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Proximate composition, phytochemical screening and anti-anaemic effect of *Aloe barbadensis* (*Aloe*) leaves in nutritionally stressed rats

Anthony U. Osagie[1] and Ehimwenma S. Omoregie[2*]

[1]*Biochemistry Unit, Department of Basic and Applied Sciences, Benson Idahosa University, Benin City. Nigeria.*
[2]*Department of Biochemistry, Faculty of Life Sciences, University of Benin, P.M.B. 1154 Benin City. Nigeria.*

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Abstract
In this study, the phytochemical screening and anti-anaemic effect of *Aloe barbadensis* leaves was investigated in nutritionally stressed rats. Preliminary phytochemical screening test of the plant revealed that it contained tannins, flavonoids, anthraquinones, saponins, alkaloids and cardiac glycosides. Sets of male albino rats of the Wistar strain, weighing between 100 and 105g, were fed on normal diet (20% protein) and low protein diet (2% protein) supplemented with the plant leaves (10% of diet) for ten (10) weeks. The results revealed that rats fed on the protein deficient diet supplemented with the leaves (PDDV) had significantly ($p < 0.05$) increased packed cell volume (PCV), haemoglobin (HB), red blood cells (RBC), platelets, total protein and albumin levels when compared with the protein deficient diet fed rats (PDD – negative control) which had significantly reduced ($p < 0.05$) levels of these parameters when compared with the normal diet fed rats (ND – positive control). The histopathological examination of the liver and kidney tissues showed evidence of severe fatty changes especially in the PDD fed rats whereas the PDDV fed rats showed moderate fatty changes relative to the control. These findings confirm local claims on the efficacy of *A. barbadensis* leaves in the treatment of anaemia as a result of its bioactive principles.

Keywords: *Aloe barbadensis*; Nutritionally-stressed; Anaemia; Protein energy malnutrition; Phytochemicals.

INTRODUCTION

Aloe barbadensis, a member of the Liliaceae family, is native to Africa but commonly cultivated worldwide. Its local names are *Aloe, Aloe vera*, 'silent healer', heaven's blessing'. It is a perennial drought-resisting succulent (cactus) plant, which historically has been used for a variety of medicinal purposes. The plant has stiff grey-green, lance-shaped leaves containing a clear gel known as aloin in a central ungelatinous pulp. Clinical evaluations have revealed that the pharmacological active ingredients of the plant are concentrated in both the gel and rind of the leaves. These active principles have been shown to possess analgesic, hypotensive, anti-microbial, immunomodulatory, anti-inflammatory activities (Sivagnanam, 2003). The clear gel is an effective healer of wounds and burns, while the aloin is useful in the cosmetics industry and also as a strong laxative for short-term constipation.

Anaemia is a disease with serious challenge to human health particularly due to poor nutrition status of the populace (Mbaka and Owolabi, 2011). The incidence of anaemia in protein energy malnutrition (PEM) is well documented in the literature (Adelusi

* Corresponding author: ehiomoregie@yahoo.co.uk; 08023397020

and Olowookere, 1985; Omoregie and Osagie, 2002; Omoregie and Osagie, 2007; Omoregie and Osagie 2011). In PEM, anaemia is characterized by changes in haematological system resulting in non-adaptive anaemia which cannot be explained only by iron and vitamin deficiencies. Nevertheless, the red cells may be normocytic and hypochromic with reduced red cells indices (El-Nawawy *et al*., 2002; Omoregie and Osagie, 2007).

Medicinal plants have been used for decades as therapeutic agents in the treatment and prevention of different diseases due to their easy access, low toxicity and for economic reasons (Ogbonnia *et al*., 2010; Saha *et al*., 2011). They also contribute predominantly to the healthcare system especially among the rural populace (Mbaka and Owolabi, 2011). The therapeutic values of these plants are linked to their biologically active secondary metabolites such as flavonoids, alkaloids, tannins, polyphenols, saponins, etc. These plants' bioactive agents have been reported to possess diverse biological roles in combating diseases (Edeoga *et al*., 2005; Mishra *et al*., 2012).

The local treatment of anaemia using herbal remedies with haematinic potential is very popular in most developing countries. This may be due to their easy access and low cost. Based on local claims on the anti-anaemic activity of *Aloe barbadensis*, this study serve to evaluate the proximate composition, phytochemical screening and anti-anaemic effect of *Aloe* plant in nutritionally stressed rats by using a protein deficient diet.

EXPERIMENTAL

Plant material. *Aloe barbadensis* leaves were collected from a private farm in Ugbowo area, Benin City, Edo State, Nigeria. The leaves were identified and confirmed by a Botanist at the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria. Sample of the plant was kept in the herbarium. The leaves were pooled, washed with distilled water and air-dried at room temperature, away from the sun. The dried leaves were then pulverized and used for the animal feeding study.

Proximate composition. The moisture, ash, fibre and protein content of the plant leaves were determined by A.O.A.C. (1990) method. The crude carbohydrate content was determined by Dubois *et al* (1956) method. The mineral content of the leaves including zinc, magnesium, calcium, iron, and phosphorus were estimated by atomic absorption spectrophotometer. Sodium and Potassium content were determined by flame emission photometry (Isaac and Kerber, 1972).

Phytochemical screening. Standard phytochemical screening techniques were used to detect the presence of alkaloids, tannins, flavonoids, cardiac glycosides, anthraquinones and saponins (Harborne, 1992; Evans, 1996).

