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# Antioxidants and Electrolyte Profile in Early Pregnancy: *In vivo* Studies

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## ABSTRACT

The effect on electrolyte profile following oral administration of antioxidants (vitamin A, C and E supplements in early pregnancy was investigated using albino rats of the Wistar strain. Eighty-five female rats weighing between 255-300 g were used for the study. They were randomly assigned to three study groups having 5 sub groups with five animals each, a control and vehicle group with five animals each. After pregnancy has been confirmed, the control group was administered 1 mL of distilled water, vehicle group 1 mL of tween 80, while test groups 1, II and III received different doses of vitamin A, C and E, respectively via the intragastric route for 11 days. The administration of vitamin A, C and E in early pregnancy for 11 days produced insignificant changes in serum $Na^+$ levels ($p>0.05$) and a significant increase in serum $Ca^{2+}$ level. With the exception of vitamin C, vitamin A and E produce a significant increase in serum $K^+$ and $Cl^-$ levels. Conclusively administration of vitamin A, C and E in early pregnancy cause no significant alteration in serum $Na^+$ levels. However, care must be taken when vitamin A and E are administered with agents that elevate serum $Ca^{2+}$ and $K^+$ levels as this may pontentiate hypercalcemia and hyperkalemia, respectively in early pregnancy.

**Key words:** Hypercalcemia, hyperkalemia, supplementation, hypertension, pre-eclampsia

## INTRODUCTION

Due to the popular usage of vitamins it is becoming increasingly important to be aware of the effects of therapeutic doses of these vitamins during pregnancy. Vitamins are important for maintaining good health, but the consumption of high doses of certain vitamins poses a great risk in pregnancy. Vitamin C has been shown to cause electrolyte disturbances in high doses; however, limited data are available to show any electrolyte alteration at therapeutic doses. Pregnancy remains a very important physiologic process in the life of a woman (Wideman *et al*., 1964; Christian *et al*., 2008). Report shows that some miscarriages are as a result of high doses of vitamin C supplements in the maternal system during early pregnancy (Samborskaia, 1977; Wilcox *et al*., 1999) and that vitamin C might be responsible for cramps that occur in early pregnancy (Chalker and Downer, 1992; Massey *et al*., 2005).

Hypertensive disorders are common medical complications of pregnancy with a reported incidence of about 10% in first pregnancies and 20-25% in women with chronic hypertension

(Kamath, 2006). According to Guyton (2006) and Hall *et al.* (1999), likely causes of intracellular swelling are; depression of metabolic systems of the tissues and lack of adequate nutrition to cell. This leads to increased vascular capacity, hypertension and development of varicose vein as seen in pregnancy. Hormonal imbalance leading to altered lipid profile in serum is attributed to be the prime factor in aetiopathogenesis of pregnancy-induced hypertension (Sahu *et al.*, 2009). Furthermore, nausea and vomiting are by far the most common medical conditions during pregnancy, with an estimated prevalence around 50-70% (Ornstein *et al.*, 1995; Weigel and Weigel, 1989; Bashiri *et al.*, 1995). The rate of its most severe form, hyperemesis gravidarum, has been estimated at 0.5-2% of all pregnancies (Bashiri *et al.*, 1995). According to Guyton (2006) and Hall *et al.* (1999), the net result of vomiting is a loss of $H^+$ and $Cl^-$ from the extra cellular fluid and the development of metabolic alkalosis. Studies suggest the aetiology of nausea and vomiting of pregnancy to be the result of placental hormonal imbalance though this is inconclusive (Kaupilla *et al.*, 1979; Soules *et al.*, 1980). In a study on prevention of neural tube defects with folic acid, women who received vitamins were less likely to experience nausea and vomiting and less severe forms, than women who received a placebo (Czeizel, 1996).

Present study seeks to investigate the effect of vitamin A, C and E supplementation on electrolyte status and its potential benefit in early pregnancy.

