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Full Length Research Paper

Ameliorative effects of *Cnidoscolus aconitifolius* on anaemia and osmotic fragility induced by protein-energy malnutrition

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This study was designed to evaluate the ameliorative effect of dietary supplementation of *Cnidoscolus aconitifolius* leaf on anaemia and changes in erythrocyte osmotic fragility in protein energy malnourished rats. Protein energy malnutrition has been associated with anaemia and changes in osmotic fragility, deformability and lifespan of erythrocytes. In this study, protein-energy malnutrition induced in weanling male Wister rats by feeding them low protein diet for 3 weeks was associated with significantly reduced (P<0.01) haematological indices: packed cell volume (PCV), red blood cell count (RBC), white blood cell count (WBC), platelet counts and mean corpuscular count (MCV). It was also associated with increased erythrocyte osmotic fragility at 0.5- 0.7% NaCl concentration. Upon introduction of recovery diets containing 20% soya protein or 20% *C. aconitifolius* in place of soya protein or 10% soya proteins with 10% *C. aconitifolius* or commercial rat feed for 4 weeks, the recovery diet containing 10% soya and 10% *C. aconitifolius* caused the most significant (P<0.01) increase in platelet count, WBC, MCV, MCH and MCHC when compared with all the other treated groups and the malnourished group. The group fed with 20% *C. aconitifolius* in place of 20% soya protein also caused the most significant elevation in RBC, PCV and Hb compared with the malnourished group. The effects of diet containing 20% soya protein and commercial feed on PCV, Hb, platelet and all other haematological indices were not significantly different from each other (p<0.05). On osmotic fragility, the recovery diet containing a mixture of 10% *C. aconitifolius* and 10% soya protein produced the highest reduction of osmotic fragility, followed closely by the diet containing 20% *C. aconitifolius* which produced greater effects than the feed containing only soya meal as the protein source or commercial rat feed which produced the lowest reduction in osmotic fragility. From the results of this study, it can be deduced that *C. aconitifolius* has haematopoetic property and by reducing osmotic fragility in protein energy malnutrition, it can increase the life span of erythrocytes.

Key words: *Cnidoscolus aconitifolius*, anaemia, osmotic fragility, protein energy malnutrition.

INTRODUCTION

Protein energy malnutrition (PEM) is a common condition in most developing countries especially in Africa including Nigeria (Nnakwe, 1995), Senegal (Idohou-Dossou et al., 2003) and in most of the war ravaged countries such as Somalia and Sudan in Africa and Thailand in Asia. It occurs as a result of lack of quality protein food, poverty, faulty weaning process, poor sanitary conditions and malnutrition in general. Chronic PEM has been shown to have short-term and long term effects such as growth retardation, lowered resistance to infection and increased mortality rates in young children (WHO, 2000). PEM has also been reported to be associated with anaemia (Borelli et al., 2007) and decreased osmotic fragility (Kaplay, 1978, 1984, Ramanadhan and Kaplay, 1982). The anaemia is due to reduced erythropoiesis (Borelli et al., 2007) and/or decreased red cell survival (Brown et al., 1978). Changes in osmotic fragility have been attributed, *in vitro*, to cholesterol enrichment of the erythrocytes membrane, lipid peroxidation (Fondu et al., 1980) and other changes

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Table 1. Composition of PEM diet.

Component	Quantity (g)
Soya meal	40
Corn starch	820
Veg. Oil	80
Vitamin	60
Total	1000

Table 2. Composition of recovery diets.

Component	Group A	Group B	Group C
Soya meal (g)	100	-	200
Corn starch (g)	660	660	660
Vegetable oil (g)	80	80	80
Vitamin (g)	60	60	60
C. aconitifolius (g)	100	200	-

Note: Group D received commercial feed containing 21% crude protein.

in the lipid composition of the membrane (Kanakaraj and Megha, 1989). The changes in lipid composition were earlier investigated by Kuypers et al. (1985). They discovered that replacement of phosphatidyl choline in rabbit and horse erythrocytes membrane with 1,2-dipalmitoyl phosphatidyl choline up to about 20% led to increased osmotic fragility and general change in shape of the erythrocytes.