Animal feeding experiment. Male albino rats of the Wistar strain weighing between 100-105g were placed on the experimental diet (Table 1) which included a normal diet (ND) and a protein deficient diet (PDD) as previously described in the literature (Adelusi and Olowookere, 1985; Omoregie and Osagie, 2002). The animals were divided into six groups, containing eight animals per group. Group 1 represents the positive control and was fed the normal diet (ND) with adequate protein (20 %). Group 2 and 3 were placed on the normal diet supplemented with 10 % pulverized leaves (NDV) and 130mg per kg body weight ferrous iron salt (Fesolate®) tablet (NDF), respectively. Group 4 animals served as the negative control and were placed on the protein deficient diet (PDD) containing only 2% protein. Whereas, groups 5 and 6 were given protein deficient diet supplemented with 10 % of the

pulverized leaves (PDDV) and 130mg per body weight ferrous iron salt (Fesolate®) tablet (PDDF), respectively. The animals in each group were fed their respective diets for ten weeks with free access to food and water throughout the duration of the study. The body weight, food intake and faecal output were recorded weekly. The ferrous salt served as the reference control drug. The animals were treated in conformity with internationally accepted guidelines for animal use and care (EEC Directive of 1986; 86/09/EEC; National Institutes of Health Publication 85-23, revised 1985).

Collection of blood samples. The animals were fasted overnight and sacrificed through cervical dislocation. Part of the blood was collected from the heart into EDTA tubes for analysis of red cell indices. Another set of heparinized tubes were used to collect blood for biochemical analysis. The heparinized blood was centrifuged, the plasma collected and used for biochemical analyses. The heart, kidney, liver and spleen were removed at once, blotted dry and weighed.

Preparation of tissue homogenates. 1.0g of the liver tissue was homogenized in 10ml of ice-cold normal saline (0.9%) to obtain 10% (W/V) homogenate. The homogenate was centrifuged at 5000g for 10 minutes and the supernatant obtained was used for determination of catalase (CAT), vitamins C and E.

Biochemical analysis. Packed cell volume (PCV), haemoglobin (HB), white blood cell (WBC), platelet (PLT) counts were estimated by standard procedures (Dacie and Lewis, 2001). Total cholesterol, HDL-cholesterol, LDL cholesterol, VLDL cholesterol and triacylglycerols were estimated using sigma diagnostic kits (Sigma Diagnostics, St. Louis, MO, USA). Plasma triacylglycerol was determined based on an enzymatic procedure by Trinder (1969). Plasma total cholesterol was estimated by an enzymatic endpoint method of Trinder (1969) and Richmond (1973). HDL cholesterol was estimated by the precipitation method of Lopes-Virella *et al*., (1977). Plasma total protein was estimated by Biuret method (according to Henry *et al*., 1957). Plasma albumin was determined by the bromocresol green (BCG) dye binding method (Doumas and Briggs, 1972). Catalase activity was estimated as residual H_2O_2 after incubation with the enzymes (Kaplan and Groves, 1992). The vitamin C content of the tissue was determined according to the method of Roe and Kuether, 1943. Vitamin E content was estimated by the Desai (1984) method.

Histopathology. The excised liver and kidney were immediately stored in 10% formol saline. All stained tissues were examined microscopically using the Leitz Watzler and Wild Heerbrugg M3 stereomicroscope for photographic documentation of all findings (Humason, 1962).

Statistical analysis. The data were expressed as means ± standard error of mean. Results were analyzed statistically by one-way Analysis of variance (ANOVA), using Graph Pad Prism version 4 software, to compare all columns. Turkey's Test was employed to determine the significant difference between the means with P values < 0.05 considered statistically significant.

RESULTS

Figure 1 presents the proximate composition of *Aloe barbadensis* leaves (g/100g dry weight) revealed that it contains moisture (90.3 ± 0.45), ash (7.0 ± 0.15), crude protein (2.4 ± 0.10), carbohydrate (3.0 ± 0.08), crude fibre (4.4 ± 0.12) and crude fat (1.4 ± 0.05). The mineral composition of the ashed leaves sample (mg/100g dry weight) showed that it contains magnesium (228.2mg/100g), potassium (52.65mg/100g), phosphorus (53.0mg/100g), iron (73.3mg/100g), sodium (59.8mg/100g), calcium (12.9mg/100g) and zinc

(45.6mg/100g) (figure 2). The phytochemical screening of the plant leaves revealed the presence of tannins, saponins, flavonoids, anthraquinones, alkaloids and cardiac glycosides (table 2).

Figure 3 shows the effect of *A. barbadensis* leaves supplement on food intake, weight gain and faecal output. Preliminary evidence of protein energy malnutrition was more obvious in rats fed the protein deficient diet (PDD – negative control) and in the malnourished rats fed on the ferrous salt supplemented diet (PDDF) when compared with the normal diet fed rats (ND - positive control). Animals in these groups had significantly ($p < 0.05$) low food intake, weight loss, as well as low faecal output when compared with the positive control. However, the supplementation of the protein deficient diet and the normal diet with the leaves (PDDV and NDV, respectively) resulted in increased food intake, weight gain and faecal output ($p < 0.05$) than their PDD counterpart. Similarly, the normal rats fed on the ferrous salt supplemented diet (NDF) consumed more food, gained weight and excreted more faeces.

