Antioxidant Activity of Aqueous Extract of *Alchornea laxiflora* (Benth) Leaf on some Selected Organs in Iron Deficient Albino Rats

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Abstract: The effects of administration of aqueous extract of *Alchornea laxiflora* leaf at doses of 100, 200 and 300 mg/kg body weight on some antioxidant enzymes and lipid peroxidation in iron deficient albino rats were investigated. Thirty six albino rats (51.17±1.02g) were used for the study. Eight rats were fed on iron-sufficient diet while the remaining twenty eight were made iron-deficient by maintaining them on iron deficient diets. After five weeks of feeding, haematological parameters (PCV, Hb, RBC, MCV, MCH and MCHC) of the iron-deficient rats were significantly reduced compared with rats fed on iron-sufficient diets ($p<0.05$). The iron-deficient rats were then treated with the extract, reference iron drug ($FeSO_4$) and iron-sufficient diets for two weeks. There was a significant ($p<0.05$) decrease in the activities of the antioxidant enzymes (SOD and CAT) in the serum, liver and kidney of rats in the iron deficient group (IDG) and significant decrease in the level of GSH in the serum, liver and kidney of rats in the iron-deficient group when compared to other treated groups. Also, there was a significant ($p<0.05$) increase in the level of malondialdehyde in liver, kidney and brain of untreated iron-deficient rats when compared with the control (iron sufficient group). The results revealed that the administration of aqueous extract of *Alchornea laxiflora* leaf at all doses to the iron-deficient rats significantly increase the activities of superoxide dismutase and catalase, increase in the level of GSH and decrease in the level of MDA. Therefore, aqueous extract of *Alchornea laxiflora* leaf reversed oxidative stress in iron deficient rats; these may be attributed to its rich phytochemical contents.
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1.0 Introduction

Despite the abundance of iron in the environment, iron deficiency is still the most common nutritional deficiency in the world and the most common cause of anaemia worldwide (John, 1995). Iron deficiency anaemia (IDA) is asystemic disorder involving multiple systems rather than a pure hematologic condition associated with anaemia. It results from lack of sufficient iron for synthesis of hemoglobin and shortening of red cell life span (Lanskowsky, 1995). Aanemia is associated with a low work capacity, a poor pregnancy outcome, as well as lasting effects on learning and cognitive function, attention, behavior, health and growth (Willoughby and Laitner, 2000; Shams *et al*., 2010).

Imbalance between oxidative stress (OS) and antioxidant system is present in iron deficient Patients (Madhikarmi and Murthy, 2012). Also, oxidative stress is associated with increased morbidity particularly in iron deficiency anaemia (Guyatt *et al.*, 1992; Willoughby and Laitner, 2000). Oxidative damage to cells can be caused by the formation of superoxide radical (O_2), hydrogen peroxide radical (H_2O_2) and hydroxyl radical (OH). Studies show that these radicals also affect the equilibrium between pro-oxidants and antioxidants in biological systems, leading to modifications in genomes, proteins, carbohydrates, lipids and lipid peroxidation (Romero *et al.*, 1998) thus inactivating antioxidant defense. OS plays an important role in the pathogenesis of IDA by prooxidant and antioxidant balance in favor of the prooxidant, leading to potential damage to biomolecules such as nucleic acids, proteins, structural carbohydrates and lipids. Free radicals are atom (e.g. oxygen, nitrogen) with at least one unpaired electron in the outermost shell, and have independent existing capability (Madhikarmi and Murthy, 2011).

Plant and its products are rich sources of a phytochemicals and have been found to possess a variety of biological activities including antioxidant potential (Craig, 1970). Natural antioxidants are in high demand for application as nutraceuticals, bio-pharmaceuticals, as well as food additive because of consumer preference. *Alchornea laxiflora* (Benth) is a forest understorey tree of about 6m high growing in Nigeria. It is also found in other part of Africa. The leaves play important role in the preservation of kolanuts, stem and branchlets are also used in Nigeria as chewing sticks. Decoction of the leaves is used in the treatment and management of inflammatory and infectious diseases as well as an important component of herbal antimalarial (Adewole, 1993), antibacterial

(Lamikanra *et al.*, 1990), anti-inflammatory and antimicrobial (Ogundipe *et al.*, 1999) formulations.

The aim of this work is to study the antioxidant potential of aqueous extract of *Alchornea laxiflora* leaf in iron deficient albino rats.

2.0 Materials and Methods

2.1 Laboratory animals

Albino rats (*Rattus norvegicus*) of both sexes weighing between 51.17±1.02g were obtained from the small animal holding unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria.

