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THE EFFECT OF SODIUM CITRATE ON HAEMOGLOBIN IN A WISTAR RAT

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ABSTRACT
The study was carried out to investigate the effect of sodium citrate on haemoglobin concentration in wistar rat, which could be useful for industrial preservatives purposes. Eighteen male matured rats of wistar strain (RattusNorvegicus), weighing between 100-150g and divided into three groups of six rats each, were used for the study. The first group (Group A) was given 10mg/kg body weight, the second group (Group B) was given 15mg/kg body weight, while the third group (Group C control) was given water and feed only. The rats were sacrificed on the 2nd-day, 6thday and 14th day following treatment, and the haemoglobin concentration was determined. Result obtained showed statistically significant differences (P<0.05) between all the three rat groups. In conclusion, sodium citrate significantly increased haemoglobin concentration.

INTRODUCTION
Heamoglobin (Hg) is the oxygen carrying pigment within the red blood cell. Its main function is the uptake and delivery of oxygen. It is a protein base component of red blood cells, which is primarily responsible for transferring oxygen from the lungs to the rest of the body. Haemoglobin is actually the reason red blood cells appear red, although oxygen rich blood is noticeably brighter than the depleted blood returning to the heart and lung. Abnormal haemoglobins are usually detected by change in charge or electrophoretic mobility (Pauling et al, 1949). In a condition known as Anaemia, there is deficiency of haemoglobin which can be caused by either too few red blood cells, or too little haemoglobin in cells.

Sodium citrate, being the sodium salt of citric acid with the chemical formula of $Na_3C_6H_5O_7$, is used as additives, preservatives and buffering agent. Research reveals that most additives are considered safe but some are known to be carcinogenic and even toxic. Preservatives also can cause

adverse reactions in children, and are potentially carcinogenic.

The accumulation of hydrogen ions is thought to cause fatigue during short term high intensity exercise (Green, 1995). There are several mechanisms by which an increase in acidity is believed to contribute to the onset of fatigue, including inhibition of key enzymes in the glycolytic pathway, and a reduction of centrality of the muscle fibres (Sutton et al, 1981). Some scientists suggest that a minimum productionof lactic acid and hydrogen ion may be needed during exercise for any significant effect of pre-exercise ingestion of alkalisers to be observed on the contribution of anaerobic glycolysis to overall energy generation in intensively working muscle (Ibanez et al, 1995). It was noted that in studies in which no effect was found after alkalosis, peak blood lactate under the placebo condition was lower (6mmol/l) than in the studies in which a significant metabolic effect was observed (9-18mmol/l).

Again, ingestion of sodium carbonate or sodium citrate has been found to have no effect on running time to exhaustion at various treadmill velocities. Although Schabart et al (2000) in their research did not find any effect of sodium citrate ingestion on performance in a 40km cycling time trial, Potteiger et al, (1996) reveal an ergogenic effect of sodium citrate ingestion on endurance performance capacity in trained subjects in cycle ergometer tests.

Hence, this study is carried out to investigate the effect of sodium citrate on Haemoglobin concentration of wistar rat owing to its widespread use as an additive in the industrial production of food and drinks.

MATERIALS AND METHOD

Materials used for this study include:
Distilled water, needle and syringe, cotton wool, dissecting set, pin (4), knife holder handle four, scissors, cages, water cane, silvano weighing balance, electric weighing, beaker, anticoagulant bottle, sawdust, wistar rat (18), foul mash feed, sodium citrate, chloroform, a dissecting board, hand gloves, surgical blade, drabkin'sfluid, caloric meter at a wave length of 540nm, blood sample, test tubes, haemoglobin pipette.

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 of animal (group A) was given 10mg/kg body weight of sodium citrate. This was dissolved in 2ml of distilled water. Two days after the administration two out of the six rats were sacrificed with the aid of chloroform anesthesia and blood sample was collected for examination. Two rats from the third cage (group C) which served as control (no administration of sodium citrate) weresacrificed also on the 2^{nd} day, the dose was repeated for the remaining four rats in the first cage (group A) that is 10mg each dissolved in 2ml of distilled water.Six days after the first administration two other rats

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were sacrificed leaving two rats in the cage (Group A) this was accompanied with two rats from cage C (Group C) control on the 6^{th} day, the dose was repeated for the remaining two rats and were sacrificed 14 days after the first administration. Two more rats were sacrificed from the control along side.

The second group of the animal (group B), six in number were given 15mg/kg body weight of sodium citrate dissolved in 2ml of distilled water. After two days of administration, two rats were sacrificed with the aid of chloroform anaesthesia and the blood sample was collected for examination that same day. The dose was repeated for the other four rats in the cages (Group B).Six days after the first administration, two other rats were sacrificed leaving two rats which were given the same dose of 15mg/kg body weight of sodium citrate. On the 14^{th} day after the first administration, the last two were sacrificed and blood sample was collected. These blood samples were collected from the axillary artery for all animals and the blood samples were put into an anticoagulant specimen bottle which contains heparin to prevent the blood sample from clotting and were examined thoroughly.

RESULTS

Table 1 shows the effect of sodium citrate on haemoglobin concentration in the two groups: A and B. The table also indicates the mean concentration of the haemoglobin 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 haemoglobin among the three rat groups ($P<0.05$) which were dose and duration dependent.

Table 1: Effect of sodium citrate on haemoglobin in mature Wistar strain rats (n=6)

Concentration Mg/kg Body weight	Mean Hb Concentration (g/dl)		
	Day 2	Day 6	Day 14
Group A 10mg/kg (n=6)	11.8g/dl	12.4g/dl	14.3g/dl
Group B 15mg/kg (n=6)	12.1g/dl	12.4g/dl	13.2g/dl
Group C Control (n=6)	10.3g/dl	10.7g/dl	10.9g/dl

$P<0.05$

DISCUSSION

A lot of additives, preservatives and buffering agentsused today in foods and drinks are known to be carcinogenic, even toxic and can cause adverse reactions in both children and adults. Hence, this study was carried out to investigate the effect of Sodium citrate on haemoglobin in wistar rat which could be usedas a preservative in industries. From the result of this study, sodium citrate at the concentration used increased concentration of haemoglobin. This increase was statistically significant ($P<0.05$) for the three haemoglobin concentrations at 2, 6, 14 days when compared with control group (absence of sodium citrate). While some studies show that sodium bicarbonate or sodium citrate have no effect on running time to exhaustion at various treadmill velocities, other studies show that the subjects are able to exercise for a longer duration on a cycle ergometer of their maximal oxygen uptake as a result of intravenous sodium bicarbonate infusion compared with a control condition.

However, other studies show an increased effect of sodium citrate ingestion on endurance performance capacity in trained subjects in cycle ergometer tests (Potteiger et al, (1996). This is related to this study that the sodium citrate concentration increase haemoglobin concentrations.

Recent studies have shown that sodium citrate and sodium chloride with moderate osmolality are effective in inducing hyperhydration and hypervolemia at rest (Greenleaf et al, 1997, Sanders et al, 2001). 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 increase plasma volume before exercise and involved less thermoregulatoryand perceived strain during exercise and increased exercise capacity in warm conditions.

CONCLUSION

In conclusion sodium citrate increased the level of haemoglobin concentration. However, more studies are needed to determine the possible chronic effect of sodium citrate.

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Short Communication
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