## MATERIALS AND METHODS

**Experimental animals:** Eighty-five adult female Wister albino rats weighing (225-300 g) were procured between November and December 2009 from the Animal House, College of Medicine, Ambrose Alli University, Ekpoma. They were housed in a stainless steel cage with plastic bottom grid and a wire screen top in a Laboratory located in the Department of Physiology, Ambrose Alli University, Ekpoma, Edo State, Nigeria. They were assigned into five groups; a control group (n = 5), vehicle group (n = 5) and three test groups (I, II and III) made up of five sub-groups with 5 rats each. They were fed *ad libitum* with tap water and pellated feeds purchased from Bendel feeds and flour meal Ewu, Nigeria Limited and allowed to acclimatize for 2 weeks. After which two male Wister albino rats were introduced into each group to allow for mating. The animals were allowed to mate for 6 days after which the male animals were removed from the cage. Pregnancy was confirmed using the palpation method and vaginal smear microscopy method. From the 7th day, administration of the different Vitamins began using orogastric tubes and syringes. This lasted for a period of 11 days. The administrations were conducted between the hours of 08.00 am and 10.00 am daily.

**Vitamins preparation:** Vitamin A, C and E were purchased from Clarion Medical Pharmaceuticals Nigeria Limited. Tween 80 vehicle was purchased from Sigma Pharmaceuticals Limited. Two hundred milligram of the powdered form of vitamin C was dissolved in 10 mL of distilled water and the appropriate dose per kg were prepared for administration. Vitamin A (25,000 IU equivalent to 6 mg retinol and vitamin E, 100 mg) was dissolved in 0.2 mL of tween 80 and water in a ratio of 0.2:0.2:9.6.

**Vitamin administration:** In addition to normal feed, group II received Vitamin C as follows; 200, 250, 300, 350 and 400 mg $kg^{-1}$ for treatment sub-groups 1, 2, 3, 4 and 5, respectively. In addition to normal feed, group I and III received vitamin A and E, respectively as follows; 0.6, 0.7, 0.8, 0.9 and 1.0 mg $kg^{-1}$ of vitamin A and 16.4, 18.4, 19.4, 20.4 and 22.4 mg $kg^{-1}$ of vitamin E administered to treatment sub-group 1, 2, 3, 4 and 5, respectively.

**Sample collection and analysis of electrolytes:** Twenty-four hours after the last administration was carried out, the animals were sacrificed after inhalation of chloroform. Cardiac and jugular vein puncture were used to collect blood samples into sterilized test tubes containing lithium heparin as anticoagulant. Collected blood samples were immediately sent to the biochemistry laboratory for analysis. Determination of serum $K^+$, $Na^+$, $Ca^{2+}$ and $Cl^-$ were analysed using standard methods as described by Tsalev and Zaprianov (1984).

**Data analysis:** The Mean±Standard Error of mean (X±SEM) and one-way ANOVA statistical test was performed using SPSS version 17 soft ware. The significance level was set at $p<0.05$. Results were presented using suitable tables.

## RESULTS

Mean±SEM (in mmol $L^{-1}$) and student t test for the electrolyte status ($Na^+$, $K^+$, $Cl^-$ and $Ca^{2+}$) is presented in Table 2 for the three vitamin groups. Na was lower in Group I and III but increased as dose increased, however, the increase was not statistically significant. Group II presented the opposite of group I and III. However, there were no statistical significant differences ($p>0.05$) in serum Na levels in the vitamin A and E groups at the treatment doses used (Na column in Table 1). On the other hand, $Ca^+$ was observed to increase compared with the control groups as doses increase in vitamin (A, C and E) groups. The highest values were observed after the 5th treatment in the entire vitamin groups with vitamin C presenting 0.83±0.01 mmol $L^{-1}$ as the highest value. This increase was statistically significant ($p<0.05$) (Ca column in Table 2). In groups I and III there was a significant ($p<0.05$) increase in serum K, with vitamin A (group I) presenting the highest value 9.56±0.16 mmol $L^{-1}$ after the 5th treatment (K column in Table 2). Finally, $Cl^-$ concentrations were significantly higher in Group I and III when compared with the control group. Although, group II (vitamin C) presented a high serum $Cl^-$ level compared with the control group, the increase was not statistically significant ($p>0.05$) ($Cl^-$ column in Table 2).

