

Ferrous Sulphate Improves Electrolyte Levels in Phenylhydrazine Induced Hemolytic Anaemia in Wistar Rats

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Abstract

Electrolyte derangement is a common feature in anaemia. This study was undertaken to elucidate the effect(s) of ferrous sulphate on the renal handling of electrolytes. 28 male albino Wistar rats were randomly assigned to four groups of 7 rats each namely: the normal control given distilled water (10ml/kg body weight), ferrous sulphate administered ferrous sulphate (75mg/kg body weight), anaemic control given phenyl hydrazine (40mg/kg body weight) and the anaemic treated group given phenylhydrazine administered (40mg/kg body weight of phenyl hydrazine and 75mg/kg body weight) and ferrous sulphate. Results show that mean Na⁺ and Cl⁻ levels in the serum (P<0.001) and urine (P<0.01 and P<0.001 respectively) of the anaemic control group was significantly higher than control and ferrous sulphate groups. K⁺ concentration in the serum of AC was significantly (P<0.001) higher than that of control and FES but significantly (P<0.05) lower in urine of AC compared with control and FES. The bicarbonate concentration of the serum and urine of AC was significantly (P<0.05 and P<0.001 respectively) lower than the FES group only. The levels of Na⁺, Cl⁻, K⁺ and HCO₃⁻ in the AFES group were not significantly different from any of the groups. **Conclusion:** We conclude that ferrous sulfate does not only restore Hb but also kidneys ability to handle electrolytes.

Keywords: Phenylhydrazine, Hemolytic Anaemia, hyperkalemia, hyponatremia, ferrous sulphate.

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INTRODUCTION

Electrolytes such as sodium, potassium, chloride and bicarbonate comprise about 1% of plasma in blood. They are essential for intermediary metabolism and play an important role in controlling fluid levels, nerve conduction, acid-base balance, blood clotting and muscle contraction [1]. The balance of electrolytes in the body is essential for the normal functioning of cells and organs [1]. Therefore, any derangement in their concentration could lead to dire consequences.

Anaemia is a disorder that affects people of all ages, especially the elderly, young women of child bearing age and the infants [2]. It is of different types namely hemorrhagic, hemolytic and anaemia due to deficiency of any one of the vitamins or minerals required for the synthesis of red blood cells, (e.g. iron deficiency anaemia) [3]. Whatever the anemia type, there is usually some electrolyte derangement peculiar to the type of anaemia. Hemolytic anemia is the one of interest in our study.

Hemolysis is the excessive destruction of red blood cells within circulation before the expiration of their life span [4]. In humans, apart from the fact that it most often presents as anaemia, another very common feature that easily distinguishes it from other anaemia types is jaundice [4]. Other studies have shown that there is derangement of electrolytes in hemolysis. For example Berger [5], demonstrated that there is hyperkalemia (which is one of the most common features) in hemolytic anaemia. Also [6, 7] have also shown that hypobicarbonatemia is predominant in hemolytic anaemia. A couple of other studies have also shown one derangement or another in other renal function parameters like decreased glomerular filtration rate [8], albuminuria and proteinuria [9, 10].

From the foregoing, it is clear that hemolytic anaemia to some extent plays a role in the derangement of some renal function parameters such as glomerular filtration rate and its ability to handle electrolytes and some solutes. The kidneys are responsible for the regulation of electrolytes such as Na⁺, K⁺, Cl⁻ which maintain the osmolarity of the extracellular fluid [11] and HCO₃⁻ which through the bicarbonate buffer, plays

a role in acid base balance [12] Alterations in the concentration of any one of the above parameters usually have dire consequences.

Most common anaemias are treated with the use of hematinics and supplements most often geared towards replenishing iron loss as well as some of the other vital vitamins required for the synthesis of red blood cells. Other more complicated ones are additionally treated based on their causes. For example, hemolytic anaemia resulting from thalassemia, is treated by blood transfusion, administration of iron chelators etc [13].

Phenylhydrazine is used to stimulate hemolytic anaemia in laboratory animals. It causes hemolysis by generating reactive oxygen species in the form of hydroxyl radicals to which the fragile red cell membranes are susceptible. As a result, lysis occurs and within 48hrs, and hemolytic anemia sets in [5].

