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EFFECT OF SODIUM CITRATE ON RED BLOOD CELL COUNT IN WISTAR RAT

[1]Oladipo G.S, [2]Okoh, P.D, [1]Osaat R.S, [3]Leko B.J.
[1]Department of Human Anatomy
Faculty of Basic Medical Sciences
University of Port Harcourt

[2]Department of Surgery
University of Port Harcourt Teaching Hospital

[3]Department of Anatomy
Madonna University, Elele

Correspondence: *Dr. Oladipo G.S,*
E-Mail: oladipogabriel@yahoo.com
GSM: +2348050428628

ABSTRACT
The effect of sodium citrate which is the sodium salt of citric acid, a preservative, an additive, an antioxidant and an anticoagulant used in blood transfusion was investigated in this study on red blood cell in wistar rat. Eighteen male adult rats of wistar strain (RattusNorvegicus) weighing between 150-200g were used. They were divided into 3 groups of 6 rats each in a group. Group A was given 10mg/kg body weight of sodium citrate. Group B was given 15mg/kg body weight of sodium citrate. Group C was given water and feed only. This served as a control test. The rats were all sacrificed on day 2,6 and 14 and blood samples were collected, investigated and tested. The Results when subjected to statistical analysis using analysis of variance (ANOVA) revealed that there were significant differences in the concentration of red blood cells counts among three rat groups i.e. there was significant increase in red blood cell counts (P<0.05) showing that sodium citrate increases the value of red blood cell counts.

INTRODUCTION
Red blood cell is a blood cell containing the red pigment haemoglobin which gives it its red colour (David, 2002) of which the main function is the transport of oxygen. It is the most numerous of the cellular components which makes up almost half of the blood's volume. The cells are filled with protein chemical called haemoglobin which also comprises element like iron that transports oxygen and carbon dioxide. Oxygen is consumed to provide energy to cells leaving carbon dioxide as waste product which the red blood cells carry away from the tissues and back to the lungs.

Any reduction of the red blood cell in the body leads to a condition known as anaemia. Anaemia means that the cells lack sufficient levels of iron. Without an iron atom, the damaged haemoglobin pigment cannot attract oxygen in the lungs very effectively which would lead to complete body dysfunction. Anaemia may be due to loss of blood (haemorrhagicanaemia) resulting from an accident, operation and after birth etc and lack of iron which is necessary for the production of haemoglobin. Haemolyticanaemia results from the increased destruction of red blood cells which contain the pigment. Anaemia can also be caused by the impaired production of red blood cells as in leukaemiaetc (David, 2002).

Sodium citrate is the sodium salt of citric acid which has a salty taste. It is used as a food additive, usually for flavor or as a preservative. It is also used as an anticoagulant to blood in transfusion. It can be used in blood collection tubes and for the preservation of

blood in blood banks. Also as a buffering agent it helps maintain pH levels in soft drinks.

Sodium loading with a sodium concentrated beverage composed of sodium citrate and sodium chloride with moderate osmolality are effective in inducing hyperhydration and hypervolemia at rest (Greenleaf et al 1997, Nose et al 1990, Sanders et al, 2001). Stacy et al (2007) shows that pre-exercise ingestion of a high sodium beverage increased plasma volume before exercise and involved less thermoregulatory and perceived strain during exercise and increased capacity in warm conditions.

Furthermore, studies show that endurance exercise relies heavily on aerobic energy production. However, the contribution of anaerobic glycolysis to the overall energy supply is shown by a significant increase in blood lactate level. For example in a well trained runner, blood lactate concentration may rise more than 20mmol/l after a 5000m run (Osnes and Hermansen, 1972). Therefore, it is evident that the use of buffering agents has the potential to enhance performance during this type of exercise. Also, other studies have investigated the use of buffering substances as ergogenic aids during endurance exercise (Potteiger et al, 1996).

Hence, this study is carried out to provide a result on the effect of sodium citrate on red blood cell count of wistar rat. This emphasizes its usefulness as a food preservative for industrial purpose.

MATERIALS AND METHOD

Materials used for this study include:

Wistar strain rats (18), cages, sawdust, growers mash feed, water canes for water, silvano weighing balance, sodium citrate, electric weighing balance, distilled water, beaker, syringes and needles, chlorophorm, cotton wool, dissecting board, scissors, anticoagulant bottles, pins (4), hand gloves, knife holder handle four, surgical blade, a cooler kit.