2.2 Feed Components

Maize (*Zea may*) and locust bean [*Parkia biglobosa* (A.) Jacq] seeds were obtained from Baboko Market, Ilorin, Nigeria while the soybean oil used was a product of Grand Cereal and Oil Mills Limited, Bukuru, Jos, Nigeria. The vitamin mix was a product of BASG Aktiengesellschaft, Germany Pantex, Holland. Component chemicals of the mineral mix used were products of Sigma Chemicals Limited, London.

2.3 Plant identification and preparation of extract

The leaves of *Alchornea laxiflora* was obtained from Faculty of Agriculture, University of Ilorin, Ilorin, Nigeria and was authenticated in the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria, where a voucher specimen (UIH 739) was deposited at the Departmental Herbarium.

The leaves of *Alchornea laxiflora* were separated from the stem and oven dried at 40°C for 72 hours to a constant weight. The dried leaves were then pulverized using Beltone Luinohun Blender/Miller III (model MS-223, Taipei, Taiwan). The powdered material was stocked in a plastic container from which 1000 g was extracted in 1.5 Litre of cold distilled water for 48 hours at 37^0C. This was then filtered with Whatman No. 1 filter paper. The filtrate was concentrated on a steam bath to give 24.7 g of the extract. The extract was reconstituted in distilled water to give the required doses of 100, 200 and 300 mg/kg body weight as used in this study. (Value arrived at from information obtained during ethnobotanical survey). The reconstituted aqueous extract was administered orally using cannula to all the animals in different groups (Yakubu *et al.*, 2005).

2.4 Composition of diet

The composition of iron deficient and iron sufficient diets per kg diet is shown in Table 1. The components of the diets were thoroughly mixed and made into pellets to ensure good handling by the animals (Oladiji *et al.*, 2007).

Table 1: Feed Components of Iron Sufficient and Iron Deficient Diets

Feed Components	Iron sufficient (g/kg)	Iron deficient (g/kg)
Locust beans	710	710
Corn starch	40	40
*Soybean oil	40	40
Sucrose	100	100
Methionine	20	20
Lysine	10	10
**Vitamin mix	10	10
***Mineral mix	30	30
Fibre	40	40

*Soybean oil: Polyunsaturated Fatty acids (58%), monounsaturated fatty acids (29%) saturated fatty acid is (13%).

**Vitamin mix (per kg of diet): vitamin A, 100,000 lU; vitamin D_3, 10,000 lU; vitamin E, 100 mg; vitamin B_1, 20 mg; vitamin B_2, 40 mg; d-calcium pantothenate, 100 mg; vitamin B_6, 15 mg; vitamin B_{12}, 10µg; vitamin C, 250 mg; vitamin K_3, 15 mg; folic acid, 5000 mcg; nicotinic acid, 200 mg; biotin, 150 mcg; choline chloride, 400µg; inositol, 80 mg, vitamin c, 250mg; folic acid, 5000mcg.

***Mineral mix (g/kg diet): $CoCl_2.6H_2O$ (0.001), $CuSO_4.5H_2O$ (0.078), $MnSO_4.2H_2O$ (0.178), KI (0.032), KH_2PO_4 (10.559), NaCl (3.573), $MgSO_4.7H_2O$ (1.292), Zn $(CO3)_2$ (1.6), $CaSO_4$ (11.61), $FeSO_4.7H_2O$ (1.078).

Iron deficient diet contains no additional $FeSO_4.7H_2O$.

2.5 Animal grouping and Administration of Plant Extract and Iron Supplement

The animals were individually housed in metabolic cages of 33cm × 20.5cm × 19cm under standard condition (12 hours light: 12 hours dark cycle; 28^OC and 40-55% humidity). Rats were then fasted for 24 hours (without food but given water) prior to the commencement of the experiment. The animal grouping consisted of an initial two groups:

A: Rats maintained on iron sufficient diet designated as ISG (iron sufficient group)

B: Rats maintained on iron deficient diet designated as IDG (iron deficient group)

Animals in groups A and B were maintained on their respective diets for 5 weeks. At the end of the 5 weeks feeding period, 4 rats each from IS and ID groups were sacrificed and their haematological indices were determined. The remaining rats in groups B were further grouped into six with four rats in each group as follows:

B1- Iron deficient rats fed on iron deficient diet for 14 days (iron deficient diet all through) designed as IDG (iron deficient group)