The body weight changes from week one to ten of the study showed that the PDD fed rats had a growth deficit from week one to six in contrast to the control. Whereas, the PDDV and PDDF groups had relatively less growth deficit when compared with the PDD fed rats. The NDV and NDF diet fed rats showed increased growth even higher than that of the control (figure 4).

Results of haematological indices showed significantly increased ($p < 0.05$) PCV, RBC and haemoglobin concentration in the PDDV and PDDF fed rats when compared with the PD fed rats. The levels of these parameters in the NDV and NDF fed rats were non-significantly different ($p>0.05$) from that of the positive control. The WBC level was significantly high ($p < 0.05$) in the NDF and PDD fed groups and reduced significantly or normal in other groups studied (figure 5). The platelet level of the PDD fed rats was markedly decreased ($p < 0.05$) when compared with the other groups (figure 6). There were no significant differences ($p > 0.05$) in the red blood cell indices (MCV, MCHC and MCH) amongst the groups (figure 7).

The results of the total protein and albumin levels are shown in figure 8. There was significant reduction ($p < 0.05$) in serum total protein and albumin concentration especially in PDD fed group, but increased in the other groups in comparison with the control. The lipid profile of the rats is presented in figure 9. The triglycerides level was significantly low ($p<0.05$) in the NDV, NDF and PDD fed rats when compared with the positive control. The PDDV group had significantly high level ($p<0.0\%$) of triglycerides in contrast with the positive control. The total cholesterol level was also significantly low ($p < 0.05$) in the PDDF, NDV, PDDV groups, but normal in the other groups in comparison with the positive control. The HDL cholesterol level was observed to be significantly high ($p<0.05$) in the PDV when compared with the negative control. The level of this parameter was normal in the other groups studied. Figure 10 shows the effect of the leaves supplement on catalase (CAT) activity. The result reveals significant increase ($p<0.05$) in CAT activity in all the groups with the exception of the PDD fed animals which shows very low catalase activity when compared with the positive control. The vitamins C and E levels (figure 11) were also significantly high ($p < 0.05$) in all the groups except for the PDD fed rats showing low levels of these vitamins in contrast with the control.

Figures 12, 13, and 14 represent histopathological micrographic sections of the liver and kidney of rats. The hepatocytes of the PDD and PDDF fed rats showed severe macrovesicular fatty changes with fatty

vacuoles all over the hepatocytes. There were no remnants of the cytoplasm with the nuclei located to the periphery of the cells (figure 12 - Plate B). In contrast, the positive control (ND), NDV and NDF fed rats revealed normal hepatocytes and sinusoidal spaces with no evidence of fibrosis (figure 12 – Plate A). The supplementation of the protein deficient diet with the plant leaves (PDDV) resulted in mild macrovesicular fatty changes with few fat vacuoles, thin cytoplasm, very prominent nuclei, normal bile ducts and portal vessels (figure 13 – Plate A) when compared with that of the PDD fed rats (Fig. 13 – Plate B). The section of the kidney of the ND, NDF, NDV, and PDDV showed glomeruli with open capillary loops, normal cellularity, well preserved tubules, normal blood vessels and apparently normal basement membrane (figure 14 - Plate A). Whereas, section of the kidney of PDD and PDDF fed rats although revealed glomeruli with open capillary loops, normal cellularity, however, the epithelial cells showed poor stain uptake suggestive of scanty cytoplasmic inclusion.

Table 1: Composition of the Experimental Diet (Omoregie and Osagie, 2002)

Dietary components	Normal diet (ND)	Protein deficient diet (PDD)
Carbohydrate (garri)[1]	63	82
Protein (defatted soya beans)	21	2
Fat (palm oil) [1]	8	8
Vitamin and Mineral salt mi[2]	8	8

[1]obtained from New Benin Market, Benin City, Nigeria [2]contained all the vitamins and minerals in recommended daily allowance, products of ABC Plus Capsule, Hollard and Barrett Ltd., Nuneaton, Warwickshire, UK.

Table 2: Phytochemical screening of *Aloe barbadensis* leaves

Phytochemicals	*A. barbadensis*
Tannins	+
Saponins	+
Flavonoids	+
Anthraquinones	+
Cardiac glycosides	+
Alkaloids	+

+ = Present; - = Absent

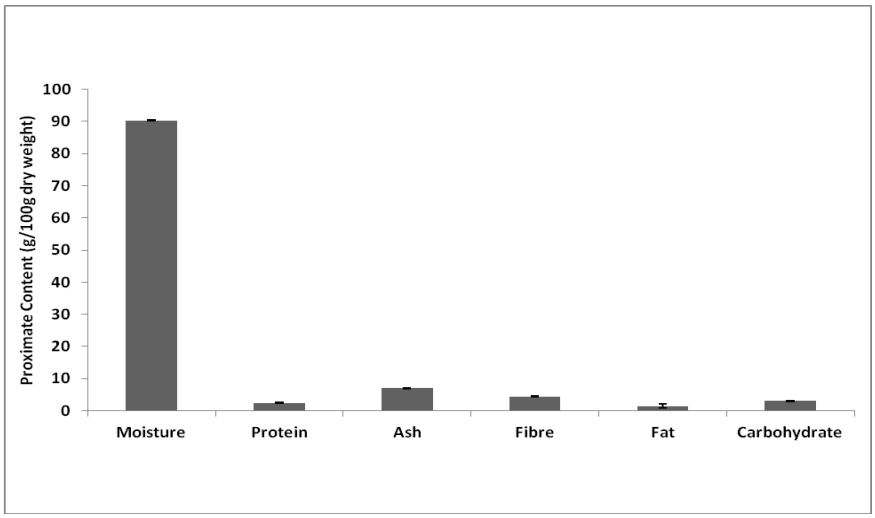

Figure 1: Proximate Composition of *Aloe barbadensis (*Values are mean ± S.E.M of triplicate determinations)