In vitro generation of free radicals especially peroxyl radicals have also been shown to produce peroxidation of unsaturated bonds in the erythrocytes membrane (Fondu et al., 1980; Brzenska-Slebodzinska, 2001), thereby resulting in increased osmotic fragility. This was further corroborated by Meurs et al. (2005) in their findings that disruption of cholesterol-phospholipids ratio due to deficiency of scavenger receptor class B type I (SR-BI), a multifunctional receptor that promotes selective uptake of cholesteryl esters from high density lipoprotein (HDL) results in increased osmotic fragility, decreased deformability and life span of erythrocytes.

Cnidoscolus aconitifolius is a perennial shrub belonging to the family *Euphorbiaceae*. It is commonly found in the tropic and sub tropical regions world wide, including Africa, south of Sahara, North and South America, India, etc. It is commonly eaten as vegetable in soup in South Western Nigeria where it is called Iyana Ipaja (Ganiyu, 2005). High fibre content and antibacterial activities of this plant have been reported (Sarmiento-Franco et al., 2003; Awoyinka et al., 2007). However, the possible beneficial effects of *C. aconitifolius* on protein energy malnutrition have not yet been reported. This research was therefore designed to investigate the effect of dietary supplementation of leaves of this plant on anaemia and changes in erythrocyte osmotic fragility associated with PEM induced in rats.

MATERIALS AND METHODS

Plant materials

C. aconitifolius leaves were harvested fresh between October and December, 2007 in Ibadan, Oyo State, Nigeria and was identified at the herbarium of the Department of Botany and Microbiology, University of Ibadan. The leaves were picked cleaned and air dried at room temperature. The dried leaves were macerated to fine powder and preserved in sealed polythene containers at 4°C before use.

Animals and experimental design

Forty weanling male albino rats (*Rattus norvegicus*) of the Wistar strain obtained from the experimental animal unit of the Department of Veterinary Physiology, Biochemistry and Pharmacology, University of Ibadan were used for this investigation. Protein Energy Malnutrition (PEM) was induced in thirty two (32) rats by feeding them with low protein diet according to the method of Adelusi and Olowookere (1985) for fourteen days. The composition of the diet is as shown in Table 1. These PEM rats were then divided into four groups A to D and fed with recovery diets containing various proportions of air dried *C. aconitifolius* leaves *ad libitum* for twenty eight days. Group A received feed containing 10% *C. aconitifolius* leaves by weight and 10% soya meal, group B received 20% *C. aconitifolius* and no soya meal, group C received feed containing 20% soya meal while group D received commercial feed obtained from Vital Feeds Ltd. As shown in Table 2.

Blood sample collection

At the end of induction of PEM and at the end of rehabilitation with test diets containing *C. aconitifolus*, blood was collected from retro-orbital venous plexus with capillary tubes into heparinized bottles and used for haematological and osmotic fragility studies.

Haematological studies

Red blood cells (RBC) and white blood cells (WBC) were counted with haemocytometer. Packed cell volume (PVC) was estimated using the micro-haematocrit method (Cole, 1974). Haemoglobin concentration (Hb) was determined by the standard cyanmethaemoglobin method of Jain (1986). The values of mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) were calculated as described by Jain (1986). Blood smears were stained with Giemsa stain for differential WBC count (Gueye et al., 1988).

Osmotic fragility test

Erythrocyte osmotic fragility was determined by diluting 0.02 ml of blood in test tubes containing 0.0 to 0.9% NaCl in 1% phosphate buffer, mixed gently and kept at room temperature (29°C) for 30 min. It was then centrifuged at 3000 rev/min as described by Oyewale (1992). Tube with the highest haemolysis was assumed to be having maximum (100%) haemolysis.