Ferrous sulphate is an anti-anaemic drug which is usually administered to treat iron deficiency anaemia [14] which is the most common cause of anaemia [15]. According to WHO, the prevalence of anaemia in the world is 24%, and this approximates to about 1620 million people [16]. Can ferrous sulphate also be used to control anaemia resulting from other causes other than iron deficiency? If so, can it reverse or at least improve the altered electrolyte levels? Is the electrolyte derangement secondary to hemolysis or the oxidative effect of phenylhydrazine?

The aim of our study is therefore to elucidate of the effect(s) of ferrous sulphate on renal handling of electrolytes in phenylhydrazine induced anaemic rats of the Wistar strain.

MATERIALS AND METHODS

Experimental Animals

A total of 28 male albino Wistar rats were purchased from the animal house of the Department of Physiology, Faculty of Basic Medical Sciences, University of Calabar. The rats were acclimatized for 7 days and maintained on normal rat chow and tap water *ad libitum*. Each animal was kept in a metabolic cage and they were maintained at an ambient temperature of 28-30 °C, and a light/dark cycle of 12/12 hours. The conduct of the experiments were approved and in accordance with the approved research guidelines on laboratory animal use of the Faculty of Basic Medical Sciences, University of Calabar, Calabar. All animals were humanely handled and their welfare respected throughout the study as stipulated in the 1964 Helsinki declaration and amended [17].

Experimental Design

After the one week acclimatization period, the animals were randomly divided into four (4) groups of

seven (7) rats each kept in different metabolic cages as follows:

Normal control group (NC): received normal rat chow + tap water + distilled water (at 10ml/kg body weight)

Hematinic (Ferrous Sulphate) group (FS): were fed normal rat chow + tap water + ferrous sulphate (using an oral gavage at 75mg/kg bodyweight).

Anemic Control Group (AC): was administered with Phenylhydrazine (PHZ) intraperitoneally at a dose of 40mg/kg of body weight + normal rat chow + tap water + distilled water (as in group one).

Anemic Treated Group (A_{FES}): Phenylhydrazine (PHZ) was administered intraperitoneally for two consecutive days to induce Anaemia in them at a dose of 40mg/kg body weight. Subsequently, they received normal rat chow + tap water + ferrous sulphate at 75mg/kg body weight

Induction of Anaemia

Before the induction of anaemia, Hb concentration was determined using Sahli's apparatus [18]. After this, anaemia was induced by injecting phenylhydrazine (PHZ) intraperitoneally at 40mg/kg bodyweight at 48hr intervals for two consecutive times [19; 20]. After the induction of Anemia, rats with Hb < 11.5g/dl were declared anemic [21].

At the end of 14 days, blood and urine samples were collected as follows:

Collection of Urine

24 hour urine samples were collected from each metabolic cage and put into urine sample bottles and stored in the freezer until when needed.

Collection of Blood Samples

The animals were anaesthetized using chloroform in an inhalation chamber with 4% isoflurane (IsoFlo, Abbott Laboratories, Berkshire, UK) regulated with a calibrated vaporizer [22]. Blood samples from each rat were collected via cardiac puncture into sample bottles. Serum was obtained by centrifuging clotted samples at 3000rpm for 10mins.

Determination of Electrolyte Concentration

The serum and urine samples were used for the estimation of serum electrolyte (Na⁺, K⁺, Cl⁻, HCO₃⁻) concentrations. Na⁺, K⁺ and Cl⁻ levels were measured using flame photometers [23], while HCO₃⁻ was measured using titration method [24].

RESULTS

Below is a graph showing the results of the comparison of hemoglobin concentration.