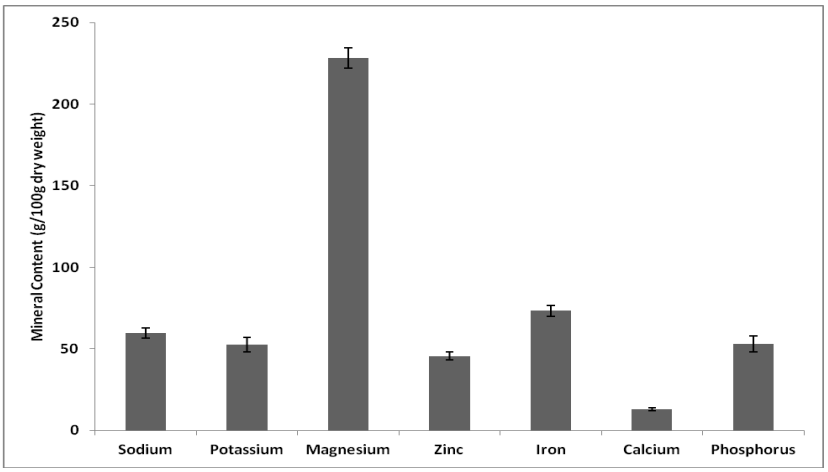

Figure 2: Mineral Composition of *Aloe barbadensis (*Values are mean ± S.E.M of triplicate determinations)

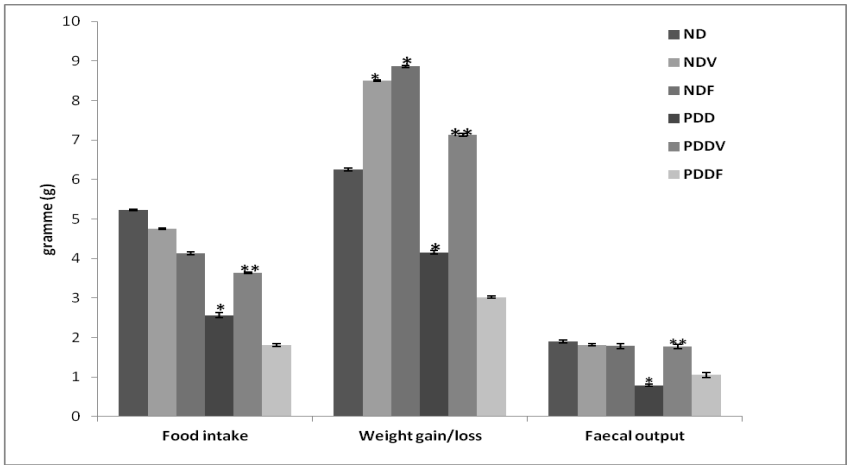

Figure 3: Effect of *A. barbadensis* on food intake, weight and faecal output in normal and in nutritionally stressed rats

All values are mean ± SEM (n = 8). * Significant (P < 0.05) compared with normal diet (ND-positive control) group. ** Significant (P < 0.05) compared with protein deficient (PDD – negative control) group.

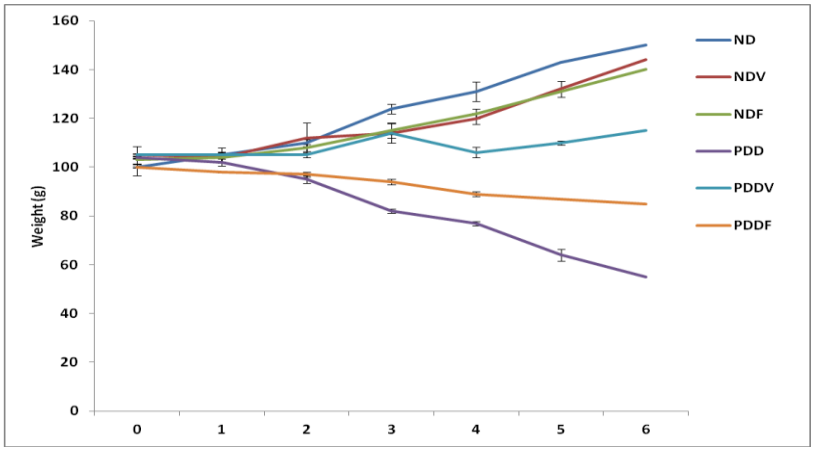

Figure 4: Effect of *Aloe barbadensis* on changes in body weight in normal and nutritionally stressed rats

All values are mean ± SEM (n = 8). * Significant (P < 0.05) compared with normal diet (ND-positive control) group. ** Significant (P < 0.05) compared with protein deficient (PDD – negative control) group.