Table 1: Grouping and treatment administered to different rat groups (n = 5 rats per group)

| Group | Treatment |
|---|---|
| CONTROL | DIST $H_2O$- Normal feed +Distilled water 1 mL |
| | VEHICLE- Normal feed + Vehicle 1 mL |
| I | Normal feed+Vehicle+Vit A 0.6 mg $kg^{-1}$ |
| | Normal feed+Vehicle+Vit A 0.7 mg $kg^{-1}$ |
| | Normal feed+Vehicle+Vit A 0.8 mg $kg^{-1}$ |
| | Normal feed+Vehicle+Vit A 0.9 mg $kg^{-1}$ |
| | Normal feed+Vehicle+Vit A 1.0 mg $kg^{-1}$ |
| II | Normal feed+distilled water+Vit C 200 mg $kg^{-1}$ |
| | Normal feed+distilled water+Vit C 250 mg $kg^{-1}$ |
| | Normal feed+distilled water+Vit C 300 mg $kg^{-1}$ |
| | Normal feed+distilled water+Vit C 350 mg $kg^{-1}$ |
| | Normal feed+distilled water+Vit C 400 mg $kg^{-1}$ |
| III | Normal feed+Vehicle+Vit E 16.4 mg $kg^{-1}$ |
| | Normal feed+Vehicle+Vit E 18.4 mg $kg^{-1}$ |
| | Normal feed+Vehicle+Vit E 19.4 mg $kg^{-1}$ |
| | Normal feed+Vehicle+Vit E 20.4 mg $kg^{-1}$ |
| | Normal feed+Vehicle+Vit E 22.4 mg $kg^{-1}$ |

Table 2: Effect of vitamin A, C and E supplementation in early pregnancy on electrolyte parameters (mmol $L^{-1}$) in albino rats

| Group | Na | K | Cl | Ca |
|---|---|---|---|---|
| Control | 139.80±2.60 | 8.60±0.24 | 99.00±2.41 | 0.66±0.01 |
| Vehicle | 141.00±0.71 | 9.70±0.10 * | 98.00±1.10 | 0.68±0.01 |
| **Vitamin A** | | | | |
| 1 | 135.60±1.29 | 8.70±0.07 | 104.00±1.84 | 0.52±0.01* |
| 2 | 135.60±1.29 | 8.90±0.12 | 106.00±2.07* | 0.48±0.02* |
| 3 | 138.00±1.41 | 9.20±0.17* | 106.00±2.51* | 0.63±0.01 |
| 4 | 138.80±1.50 | 9.62±0.15* | 106.00±1.76* | 0.66±0.02 |
| 5 | 138.60±1.78 | 9.56±0.16* | 110.00±1.70* | 0.82±0.01* |
| **Vitamin C** | | | | |
| 1 | 139.00±0.89 | 8.44±0.13 | 98.00±2.17 | 0.68±0.01 |
| 2 | 138.80±2.18 | 8.64±0.21 | 102.00±1.10 | 0.81±0.01* |
| 3 | 135.80±1.16 | 8.60±0.07 | 104.00±1.79 | 0.80±0.01* |
| 4 | 137.80±1.43 | 8.70±0.09 | 102.60±0.60 | 0.81±0.01* |
| 5 | 135.00±1.48 | 8.92±0.20 | 105.00±1.76 | 0.83±0.01* |
| **Vitamin E** | | | | |
| 1 | 135.60±1.12 | 8.50±0.12 | 102.00±0.55 | 0.62±0.01* |
| 2 | 137.0±1.62 | 9.24±0.0* | 106.00±1.38* | 0.74±0.01* |
| 3 | 136.60±1.72 | 9.00±0.11* | 107.00±0.71* | 0.58±0.02* |
| 4 | 137.20±1.62 | 9.20±0.07* | 107.00±0.71* | 0.56±0.02* |
| 5 | 138.60±2.60 | 9.20±0.06* | 110.00±1.00* | 0.78±0.01* |

Values are Mean±SEM of five rats; * = level of significance ($p<0.05$) compared with the control.

## DISCUSSION

Cell culture studies have linked increased bone resorption and decreased bone formation with high vitamin A intake. This interaction occurs because vitamins A and D may compete for the same receptor and then interact with parathyroid hormone, which regulates serum calcium concentration (Penniston and Tanumihardjo, 2006). Indeed, a study by Forsmo *et al.* (2008). Showed a correlation between low bone mineral density and high intake of vitamin A (Forsmo *et al.*, 2008). In present study vitamin A increased serum Ca levels in early pregnancy this may be of benefit in mataining bone density in pregnant mothers and the developing fetus.

