutrition, malaria is responsible for over 70% of deaths in young children especially in developing countries [5].

Fansidar® is an antimalarial agent each tablet contains 500 mg sulfadoxine and 25 mg pyrimethamine [6]. The drug combination of fansidar® (sulfadoxine-pyrimethamine) acts synergistically by sequential blockade of two enzymes involved in the biosynthesis of folinic acid in the parasites [6]. A single dose therapy eliminates trophozoites and schizonts from the blood. According to world health organization (WHO), fansidar® is used for the prophylaxis or treatment of chloroquine resistant *Plasmodium falciparum* [6]. The clinical treatment with fansidar® is often accompanied by serious side effect such as allergic reactions including skin eruptions, toxic epidermal necrolysis, urticaria, serum sickness, pruritus, and allergic myocarditis that could be fatal [6].

An increasing number of serious complications of antimalarial drug have been reported, this has raised some doubt about the safety of fansidar® [6].

Fansidar® has approval for use in the treatment and prophylactic measure against malaria and is considered to be more effective in treating malaria caused by *Plasmodium falciparum* than that caused by *Plasmodium vivax* [7], for which chloroquine is considered more effective, though in the absence of a species-specific diagnosis the sulfodoxine-pyrimethamine combination may be indicated [7]. Fansidar® is cheap and widely used drug among the poor people of the third world countries where malaria is predominant and used to treat serious malaria infections in areas where other drugs may not work [7]. The increase in the prevalence of malaria resistance to drugs also poses the threat of fansidar® abuse among patients which may possibly result to unhealthy alterations in serum electrolyte level. In this study, based on the already known health benefits of vitamin C such as an effective donor antioxidant, protective role against development of cancer with increased plasma concentrations, wound healing and formation of collagen and also the fact that preparations containing vitamin C have been found to stabilize electrolyte changes among others [8,9], it becomes imperative that its co-administration with fansidar® be exploited to possibly reduce some of the adverse effects of fansidar® especially on serum electrolytes and body weight changes in Wistar albino rats.

2. MATERIALS AND METHODS

2.1 Equipment, Chemicals and Reagents

Materials used in laboratory included dissecting set, cage, glass wares such as syringes (2 mL, 5 mL), test tubes, pipette and micro-pipette slide from Singer Company Nigeria Ltd. Centrifuge, microscope were from Olympus, Japan while triple beam weighing balance, specimen jars and beakers were from Ohaus, USA, Sigma Chemical Co. Ltd, USA and M & G Scientific Co. Ltd, USA respectively. Water bath and spectrophotometer were also included among others. All chemicals and reagents used for this work were of analytical grade and included chloroform, hydrochloric acid, ethanol, magnesium ion, thioburbitric acid, sodium hydroxide, glacial acetic acid all from Sigma Chemical Co. Ltd, USA while hydrogen peroxide and formalin were from Aldrich Chemical Co. Ltd, USA. Teco Diagnostic kits were also used.

2.2 Experimental Animals

Adult sixty (30 males and 30 females) strains of Wistar albino rats were obtained from a disease-free stock of the Department of Biochemistry, University of Calabar, Calabar and used for the study. The animals were allowed two weeks for acclimatization and weighed between180-200 g before treatment commenced. The rats were assigned on the basis of their weight into three study groups of twenty rats each (10 males and 10 females), normal feeds and clean tap water were given to the animals *ad libitum*. They were kept in plastic cages, ten rats per cage, placed in a well-ventilated animal room at temperature and relative humidity of 28±2℃ and 70% respectively with 12 h light and dark cycle. Each of the animals was given an identification mark and was handled according to the United States National Institute of Health (NIH) guide for Care and Use of animals and in accordance with the recommendation of IASP [10].

2.3 Experimental Design

The design consisted of sixty 60 Wistar albino rats randomly assigned into three study groups of 20 rats each (10 male and 10 female). The male and female rats in each group were kept in separate cages in order to avoid mating.

Group I: Control animals allowed free access to rat feed and water only.

Group II: Animals treated with fansidar® and allowed free access to rat feed and water.

Group III: Animals treated with fansidar® and vitamin C co-administration and allowed free access to rat feed and water.

2.4 Drug Administration

Two (2) packs of fansidar® containing three tablets each of 500 mg per tablet and fifty 50 tablets of vitamin C containing 100 mg each were obtained from Edoh Pharmacy, Ogoja. Stock solutions of 1000 mg of fansidar® tablet and 1000 mg of vitamin C tablet each dissolved in 5 mL of distilled water was prepared and used for the study. Group II was administered 14.29 mg/kg body weight of fansidar®. Group III was also administered 14.29 mg/kg body weight of fansidar® and 14.29 mg/kg body weight of vitamin C. Group I was allowed free access to tap water only for the period of 14 days. Note that the calculated value 14.29 mg/kg body weight used for the test groups was based on the average weight of an adult human of 70 kg.