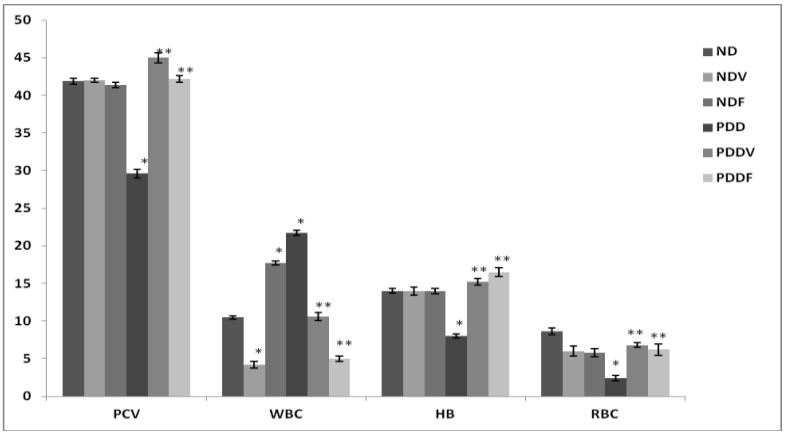

Figure 5: Effect of *Aloe barbadensis* on haematological indices in normal and nutritionally stressed rats
Values are mean ± SEM (n = 8). PCV in (g/dl); WBC × 10^3(µL); HB (g/dl); RBC × 10^6(µL). *Significant (P < 0.05) compared with normal diet (ND) group. **Significant (P < 0.05) compared with protein deficient (PDD)

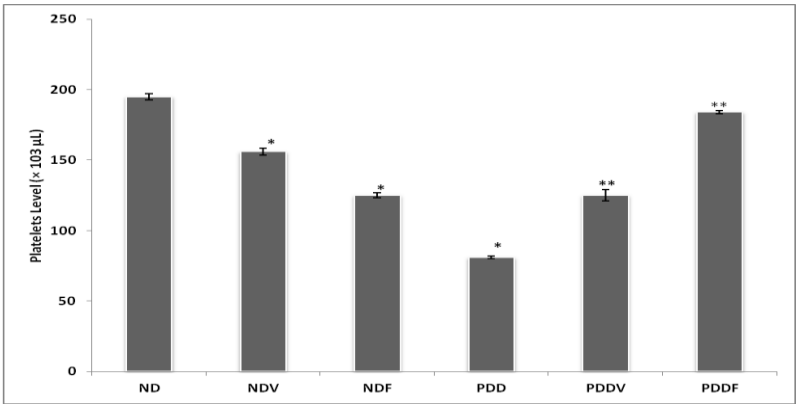

Figure 6: Effect of *Aloe barbadensis* on platelet level in normal and nutritionally stressed rats
Values are mean ± SEM (n = 8). * Significant (P < 0.05) compared with normal diet (ND-positive control) group. ** Significant (P < 0.05) compared with protein deficient (PDD – negative control) group.

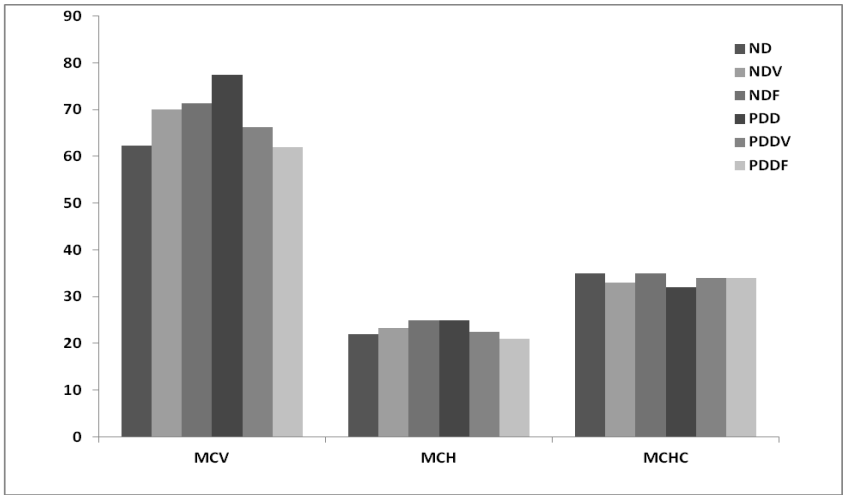

Figure 7: Effect of *Aloe barbadensis* on Red Cells Indices in Normal and in Nutritionally Stressed Rats
All values represent mean ± SEM (n = 8). MCV (FL); MCH (pg); MCHC (g/dl). * Significantly different (P < 0.05) when compared with normal diet (ND-positive control) group. ** Significantly different (P < 0.05) when compared with protein deficient (PDD – negative control) group.

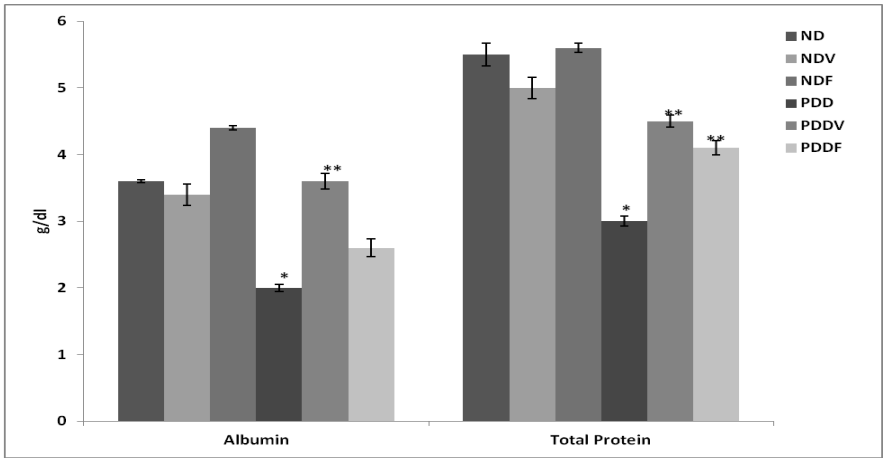

Figure 8: Effect of *Aloe barbadensis* on total protein and albumin in normal and nutritionally stressed rats
Values are mean ± SEM (n = 8). *Significant (P < 0.05) compared with normal diet (ND-positive control) group.
**Significant (P < 0.05) compared with protein deficient (PDD – negative control) group.