Eighteen male wistar strain rats weighing 100g each were purchased and acclimatized to housing conditions for two weeks prior to the commencement of the experiment in the experimental animal house of the college of Health Sciences, University of Port Harcourt. The animals were housed six each in three separate cages under temperature 22 30^{O}C and a 12 light, 12 hours dark cycle. The animals were well fed with growers mash feed every day because they are carnivorous. Their weights were checked two times, first on their arrival time and secondly before the experimental time with silvano weighing balance which has to be at zero before weighing.

The rats were divided into three groups of six rats each. The first group (Group A) was given 10mg/kg body weight of sodium citrate for three days. The second group (Group B) was given 15mg/kg body weight of sodium citrate for three days. The third group (Group C) was given water and feed only, no administration of sodium citrate because they are on the control side. The three does were given at first day, two days interval and six days intervals to each rat in group A and B.

The rats were sacrificed two each both from group A, B and C on the first day, fourth day and eight days intervals and 2m/s of blood from each rat was collected for exterminations, through a dissection or an opening made at the upside down at the right axillary artery arm side of the rats. The blood samples were put into an anticoagulant specimen bottle which contains heparin to prevent the blood samples' from clotting and were examined through Neubauer counting chamber.

The rats' blood sample was diluted by washing 20µl of blood taken into a shell back pipette into 4.0ml of formal citrate to give a final dilution of 1 in 201. The diluted sample is then mixed and loaded into the counting chamber.

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When the cells have settled out of suspension, the number lying on 5 of the 0.04mm^2 areas are counted as visual red cell counts.

RESULTS

Table 1 shows the effect of sodium citrate on red blood cell count in the two groups: A and B. The table also indicates the mean concentration of the blood cell count of the control group. The results when subjected to statistical analysis using analysis of variance (ANOVA) revealed that there were significant differences in the concentration of red blood cell counts among the three rat groups ($P<0.05$) which were dose and duration dependent.

Table 1: Effect of sodium citrate on red blood cell counts in mature wistar strain rats (n=6)

Concentration	Mean red blood cell counts /L		
	Day 2	Day 6	Day 14
Group C control (Drug free)	$8.5x10^{11}$/L	$8.7x10^{11}$/L	$9.3x10^{11}$/L
Group A=C_1 10mg/kg n=6 Therapeutic dose	$7.8x10^{11}$/L	$9.1x10^{11}$/L	$10.9x10^{11}$/L
Group B=C_2 15mg/kg n=6 Double Therapeutic Dose	$8.7x10^{11}$/L	$9.3x10^{11}$/L	$9.6x10^{11}$/L

DISCUSSION

This study was carried out to provide the effect of sodium citrate on Red blood cell count in wistar rat. As shown in table 1 in this study, sodium citrate at the concentration used increased the concentration of red blood cell counts. This increase was statistically significant ($P<0.05$) for the three red blood cell counts concentrations at 2,6,14mg concentrations when compared with control group (Sodium citrate free).

Some research studies showed effect of sodium citrate on red blood cell while some did not. In Oopik et al (2003) study, there is an improved performance in well trained college runners after an ingestion of sodium citrate/kg body mass shortly before a 5km running time trial.

Also other research reveals that drinking fluids with higher sodium concentration (with sodium citrate) than in regular sports drinks, before exercise, can elicit a transient hypervolemic response that is partly preserved in exercise and is associated with improved physiological tolerance to exercise in warm conditions in trained males (Stacy et al 2007). Infact according to them pre-exercise ingestion of high sodium beverage increases plasma volume before exercise and involved less thermoregulatory and perceived strain during exercise and increased exercise capacity in warm conditions.

Also Mitchell et al (1990)found that in exercise performed at 80% VO2 max, intravenous with control conditions (no infusions), although only sodium bicarbonate infusion of both sodium bicarbonate and sodium chloride improved performance compared prevented the development of acidosis. In this case the ergogenic effect may be attributed to not only the enhanced buffering capacity of the body, but also to the increased plasma volume

resulting from the infusion of sodium containing fluids which would result in better perfusion of the exercising skeletal muscle. The precise mechanism by which sodium citrate ingestion before the 5km time trial improved performance in well trained college runners remains obscure. Ingestion of sodium citrate has been shown to increase blood, pH, HCO3 concentration and base excess (Linossier et al, 1997, Tiryaki et al, 1995).

CONCLUSION

In conclusion, sodium citrate increased the level of red blood cell counts. However, more studies are needed to determine the possible chronic effects of sodium citrate in rats' tissues.

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