2.5 Body Weight Measurement

The weights of the experimental animals were measured before drug administration commenced, this served as the initial weight while that measured after drug administration served as the final weight. This was done with the aid of an Ohaus triple beam weighing balance gotten from the Department of Medical Biochemistry, Cross River University of Technology - Okuku campus.

2.6 Blood Samples Collection for Serum Electrolyte Analysis

Two weeks after drug administration, the rats were subjected to a 16h overnight fast prior to sacrifice and anaesthetized by chloroform (5%) inhalation. They were later placed on a dissecting slab and the limbs pinned down with the aid of dissecting pins. A longitudinal incision was made abdominally to the rib followed by a transverse incision to the limbs. Blood samples were collected with the aid of sterile syringes and needles through cardiac puncture from the left ventricle and were transferred into well labeled heparin-treated tubes and centrifuged in order to separate the serum from blood cells. The heparin-treated tubes containing serum and packed blood cells were stored at optimal temperature (4℃) until when needed for use.

2.6.1 Determination of serum electrolytes

The serum electrolytes sodium, potassium and chloride were carried out by automated

techniques, adopting the dry chemical method after preparation of slides and their amounts measured using Vitros DT 60 II Chemistry System. The serum carbondioxide was measured through the principle of dissolved carbondioxide determination using Teco Diagnostic kits. The prepared sample was then measured using Spectrumlab 23A spectrophotometer.

2.7 Statistical Analysis

Data obtained was analysed using Microsoft Office Excel 2007 and expressed as mean ± SEM. The statistical package SPSS version16.0 was used to establish statistical significance at $P<0.05$.

3. RESULTS AND DISCUSSION

In Table 1, serum sodium levels for the males of groups II and III showed no significant ($P\geq0.05$) difference but that of female in group II was significantly ($P<0.05$) increased compared with the control.

Male serum potassium levels in group II and III were significantly ($P<0.05$) higher than that of control. There was no significant ($P\geq0.05$) difference between the males in the test groups, however, there was increased female serum potassium levels in the test groups but only group III was significantly ($P<0.05$) higher than groups I and II when compared.

The male serum carbondioxide levels in the test groups showed no significant ($P\geq0.05$) difference compared with the control. However, the female serum carbondioxide level in group II was significantly ($P<0.05$) increased compared to I and II.

Alterations in serum chloride level occurred only in the experimental males as groups II and III were significantly ($P<0.05$) lower compared with the control. There was a further significant ($P< 0.05$) decrease in serum chloride level of group III compared to II.

In Table 2, the male mean weight increase (MWI) recorded by groups II and III was significantly ($P<0.05$) lower than that of control. However, MWI of group III was significantly ($P< 0.05$) higher than that of group II. This pattern was similar for the growth rate (GR) of males.

The MWI and GR for the females followed a similar pattern as the males.

3.1 Discussion

There was a marked increase in serum concentration of sodium, potassium and carbondioxide, but decrease in serum concentration of chloride. The increase in serum sodium concentration or hypernatraemia was only found in females administered fansidar®, while co-administration with vitamin C normalized serum sodium concentration hence demonstrating its ameliorative capacity. The effect of hypernatraemia by fansidar® on females may have been due to alteration in female hormones, resulting in the inhibition of the hypothalamus towards secretion of antidiuretic hormone (ADH), thus allowing for increased sodium ion concentration in the serum [11].

Sodium is a dominant extracellular cation and hypernatraemia is an electrolyte disturbance that is defined by elevated sodium (Na^+) level in the blood [12], which results in a relative deficit of free water in the body. Clinical manifestations of hypernatraemia can be subtle, consisting of lethargy, weakness, irritability, neuromuscular hyper-excitability, and oedema [12]. With more severe elevations of the sodium level, seizures and coma may occur [13].

Potassium is the dominant intracellular cation. Its serum level can be increased or decreased; hyperkalaemia therefore defines a decrease in intracellular potassium and an increase in serum potassium concentration ($[K^+]$). Clinical manifestations associated with hyperkalaemia are fairly non-specific and generally include malaise, palpitations and muscle weakness [14]. The significant elevation in potassium concentration in the serum of male experimental rats, possibly as a result of fansidar® administration, may have caused associated effects of hyperkalaemia mentioned above. A similar pattern was also observed with the serum of female rats. However in both male and female rats, the co-administration of fansidar® with vitamin C could not reverse the effect of hyperkalaemia as it did in the case of hypernatraemia. Since it is known that vitamin C interacts with certain micro-minerals such as iron, copper, cobalt, manganese etc. thereby altering their concentration in blood [15], this fact leads to the assumption that vitamin C may have possibly interacted with potassium, acting synergistically with fansidar® to cause enhanced efflux of potassium from the intracellular compartment into the serum as seen in group III females.