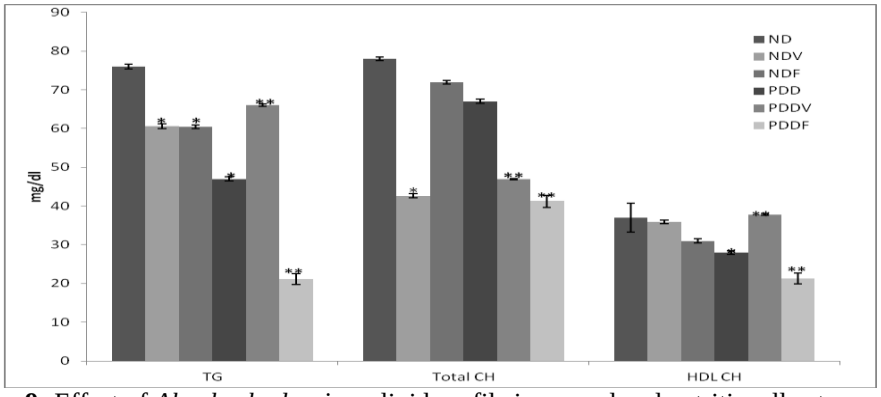

Figure 9: Effect of *Aloe barbadensis* on lipid profile in normal and nutritionally stressed rats
Values are mean ± SEM (n = 8). *Significant (P < 0.05) compared with normal diet (ND-positive control) group.
**Significant (P < 0.05) compared with protein deficient (PDD – negative control) group.

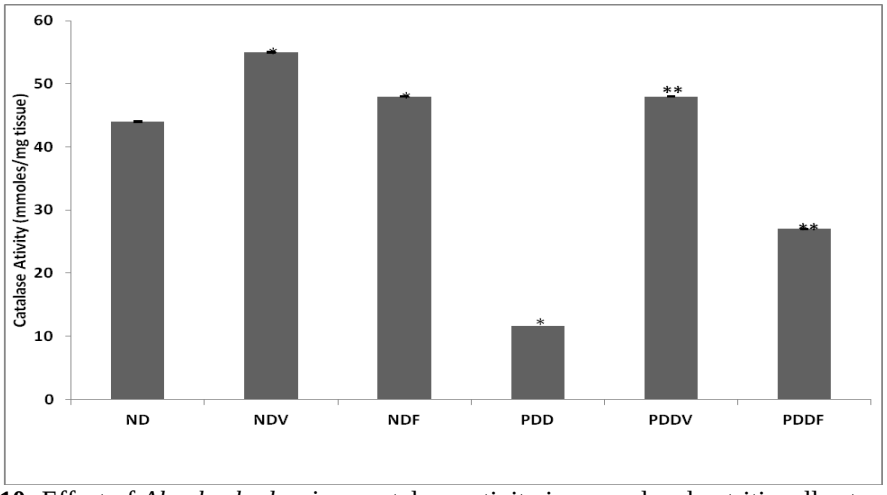

Figure 10: Effect of *Aloe barbadensis* on catalase activity in normal and nutritionally stressed rats
Values are mean ± SEM (n = 8). *Significant (P < 0.05) compared with normal diet (ND-positive control) group.
**Significant (P < 0.05) compared with protein deficient (PDD – negative control) group.

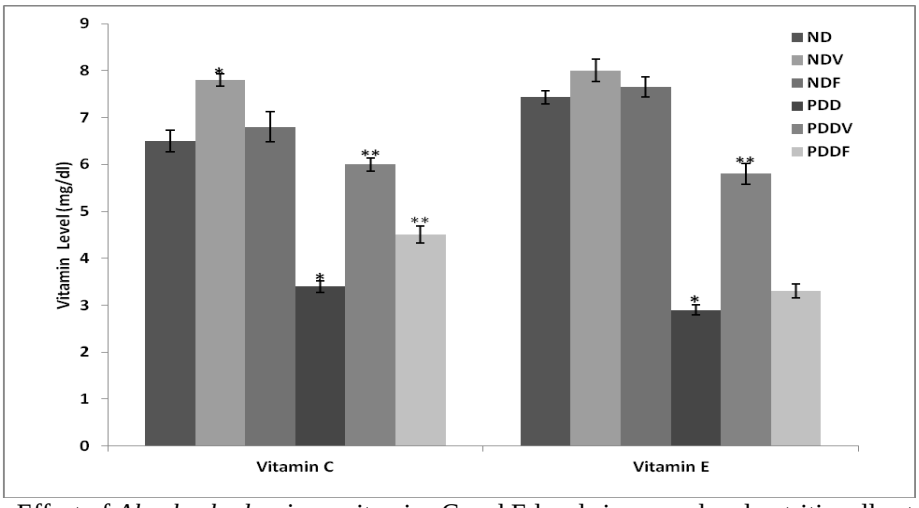

Figure 11: Effect of *Aloe barbadensis* on vitamins C and E levels in normal and nutritionally stressed rats Values are mean ± SEM (n = 8). *Significant (P < 0.05) compared with normal diet (ND-positive control) group. **Significant (P < 0.05) compared with protein deficient (PDD – negative control) group.