The effect of vitamin C on electrolyte profile in this study was not significant.. This is corroborated by the study of Eteng *et al.* (2006), who also obtained a non-significant increase in serum $Na^+$ following administration of vitamin C to Wistar rats. The study is also in agreement with the findings by Seller (1996), which revealed an insignificant change in serum $Na^+$ and $K^+$ following vitamin C administration. However, this study revealed a significant increase in serum $Ca^{2+}$ level. This is in agreement with the study of Abt and Farmer (1983), which revealed that vitamin C, prevents the loss of serum $Ca^{2+}$ which is accentuated by pregnancy. Sodium and chloride are the most abundant electrolyte in the extra-cellular fluid and to a large extent determine plasma osmolarity (Guyton, 2006; Hall *et al.*, 1999), vitamin C administration in pregnancy does no harm neither does it cause unfavorable alteration in plasma electrolyte, hence it does not predispose to hypertension. Vitamin C has also been promoted as efficacious against a vast array of diseases and syndromes. Research has been done on the effects of Vitamin C on a variety of disorders and diseases including heart disease (Rath and Pauling, 1994) and pre-eclampsia (Rumbold *et al.*, 2008). Its uses are poorly supported by a few experimental evidence and sometimes contraindicated by others (Brzozowska *et al.*, 2008; Bjelakovic *et al.*, 2008). In a study conducted in rats, during the first month of pregnancy, high doses of vitamin C may suppress the production of progesterone

from the corpus luteum (Ovcharov and Todorov, 1974). Progesterone, necessary for the maintenance of a pregnancy, is produced by the corpus luteum for the first few weeks, until the placenta is developed enough to produce its own source. By blocking this function of the corpus luteum, high doses of vitamin C (>1000 mg) are theorized to induce an early miscarriage. However, the authors do state: 'This could not be interpreted as an evidence of causal association (Vobecky *et al*., 1976). This effect may be due to metabolic acidosis due to calcium resorption reported in this study. However, further research is important in this respect.

While, there was no significant difference in serum sodium (Na) following vitamin E supplementation in early pregnancy, potassium (K), chloride (Cl) and calcium presented significant changes. These findings disagree with the study by Prasad (2010) who reported that vitamin E had no affect on serum electrolytes ($Na^+$ and $Cl^-$) during hypercholesterolemic state. However, present study is in agreement with the findings by Suleyman *et al*. (2010) who reported a significant increase in $Na^+$ in athletes on vitamin E supplementation. Bertoni *et al*. (1981) revealed that vitamin E deficient rats had a decreased serum level of $Na^+$, $K^+$ and $Cl^-$, this suggest that vitamin E plays an important role in electrolyte homoestasis. However our study revealed that vitamin E supplementation in early pregnancy is associated with a significant increase in serum $K^+$, $Cl^-$ and $Ca^{2+}$ levels.

Sodium ($Na^+$) ion and chloride (Cl-) ion excretion from the body is a function of arterial blood pressure (Guyton, 2006; Hall *et al*., 1999). Sodium ($Na^+$) ion depletion stimulates rennin release with subsequent production of Angiotensin II, a potent vasoconstrictor (Hall *et al*., 1999; Guyton, 2006; Hall *et al*., 1999). Increased plasma sodium ($Na^+$) ion levels inhibit rennin release from the juxtaglomerular cells with consequent decrease in angiotensin II release (Hall *et al*., 1999; Guyton, 2006; Hall *et al*., 1999). When modulation of the rennin-angiotensin system is pharmacologically prevented, changes in salt intake markedly influence long-term levels of arterial blood pressure (Hall *et al*., 1999).

## CONCLUSION

No study till date has documented the effect of vitamin A and E on electrolyte profile in early pregnancy. Present study revealed significant alteration in serum $K^+$, $Cl^-$ and $Ca^{2+}$ levels with no significant change in $Na^+$. Worthy of note is the effect observed for $K^+$. However, care must be taken when using these supplements with agents that cause elevation of serum $K^+$ level as this may result in interactions that can potentiate hyperkalemia.

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