B2- Iron deficient rats fed on iron sufficient diet for 14 days (change of diet) designed as CDG (change of diet group)
B3- Iron deficient rats orally administered on daily basis for 14 days with reference iron supplement tablet ($FeSO_4$) designated as RDG (reference drug group)
B4- Iron deficient rats orally administered with *A. laxiflora leaf* extract (100 mg/kg/rat/day) for 14 days designed as IDA-100 mg.
B5- Iron deficient rats orally administered with *A. laxiflora leaf* extract (200 mg/kg/rat/day) for 14 days designed as IDA-200 mg.
B6- Iron deficient rats orally administered with *A. laxiflora leaf* extract (300 mg/kg/rat/day) for 14 days designed as IDA-300 mg.
The rest of the rats in group A were still fed on iron sufficient feed for 14 days (iron sufficient all through) designated as ISG (iron sufficient group).
The aqueous extracts of *Alchornea laxiflora* leaf at various doses were administered to the various groups using cannula.

2.6 Collection of Blood Sample and Preparation of serum and tissue homogenates

The rats were placed under diethyl ether anaesthesia; the neck area was shaved to expose the jugular veins. The veins after being slightly displaced (to avoid contamination with interstitial fluid) were then sharply cut with a sterile scalpel blade. Blood was collected into EDTA sample bottles for haematological assay and also collected into clean sterile sample bottles which were allowed to clot for 30 minutes. This was then centrifuged at 33.5 g for 15 minutes using a Uniscope Laboratory Centrifuge (model SM800B). The sera were aspirated with Pasteur pipettes and stored frozen overnight at -20^0C before being used for the biochemical analyses. The animals were quickly dissected and the liver, kidney, heart, brain and lungs were removed, after which the tissues were blotted with clean tissue paper, weighed, and homogenized in 0.25M sucrose solution (1:5 w/v). The homogenates were kept frozen for 24 hours before being used for the analyses (Yakubu *et al.*, 2005).

2.7 Estimation of Haematological Parameters

The haemoglobin concentration was determined using the method described by Jain (1986). PCV was estimated using a Hawksley microhaematocrit centrifuge at 40-2 x g for 5min while red and white blood cells counts were determined using the Naubeaur haemocytometer, mean corpuscular volume (MCV) mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated as described by Dacie and Lewis (1991).

2.8 Determination of biochemical parameters

The Protein content of homogenates was determined using the Biuret method of Plummer (1974). Activities of Superoxide Dismutase (SOD) (EC 1.15.1.1) was determined based on the method described by Mistra and Fridovich (1972), Catalase (CAT) (EC 1.11.1.6) activity was determined as described by Sinha (1972), the level of Malondialdehyde (MDA) was determined according to the method of Gutheridge and Wilkin (1982) and the level of Reduced Glutathione (GSH) was determined as described by Ellman (1959). All measurements were done using Spectronic 21 spectrophotometer (Bausch and Lomb, NY).

2.9 Statistical Analysis

The data were expressed as mean ± standard error of mean (SEM). Statistical analysis was performed using analysis of variance (ANOVA) and Duncan multiple range test at 5% level of confidence ($p<0.05$).

3.0 Results

Table 2 shows the haematological parameters of rats fed with iron sufficient diet and iron deficient diet for the period of five (5) weeks. At the end of the five (5) weeks of feeding with iron deficient and sufficient diet, the level of haematological parameters of rats placed on iron deficient diet (IDD) decreases significantly ($p<0.05$) when compared with rats fed on iron sufficient diet (ISD) (Table 2). This is an indication that the rats fed on iron deficient diet were anaemic.

Table 3 shows the effect of aqueous extract of *Alchornea laxiflora* leaf on the activity of Superoxide dismutase (SOD) in the serum and some organs of iron-deficient rats. This was compared with the iron sufficient group (ISG) which is the control group and other treated groups. Result shows that there was a reduction in the activity of the enzyme in serum, liver and kidney in rats fed on iron deficient diet when compared with that of rats fed on iron sufficient diet, but the reduction was not significant. The oral administration of aqueous extract of *Alchornea laxiflora* leaf at all doses significantly ($p<0.05$) increase the activity of the enzyme and this was showed to be dose dependent with 300 mg/kg bwt of the extract having the highest value for superoxide dismutase activity in serum and kidney. In serum and kidney, there was no significant ($p>0.05$) difference in the activity of SOD of rats in the CDG and RDG but there was a significant ($p<0.05$) decrease in the activity of SOD in IDG when compared with the CDG and RDG. Hence reference iron drug and change of diet also increase SOD activity when given to the iron deficient group (Table 3).