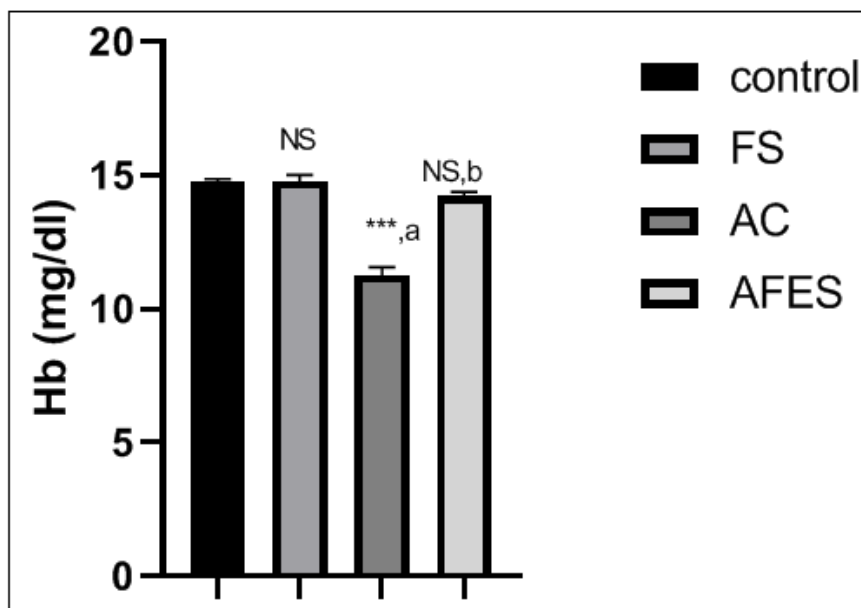


Fig-1: Comparison of hemoglobin concentration of the control, FS, AC and AFES groups

Table-1: Comparison of the mean concentration of Na⁺, Cl⁻, K⁺, and HCO₃⁻ in the serum of control, FS, AC and AFES groups

Parameter	Control	FS	AC	AFES
Na ⁺	141.36±1.42	142.16±0.63 ^{NS}	134.16±1.23 ^{***,c}	138.06±1.50 ^{NS,r,s}
Cl ⁻	110.13±0.52	109.13±1.66 ^{NS}	102.05±1.45 ^{***,c}	99.34±1.38 ^{NS,r,s}
K ⁺	4.51±0.51	4.45±0.61 ^{NS}	7.42±0.49 ^{***,c}	5.42 ±0.34 ^{NS,r,s}
HCO ₃ ⁻	24.16±0.49	24.20±1.32 ^{NS}	23.60±0.63 ^{NS,r}	10.50±0.22 ^{NS,r,s}

Key

NS; *,**,***=not significant; P<0.05; P<0.01; P<0.001 vs control
 r; d; c; a= not significant, P<0.05; P<0.01; P<0.001 vs FS
 s;b;z , x = not significant; P<0.05; P< 0.01, 0.001 vs AC

Table-1 shows the comparison of the mean concentration of Na⁺, K⁺, Cl⁻, and HCO₃⁻ in the serum of control, FS, AC and AFES groups.

There was no statistically significant difference in all the electrolytes between the ferrous sulphate (FS) group and the normal control (NC) groups.

Na⁺ concentration in the anaemic control (AC) group was significantly lower than the normal control (P<0.01) and FS (P<0.01) groups. There was no

significant difference in Na⁺ concentration when AFES was compared with NC, FS and AC groups.

Mean chloride concentrations followed the same trend as Na⁺.

Mean K⁺ concentration was significantly (P< 0.01; P< 0.05) higher in the AC group than NC and FS groups.

HCO₃⁻ levels in all the groups were not significantly different from each other.

Table-2: Comparison of the mean concentration of Na⁺, Cl⁻, K⁺, and HCO₃⁻ in the urine of control, FS, AC and AFES groups

Parameter	Control	FS	AC	AFES
Na ⁺	28.18±1.42	27.16±0.63 ^{NS}	35.15±1.03 ^{***,a}	32.15±1.50 ^{NS,d,s}
Cl ⁻	20.13±0.21	21.10±1.59 ^{NS}	30.05±1.45 ^{***,c}	26.33±0.37 ^{*,r,b}
K ⁺	33.13±0.51	34.45±1.21 ^{NS}	28.56 ±1.10 ^{*,d}	30.15±0.34 ^{NS,r,s}
HCO ₃ ⁻	13.16±0.42	13.01±0.32 ^{NS}	10.28±0.63 ^{NS,c}	11.50±0.22 ^{NS,r,s}

Key

NS; *,**,***=not significant; P<0.05; P<0.01; P<0.001 vs control
r; d; c; a = not significant, P<0.05; P<0.01; P<0.001 vs FS
s, b = not significant; P<0.05 vs AC

Table-2 shows the comparison of the mean concentration of Na⁺, Cl⁻, K⁺, and HCO₃⁻ in the urine of control, FS, AC and A_{FES} groups.