Statistical analysis

The data were expressed as Mean ± standard error of means (SEM). The test of significance between groups was determined by the student t-test at $P < 0.05$ (Bailey, 1992).

Table 3. Effects of recovery diet on erythrocyte indices in PEM rats before and after recovery diets containing *C. aconitifolius* and soya meal.

Group	PCV (%)	Hb (g/dl)	RBC (x 10^6 /µL)	Platelet (x10^3)	MCV (Fl)	MCH (g/dl)	MCHC (%)
Normal control	49.00±4.83 ^{b**}	11.10±0.78	7.00±0.21 ^{b**}	809.60±68.50 ^{b**}	69.00±3.96 ^{b*}	18.40 ±0.90 ^{b*}	26.40 ± 1.62 ^{b**}
PEM Control	29.00±2.16^{b**}	10.03±1.28	4.80±0.74^{b**}	406.30±54.39^{b**}	60.50±6.80^{b*}	22.00±3.22^{b*}	34.50 ± 2.51^{b**}
PEM+Com. feed	36.40±7.40^{d**,c**}	11.60±2.69^{c*,d*}	6.55±0.55 $^{b**, d*}$	115.12±15.96^{c**}	55.29±7.26 $^{d*, b*, c**, d**}$	17.61± 2.91 ^{c**,d*}	31.72±1.37
PEM + 20% soya	36.00±7.37	12.64±2.46	8.62±1.35 ^{b**,}	116.80±11.37	43.44±3.00 ^{b*}	15.27± 4.94	34.61± 5.44
PEM +10% soya, 10% *Ca*	42.00± 8.86 ^{c**}	16.20±2.60 ^{c*}	5.63± 0.93	422.80±27.48^{c**}	72.44± 19.85^{c**}	29.25± 6.23 ^{c**}	29.25± 6.23
PEM+ 20% *Ca*	52.00±8.80^{d**}	19.40±5.50^{d*}	7.92±1.14 ^{d*}	109.60±27.90	67.12±15.21^{d**}	22.00±3.22^{d*}	34.50±2.51

Com. Feed = Commercial feed; *Ca* = *Cnidoscolus aconitifolius*.
Values are mean ± S.D.
Different superscripts within each column are statistically significant.
* = P < 0.05, ** = P < 0.01.

Table 4. Effects of recovery diets containing *C. aconitifolius* and soya meal on total and differential WBC counts.

Group	WBC(10^3 /µL)	Neut. (10^3 /µL)	Lymphocytes (10^3 /µL)	Mono. (10^3 /µL)	Eos. (10^3 /µL)
Normal control	5.00±0.69 ^{b**}	33.00±8.41 ^{b**}	64.90±12.46	-	2.50±1.30
PEM Control	3.00±0.83^{b**}	11.20 ±3.43 ^{b**}	88.80±3.43	-	-
PEM + Com. Feed	9.28±1.58 ^{b*,d*}	42.20±2.68^{c**,d*}	62.80±2.17	1.40 ± 0.55	1.50 ±0.84
PEM + 20% Soya	7.48± 0.65 ^{b*}	44.00±7.91	55.20 ± 7.53	1.25 ± 0.85	1.00 ±0.52
PEM +10% Soya, 10% *Ca*	9.54± 0.17	28.40±4.16 $_{c}$ **	69.60± 6.65	1.30 ± 0.40	1.70±0.81
PEM + 20% *Ca*	6.16±2.56 ^{d*}	35.00±3.74^{d*}	63.20± 3.56	1.30 ± 0.40	1.70 ±0.81

Com. Feed = Commercial feed; *Ca* = *Cnidoscolus aconitifolius*.
Values are mean ± S.D.
Different superscripts within each column are statistically significant.
* = P < 0.05, ** = P < 0.01.