Table 4 shows the effect of aqueous extract of *Alchornea laxiflora* leaf on the activity of Catalase

(CAT) in some organs of iron-deficient rats. This was compared with the iron sufficient group (ISG) which is the control group and other treated groups. Result shows that there was a significant decrease in the activities of catalase in liver and kidney of the iron deficient rats. The oral administration of aqueous extract of *Alchornea laxiflora* leaf at all doses significantly ($p<0.05$) increase the activity of the enzyme and this was showed to be dose dependent with 300 mg/kg bwt of the extract having the highest value for catalase activity in liver. In kidney, the highest value of catalase activity is seen in the 100 mg/kg body weight group. In the liver and kidney, there was no significant ($p>0.05$) difference in the activity of CAT of rats in the CDG and RDG but there was a significant ($p<0.05$) decrease in the activity of CAT in IDG when compared with the CDG and RDG. Hence, reference iron drug and change of diet also increase catalase activity when given to iron deficient group (Table 4).

Table 5 shows the effect of aqueous extract of *Alchornea laxiflora* leaf on the level of malondialdehyde (MDA) in some organs of iron-deficient rats. This was compared with the iron sufficient group (ISG) which is the control group and other treated groups. Result shows that in the heart of rats in the iron deficient group, there was no significant ($p>0.05$) difference in the level of malondialdehyde when compared with those of rats in the iron sufficient group and other treated groups but in the liver, kidney and brain of untreated iron deficient rats, there was a significant ($p<0.05$) increase in the level of malondialdehyde (MDA). Also, in the lungs of the iron deficient rats, there was an increase in the level of malondialdehyde but this was not significant (Table 5). The administration of the extract to iron deficient rats significantly decrease the level of MDA in liver, kidney and brain (Table 5).

Table 6 shows the effect of aqueous extract of *Alchornea laxiflora* leaf on the level of Reduced Glutathione (GSH) in the serum and some organs of iron-deficient rats. This was compared with the iron sufficient group (ISG) which is the control group and other treated groups. Result shows that in the serum of iron deficient rats (IDG), there was a significant ($p<0.05$) decrease in the level of GSH of rats in the iron deficient group when compared to that of rats in the iron sufficient group (control). The GSH level in the liver, kidney and intestine of the IDG shows no significant ($p>0.05$) difference when compared with the rats in iron sufficient group (control). The oral administration of aqueous extract of *Alchornea laxiflora* leaf at all doses significantly ($p<0.05$) increase the level of GSH in the serum (Table 6).

Table 2: Haematological Parameters of rats fed with iron deficient and sufficient diets for five weeks

	Iron Sufficient Group	Iron Deficient Group
PCV (%)	34.95 ± 3.85^{a}	28.75 ± 6.15^{b}
Hb (g/dL)	9.15 ± 0.750^{a}	6.70 ± 2.60^{b}
RBC ($10^{6}/\mu L^{3}$)	5.41 ± 0.08^{a}	4.60 ± 1.08^{b}
MCV (fL)	64.15 ± 5.85^{a}	62.90 ± 1.30^{b}
MCH (pg)	16.5 ± 1.50^{a}	14.00 ± 2.40^{b}
MCHC (g/dL)	26.25 ± 1.06^{a}	22.35 ± 6.01^{b}

Values are expressed as Mean ± SEM (n = 4). Values in each column with different superscript are significantly different ($P<0.05$). PCV: Packed Cell Volume; Hb: Haemoglobin; RBC: Red Blood Cell; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Haemoglobin; MCHC: Mean Corpuscular Haemoglobin Concentration

Table 3: Activity of Superoxide dismutase (SOD) in iron deficient rats administered with aqueous extract of *Alchornea laxiflora* leaf

	Specific Activity (µmol/min/mg protein)		
Groups	Serum	Liver	Kidney
ISG (Control)	10.97 ± 0.42^{c}	6.86 ± 0.48^{c}	11.53 ± 0.63^{bc}
IDG	10.91 ± 0.23^{c}	6.84 ± 0.29^{c}	11.14 ± 0.46^{c}
CDG	13.96 ± 1.02^{ab}	6.95 ± 0.44^{c}	13.88 ± 1.10^{ab}
RDG	12.91 ± 0.19^{bc}	8.54 ± 0.29^{b}	14.85 ± 0.23^{a}
IDA-100mg/kg b.wt	12.11 ± 1.03^{bc}	8.52 ± 0.51^{b}	15.72 ± 1.23^{a}
IDA-200mg/kg b.wt	13.58 ± 0.52^{ab}	11.59 ± 0.15^{a}	11.44 ± 0.48^{bc}
IDA-300mg/kg b.wt	15.38 ± 1.21^{a}	9.26 ± 0.55^{b}	12.24 ± 0.73^{bc}