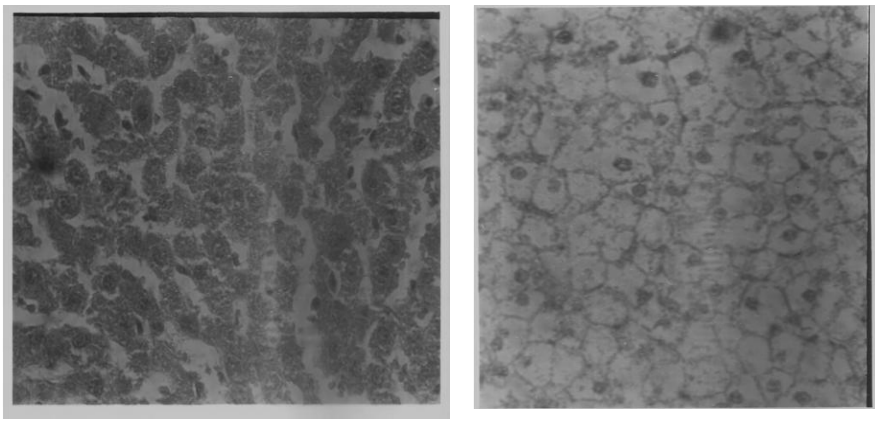

Plate A **Plate B**

Fig. 12: Micrographic section (H & E Staining) of the hepatocytes of rats fed on ND, ND+DR, ND+F, PD and PD+F supplemented diets. The hepatocytes of ND, ND+DR and ND+F fed rats showed normal architecture (Plate A) when compared with the rats fed on PD and PD+F diets (Plate B) which showed severe fatty changes indicative of fatty infiltrations and poor stain uptake.

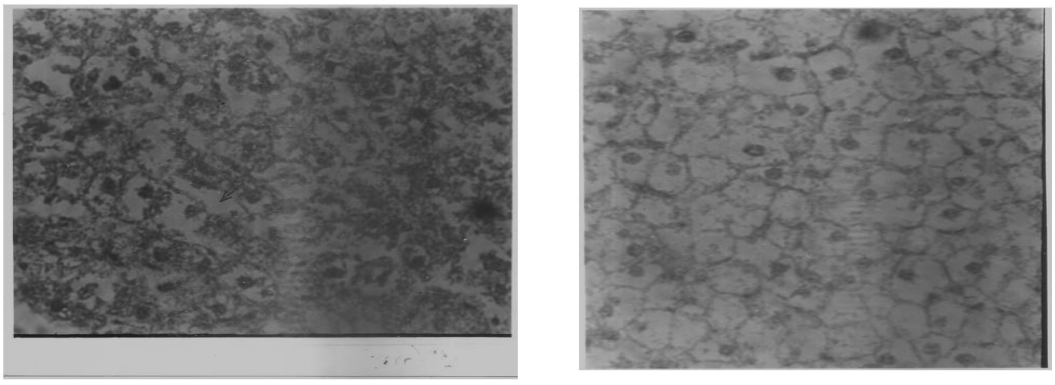

Plate A **Plate B**

Fig 13: Micrographic section (H & E staining) of the liver of rats fed on PD+DR diet (Plate A) showing moderate fatty changes in the liver when compared with the PD fed rats (Plate B).

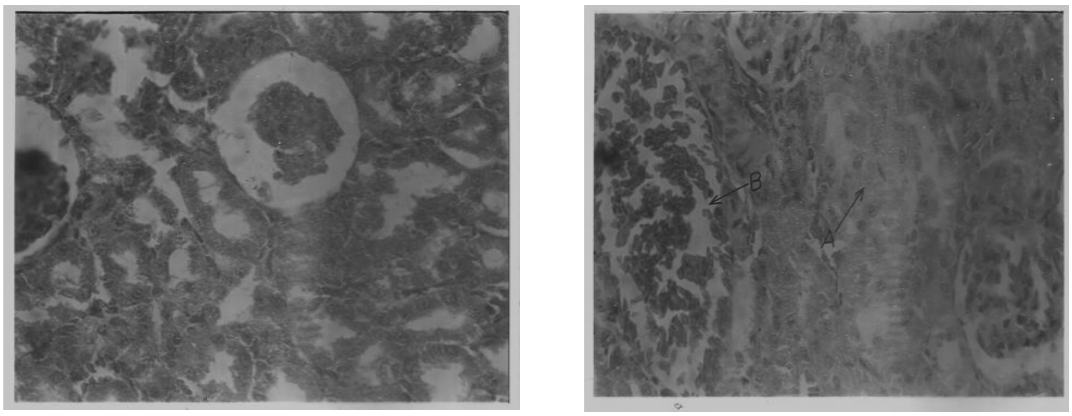

Plate A **Plate B**

Fig. 14: Micrographic section representing the kidney glomerular cells (H & E staining) of rats fed on ND, ND+F, ND+DR, PD+DR diets (Plate A) and PD, PD+F diets (Plate B). The glomerular cells from the PD and PD+F fed rats (Plate B) showed severe fatty changes with hyaline casts (A) and congested blood vessels (B) in contrast to the other groups which had normal kidney architecture (Plate A).