RESULTS

Effects of protein deficient diet in inducing PEM in rats

The effects of protein deficient diet on haematological parameters are shown in Table 3. The result showed that the diet caused significant decreases (p<0.05) of 48.8, 31.4 ,40.0 and 19.6% in the values of PCV, RBC, WBC and Platelet count, respectively, when compared to values of these parameters in the control rat fed on standard laboratory diet. The MCH and MCHC values of protein deficient rats also increased significantly (P<0.05) compared to the normal controls. The decrease in haemoglobin concentration (Hb) observed in protein deficient rat was not statistically significant (p< 0.05).

Effect of recovery diets on PEM rats

Table 4 shows the effect of feeding PEM rats with recovery diets for 28 days on haematological parameters. All the diets caused significant improvement in the blood indices studied. However, rats fed with diet containing 20 and 10% *C. aconitifolius* had the highest increases of 79.3 and 44.8%, respectively in PCV and 93.4 and 61.5% in Hb concentration when compared with values in the protein deficient control. Rats fed with 20% soya protein supplemented diet and those on commercial feed had increases of 25.0 and 25.5% in PCV, respectively. The diet containing 10% *C. aconitifolius* and 10% soya protein caused the most significant elevation in the levels of WBC,

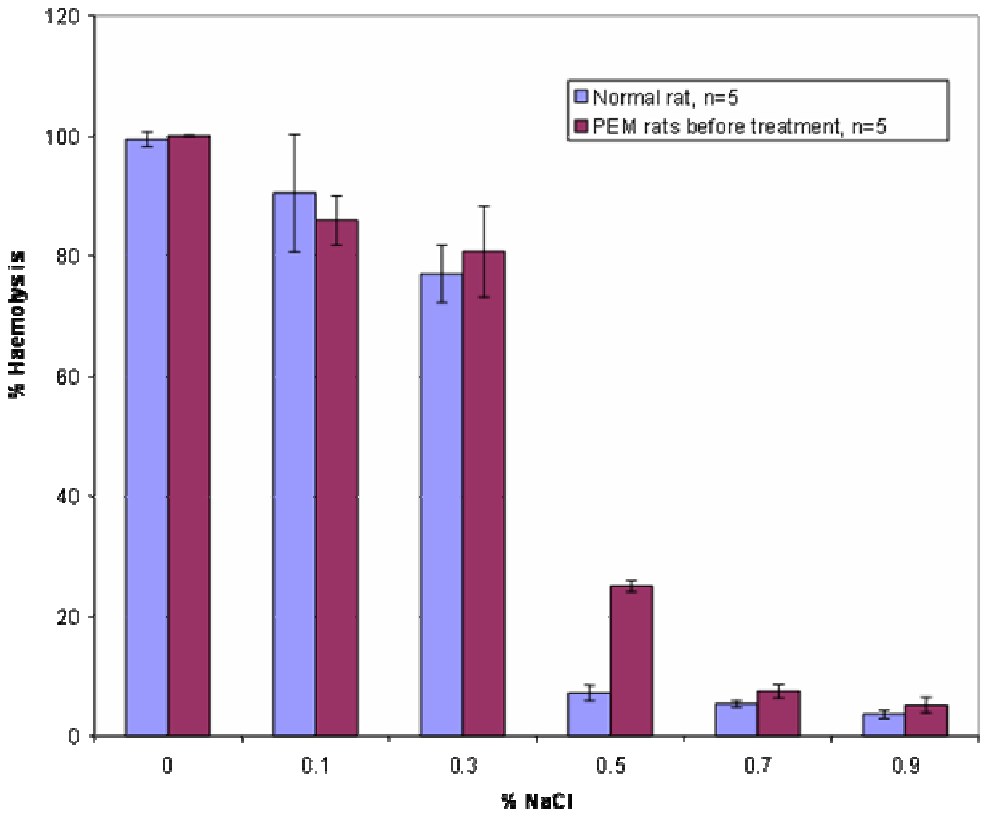

Figure 1. Erythrocyte osmotic fragility of normal and PEM induced rats.

platelet count and MCV compared with all the other treatment groups. The response of the PEM rats on 20% soya protein and commercial feed was not significantly different in most of the parameters studied.