Table 1. Fansidar® and vitamin C co-administration on serum sodium, potassium, carbondioxide and chloride levels in male and female rats

Groups	Parameter							
	Na^+ (mmol/L)		K^+ (mmol/L)		CO_2 (mmol/L)		Cl^- (mmol/L)	
	Male	Female	Male	Female	Male	Female	Male	Female
I	183±0.23	183.4±0.14	2.73±0.03	2.98±0.06	32.9±0.18	36±0.23	115±0.17	109.6±0.19
II	182.6±0.39	185±0.16[a]	4.17±0.03[a]	3.24±0.01	35.9±0.27[a]	40.9±0.23[a]	111.4±0.29[a]	109±0.15
III	181.1±0.19	183.3±0.11[b]	4.00±0.02[a]	4.21±0.01[a,b]	33.5±0.16[b]	35.00±0.15[b]	108±0.09[a,b]	110±0.09

Values presented as mean ± SEM (n=10)
a = significantly different from group I at P<0.05
b = significantly different from group II at P<0.05

Table 2. Fansidar® and vitamin C co-administration on body weight parameters of male and female rats

Groups	Parameter							
	Initial weight (g)		Final weight (g)		Mean weight increase (MWI) (%)		Growth rate (GR) (g)	
	Male	Female	Male	Female	Male	female	Male	Female
I	189.9±0.73	192.1±0.63	232.5±0.48	224.9±0.69	42.6± 0.87	32.8±0.78	3.04±0.06	2.34±0.06
II	189.9±0.73	192.1±0.63	154.2±1.47	160±0.70	-35.7± 1.91[a]	-32.1±1.01[a]	-2.55±0.14[a]	-2.29±0.07[a]
III	189.9±0.73	192.1±0.63	193.3±0.89	204.7±0.59	3.4 ±1.12[a,b]	12.6±0.95[a,b]	0.24±0.08[a,b]	0.9±0.07[a,b]

Values presented as mean ± SEM (n=10)
a = significantly different from group I at P<0.05
b = significantly different from group II at P<0.05

Hypercapnia is a condition of abnormally high carbon dioxide (CO_2) levels in the blood. Carbon dioxide is a gaseous product of the body's metabolism and is normally expelled through the lungs [16]. The clinical manifestations of hypercapnia include flushed skin, full pulse, tachypnea, dyspnea, extra systoles, muscle twitches, reduced neuronal activity, and possibly raised blood pressure. Symptoms of mild hypercapnia might include headache, confusion, and lethargy. It has been reported that hypercapnia may increase susceptibility to lung infections or pneumonia mortality similar to respiratory acidosis [17], a result of increased hydrogen ion concentration. The hypercapnia observed in group II males and females possibly may have been elicited by fansidar®. The co-administration of fansidar® with vitamin C is likely to be the cause of the normalization in dissolved CO_2 concentration observed in both males and females of group III, which strongly indicates the ameliorative activity of vitamin C against fansidar®-induced hypercapnia. Hypercapnia may lead to high levels of bicarbonate which in people with chronic kidney disease have been correlated with heart failure [18].

Hypochloraemia is an imbalance in electrolyte where there is abnormal chloride ion (Cl^-) depletion in the blood. It is often associated with hyponatraemia or hypercapnia, and metabolic alkalosis. Chloride is the major anion seen in both the blood and extracellular fluid. The clinical manifestations of hypochloraemia include dehydration, muscle hypertonicity (spasticity), muscle weakness and twitches [19,20]. Hypochloraemia was observed in groups II and III males, which may have been as a result of fansidar® administration. However, this was worsened in group III of male rats, thereby suggesting that vitamin C has the potency of decreasing serum chloride level in male rats. Fansidar® with or without vitamin C co-administration showed no effect on the serum chloride concentration of female rats.

The effects of fansidar® and vitamin C co-administration on mean weight increase and growth rate were also assessed. The results of body weight changes agree with published reports [21].

It was observed in this research that administration of fansidar® caused a significant decrease in the MWI of male and female rats. This weight reduction effect of fansidar® may possibly have been caused by increased tissue fat breakdown. On co-administration of vitamin C, there was a reverse effect on weight reduction caused by fansidar® administration resulting in weight gain. Vitamin C has been known to reduce weight gain [22,23] but in this case, it acted antagonistically with fansidar® to reverse possible tissue fat break down hence stabilizing weight. The administration of fansidar® also caused a concomitant significant decrease in growth rates of male and female rats, while the co-administration of fansidar® with vitamin C in male and female rats brought about a significant increase in growth rate, suggesting the activity of vitamin C against decreased growth rate caused by fansidar®.

4. CONCLUSION

The co-administration of vitamin C appeared to reverse the adverse alterations in serum electrolytes and body weight changes caused by fansidar® administration. Hence it is advised that fansidar® be co-administered with vitamin C in order to ameliorate the adverse health effects that fansidar® may cause.

CONSENT

It is not applicable

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the Faculty of Basic Medical Sciences, Cross River University of Technology (CRUTECH) Research and Ethics Committee.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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