The tubules appeared pale with enlarged nuclei and vacuolation most probably due to fat deposits with areas of hyaline casts and congested blood vessels (figure 14 – Plate B).

DISCUSSION

In this study, the phytochemical screening, proximate composition and anti-anaemic effect of *Aloe barbadensis* leaves were evaluated in nutritionally stressed rats. Anaemia in PEM is associated with several factors such as deficiency of some micronutrients, blood loss, haemolysis, erythroid hypoplasia, infections and an adaptation to lower metabolic oxygen requirement. Results from this study showed early physical signs of malnutrition which was more obvious in the rats placed on protein deficient diet (PDD) and the rats placed on protein deficient diet supplemented with iron salt (PDDF). These signs were characterized with reduced weight, anorexia (poor appetite), scanty hair, RBC, PCV, HB and platelet levels and increased WBCs levels from the third week of feeding up to the tenth week. These signs were less visible in the rats placed on protein deficient diet supplemented with the *Aloe* plant (PDDV) and absent in the normal rats supplemented with only *Aloe* (NDV) or ferrous salt supplemented diet (NDF).

The increased level WBC in the PDD and PDDF fed rats may suggest infection due to a compromised immune system as a result of PEM. Infection may contribute to anaemia by reducing red blood cell survival, impairing iron bioavailability, impairing the response to erythropoietin and encouraging production of free radicals (Omoregie and Osagie, 2007; 2011). On the other hand, the reduced level of WBC and increased levels of other haematological indices in the PDDV and NDV fed rats may be due to the protective effect of the plant bioactive principles. In this regard, several studies have suggested that bioactive principles from plant sources may stimulate the release of the hormone erythropoietin in the kidney thereby increasing erythropoiesis in blood cells (El-Nawawy *et al*., 2002; Omoregie and Osagie, 2007, Mbaka and Owolabi, 2011).

In this study, iron salt (Fesolate®) served as the reference standard drug for treating anaemia. We noticed that there were low levels of the hematological indices in the ferrous salt treated animals which imply that iron supplementation alone may not alleviate anaemia in PEM. Excess iron has been

suggested to enhance microbial proliferation and suppress immunity resulting in the production of reactive oxygen species (ROS) and eventually leading to tissue damage (Evans and Halliwell, 2001). Moreso, several cases of increased mortality and morbidity have been linked with oral iron supplementation especially during the early phases of treatment of PEM (Golden and Ramdath, 1987; Omoregie and Osagie, 2011).

One of the important factors in the pathophysiology of anaemia in PEM is low concentrations of plasma protein and albumin which affect the bone marrow erythroid activity and decrease haemoglobin content (Omoregie and Osagie, 2007). Similarly, in this study there was significant reduction in protein and albumin levels of rats fed the PDD and PDDF diets. Again, supplementation of the protein deficient diet with the *Aloe* plant resulted in high levels of these parameters. The incidence of fatty liver is widely reported as one of the hallmarks of PEM. In this study, the histopathological of the liver of the PDD and PDDF fed groups revealed extensive macrovesicular fatty changes with fatty vacuoles spread all over. Also, sections of the kidney showed some focal areas of hyaline casts, congested blood vessels containing hemosiderin pigments suggestive of iron overload. These manifestations were mild in the malnourished rats fed with the *Aloe* plant supplemented diet suggestive of the protective effect of the plant' bioactive principles on the liver and kidney. This was further confirmed in the normal rats that were fed the leaves supplements which showed normal histology of these tissues.

Several papers have reported the effect of PEM on antioxidant status (Ashour *et al*., 1999; Shaaban *et al*., 2002; Omoregie and Osagie, 2007, 2011). Oxidative stress in PEM may result from either increased production of free radicals or depletion of the antioxidant defense mechanism. In the malnourished state, the body's own antioxidant defence system may not be strong enough to protect cells from damage by ROS (Waterlow, 1992; Golden and Ramdath, 1987; Shaaban *et al*., 2002; Ashour *et al*., 1999; Omoregie and Osagie, 2011). The levels of antioxidant enzymes, vitamins, etc have been reported to be compromised in PEM. In the present study, a similar compromised state was observed in the PDD fed rats which shows low levels of antioxidant vitamins (C and E) as well as antioxidant enzyme catalase. However, supplementation of the protein deficient diet with the plant leaves ameliorated the effect of PEM in these animals. The bioactive agents in the leaves may possess antioxidant activity by acting as scavengers of singlet oxygen and free radicals (Siriwadhana *et al*., 2003), as well as stimulate the synthesis and release of the hormone erythropoietin in the kidney. Erythropoietin is known to enhance RBC production (erythropoiesis) (Mbaka and Owolabi, 2011).

This study has therefore provided evidence of the anti-anaemic property of *Aloe barbadensis* leaves in nutritionally stressed rats. The plant was also found to have protective effect on the liver and kidney tissues against the deleterious effect of PEM in rats. The bioactive principles in the plant possess antioxidative effect which may be useful for rapid haemopoiesis and erythropoiesis in the bone marrow. The results from this study support local claims on its efficacy in the treatment of anaemia.

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