Effect of protein deficient diet and recovery diets on erythrocyte osmotic fragility

As shown in Figure 1, the erythrocytes osmotic fragility was higher in PEM rats than in normal rat with particular statistical significance at 0.5% NaCl ($P<0.01$), 0.7% NaCl ($P<0.01$) and at 0.9% NaCl ($P<0.05$). Following introduction of recovery diets (Figure 2), the erythrocytes osmotic fragility was significantly reduced at 0 .1 to 0.5% NaCl ($P<0.01$) in the group that received 10% soya meal and 10% *C. aconitifolius*. But at 0.7 to 0.9% NaCl concentration, the osmotic fragility was higher than those obtained in PEM rats at the same NaCl concentration. Of all the various recovery diets, reduction in osmotic fragility was significantly higher ($P<0.01$) in the group which consumed 10% *C. aconitifolius* and 10% soya meal than in the other three groups. This was closely followed by the group that consumed 20% *C. aconitifolius* leaves with reduction in osmotic fragility at 0.1, 0.3, 0.7 and 0.9% NaCl greater than that of group on 20% soya meal ($P<0.05$) and group on commercial feed ($P<0.01$). The least effects were seen in group D rats that consumed commercial feed.

DISCUSSION

Anaemia is one of the common complications of protein-energy malnutrition (Borelli et al., 2007). Other symptoms of PEM include oedema, diarrhoea, weight loss, alopecia, retinopathy and opportunistic infections. The results of this study showed that anaemia with reduced RBC, PCV and Hb concentration developed in the protein malnourished rats. The results also indicate leucopenia and thrombocytopenia with increased neutropenia and lymphocytic infiltration in malnourished rats compared with the normal control.

This study showed that the PCV and Hb values of malnourished rats that received 20 and 10% *C. aconitifolius* leaves meal were boosted more than those that received 20% soya meal or commercial feed 28 days post treatment of malnutrition. This elevation in the PCV and Hb strongly indicate the haematinic property of *C. aconitifolius*. The fact that the effect of *C. aconitifolius* leaves meal was also concentration dependent would seem to confirm this haematinic property.