There was no significant difference in all the electrolytes whe FS was compared with the NC.

The mean Na⁺ concentration in the urine of the AC group was significantly (P<0.01 ; P<0.001 respectively) higher than that of the control and FS groups. That of the A_{FES} group was significantly (P<0.05) higher than the FS group but was not significantly different from the AC and control groups.

Chloride level in the urine of A_{FES} group was significantly (P<0.05) higher than the NC and FS. There was no significant difference between A_{FES} and AC groups.

K⁺ levels in the urine of AC and group was significantly (P<0.05) lower than those of NC and FS groups. There was no significant when A_{FES} was compared with all the groups.

No significant difference in HCO₃⁻ urine levels were seen in the AC group when compared with AFES. However, there was a significant (P<0.001 and 0.01 respectively) decrease in mean urine bicarbonate concentration of AC group compared with NC and FS.

DISCUSSION

Ferrous sulphate is an anti-anaemic drug which is often used to treat iron deficiency anaemia. Iron supplements are usually administered to treat iron deficiency anaemia particularly in chronic diseases such as kidney failure [25], heart failure [26] or inflammatory bowel disease [27]. If RBC destruction rate is high enough to determine a decrease in hemoglobin values below the normal range, hemolytic anemia occurs [28]. Hemolytic anaemia is also characterised by electrolyte level derrangement [29, 30]. This study was undertaken to find out whether ferrous sulphate can reverse the low Hb, RBC count as well as the enhance the renal handling of electrolytes.

This study shows that after inducing anaemia, the Hb and red blood cell count reduced by 34% in the AC group. But when ferrous sulphate was administered, the anemia was reversed, for there was only 5% decrease when this group was compared with the normal control (NC) and about 29% increase when compared to the AC group. This shows that ferrous sulphate does not only improve on red cell parameters

in iron deficiency anemia but also in hemolytic anaemia.

The sodium, chloride, potassium and bicarbonate levels in the AC group are typical of hemolytic anaemia irrespective of the cause [29, 30, 31].

The hyponatremia seen in this study is also seen in iron deficiency anemic patients as shown in a study by [1]. In his study, there was hyponatremia, hyperkalemia and hyperchloridemia in iron deficiency anemic patients. In the present study on animals induced with hemolytic anemia, there was hyponatremia and hyperkalemia but the chloride levels however followed the same trend as the sodium levels. The results of this study are in line with our previous study [29] where chronic consumption of oxidized palm oil resulted in hemolytic anemia.

However, administration of ferrous sulphate in the A_{FES} group improved the serum concentrations of sodium, chloride, and potassium towards normal. The urine concentration of the above electrolytes were also restored towards normal. We thought that the derrangement in electrolyte levels secondary to the anemic state had nothing to do with the direct oxidative effects of phenylhydrazine on the kidneys .

The results of the present study show that ferrous sulphate did not only improve the Hb concentration but also restored serum electrolyte levels. Some previous studies have shown that the low sodium and high potassium levels seen in our study are peculiar to hemolytic anaemic states [30]. One would have expected that the hyperkalemic state would lead to increased excretion of potassium. In this study, however, the potassium excretion was significantly reduced in AC compared to the NC and FS groups. Studies have shown that the reduced excretion of potassium despite high concentrations in serum may be as a result of one or more of three abnormalities: decreased distal delivery of Na⁺, mineralocorticoid deficiency and/or abnormal cortical collecting tubule function [32]. [33], 1994 had shown that chronic consumption of thermoxidized palm oil, which leads to hemolytic anemia [34] also caused destruction of the kidneys. In our previous study [35], we showed that the chronic consumption of thermoxidized palm oil led to reduced GFR which could result to reduced distal delivery of sodium. From the foregoing, the reduced potassium excretion may have resulted from either one of the above reasons outlined by [32].

The most important finding however is that the electrolyte levels were restored by the administration of ferrous sulphate. This could mean that it is either that the deranged state of the electrolytes in the serum was temporary due to the acute hemolytic anaemic state or the ferrous sulphate was able to restore the kidneys function.

CONCLUSION

Ferrous sulphate alleviated the hemolytic anaemic state in rats and also restored the deranged electrolyte concentrations in the ECF. Therefore, the electrolyte derangement is secondary to hemolytic anemia.

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