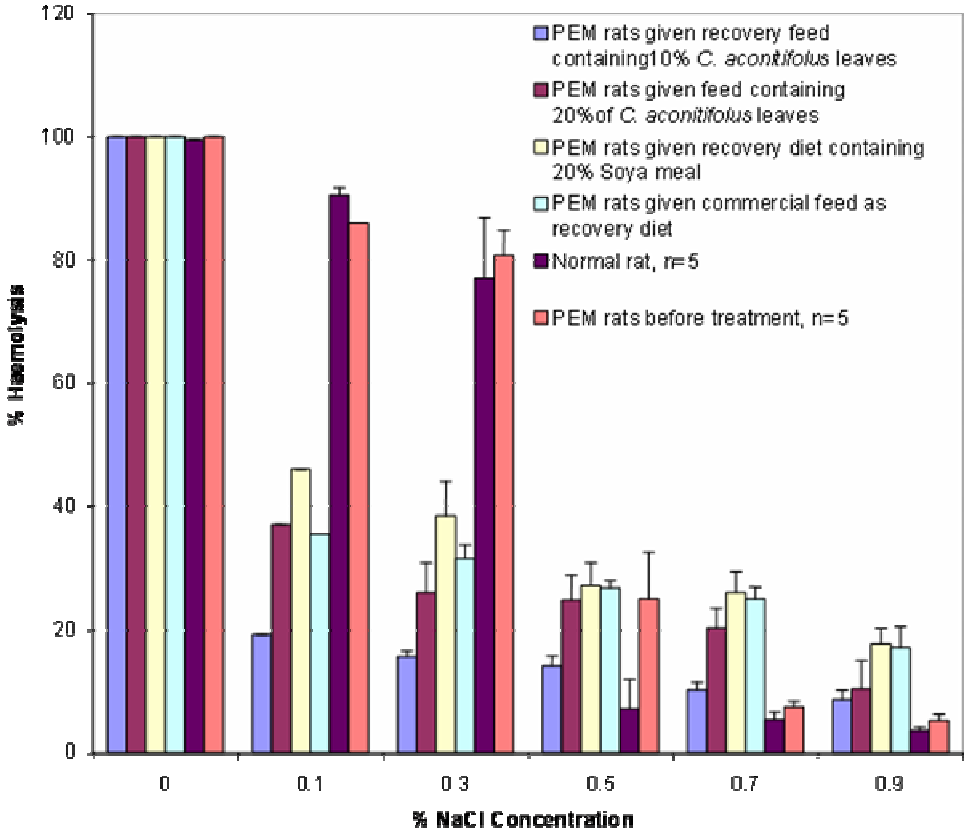

Figure 2. Comparition of erythrocyte osmotic fragility in normal and PEM rats before and after.

Analysis has shown that *C. aconitifolius* contains various substances such as iron, vitamins and protein (Kuti and Kuti, 1999). The haematinic property of this plant may therefore be due to the presence of the constituents in the plant. Result of this work is in line with earlier reports of haematinic potential of plants (Agbor and Odetola, 2001; Alada, 2000). It also strengthens the beneficial effects of various plants as sources of protein (Castellanos et al., 1994; Fasuyi et al., 2000; Agbede, 2004; Fasuyi and Aletor, 2005; Fasuyi et al., 2007).

The significant elevation of WBC counts in the treatment groups is indicative of cell-mediated immune response to opportunistic infections which might have resulted from malnutrition. The results of this study showed that the group on 20 and 10% *C. aconitifolius* leaves meal diets had significantly higher values of MCV than the groups on 20% soya meal and commercial feed which is an indication of increased reticulocytosis in these groups since it is known that reticulocytosis leads to an increase in the MCV. In addition, since it is known that macrocytic erythrocytes of severe acute anaemia in remission have a greater quantity of haemoglobin on a weight basis (MCH) than erythrocytes that are a product of normal maturation, the observed elevated values of MCH in groups on 20 and 10% *C. aconitifolius* leaves meal above those in the 20% Soya meal and commercial feed suggests that the groups on *C. aconitifolius* leaves meal are under going higher remission than those on 20% soya and commercial feed. The possibility of higher reticulocytosis and remission is also reflected in the reduced osmotic fragility of the erythrocytes of rats on the *C. aconitifolius* leaves meal diets compared to those on 20% soya and commercial feed.

From the result obtained before introduction of recovery diets, the erythrocytes osmotic fragility in protein energy malnutrition rats was higher than in normal rats. This is contrary to the reports of Kaplay (1978, 1984) and Ramanadhan and Kaplay (1982). The osmotic fragility however reduced considerably, after introduction of recovery diets that were rich in protein as earlier reported in PEM (Brown et al., 1978). However, of all the recovery diets introduced, the combination of *C. aconitifolius* and soya meal produced the best result in reduction or restoration of erythrocytes osmotic fragility. This was closely followed by total or complete use of *C. aconitifolius* leaves as the only protein supplement, and lastly the use of soya alone as protein source.

The results of this study therefore, suggest that *C. aconitifolius* dietary supplementation has potential haematinic property and could be of immense benefit as a dietary supplement to alleviate anaemia due to protein energy malnutrition. Furthermore, apart form its protein

contents, it can be deduced from this experiment that *C. aconitifolius* possesses some active materials that confer resistance to osmotic lysis and stability to red cell membrane. This is however open for further investigation.

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