Values are expressed as Mean ± SEM (n = 4). Values in each column with different superscript are significantly different ($P<0.05$). IDG: Iron Deficient Group; ISG: Iron Sufficient Group; CDG: Change of Diet Group; RDG: Reference Drug Group; IDA-100 mg: 100 mg/kg b.wt of *Alchornea laxiflora* leaf group; IDA-200 mg: 200 mg/kg b.wt of *Alchornea laxiflora* leaf group; IDA-300 mg: 300 mg/kg b.wt of *Alchornea laxiflora* leaf extract group

Table 4: Activity of Catalase (CAT) in iron deficient rats administered with aqueous extract of *Alchornea laxiflora* leaf

Groups	Specific Activity (µmol/min/mg protein)	
	Liver	Kidney
ISG (Control)	0.084±0.015^{ab}	0.130±0.022^{ab}
IDG	0.038±0.002^{c}	0.100±0.003^{c}
CDG	0.072±0.025^{b}	0.123±0.011^{b}
RDG	0.087±0.005^{ab}	0.123±0.021^{b}
IDA-100mg/kg b.wt	0.093±0.007^{a}	0.147±0.003^{a}
IDA-200mg/kg b.wt	0.101±0.007^{a}	0.113±0.013^{bc}
IDA-300mg/kg b.wt	0.102±0.006^{a}	0.128±0.011^{ab}

Values are expressed as Mean ± SEM (n = 4). Values in each column with different superscript are significantly different (P<0.05). IDG: Iron Deficient Group; ISG: Iron Sufficient Group; CDG: Change of Diet Group; RDG: Reference Drug Group; IDA-100 mg: 100 mg/kg b.wt of *Alchornea laxiflora* leaf group; IDA-200 mg: 200 mg/kg b.wt of *Alchornea laxiflora* leaf group; IDA-300 mg: 300 mg/kg b.wt of *Alchornea laxiflora* leaf extract group

Table 5: Malondialdehyde (MDA) level in iron deficient rats administered with aqueous extract of *Alchornea laxiflora* leaf

Groups	(µmole/mg protein) ×10^{-6}				
	Liver	Kidney	Heart	Brain	Lungs
ISG	12.85±1.08^{ab}	4.79±0.99^{a}	17.80±3.64^{a}	24.08±8.37^{a}	19.84±0.79^{ab}
IDG	22.23±2.46^{c}	15.03±2.76^{b}	30.55±4.10^{a}	188.84±72.49^{b}	27.99±9.61^{b}
CDG	11.89±2.99^{a}	8.09±0.81^{a}	29.57±7.07^{a}	86.52±43.05^{ab}	16.31±3.59^{ab}
RDG	15.52±1.20^{abc}	5.43±0.88^{a}	28.59±6.89^{a}	73.21±18.78^{ab}	20.42±1.34^{ab}
IDA-100 mg	20.38±3.14^{bc}	4.71±0.27^{a}	20.89±4.32^{a}	130.37±32.80^{ab}	15.09±1.25^{ab}
IDA-200 mg	21.06±2.02^{c}	6.07±0.63^{a}	23.57±6.91^{a}	109.61±8.46^{ab}	12.94±1.01^{a}
IDA-300 mg	16.33±2.92^{abc}	5.77±1.12^{a}	31.69±6.77^{a}	107.76±27.31^{ab}	12.70±1.30^{a}

Values are expressed as Mean ± SEM (n = 4). Values in each column with different superscript are significantly different (P<0.05). IDG: Iron Deficient Group; ISG: Iron Sufficient Group; CDG: Change of Diet Group; RDG: Reference Drug Group; IDA-100 mg: 100 mg/kg b.wt of *Alchornea laxiflora* leaf group; IDA-200 mg: 200 mg/kg b.wt of *Alchornea laxiflora* leaf group; IDA-300 mg: 300 mg/kg b.wt of *Alchornea laxiflora* leaf extract group

Table 6: Reduced Glutathione (GSH) level in iron deficient rats administered with aqueous extract of *Alchornea laxiflora* leaf

Groups	µmol/mg protein			
	Serum	Liver	Kidney	Intestine
ISG (Control)	107.45±14.01^{ab}	8.25±1.17^{bc}	58.10±8.21^{a}	5.40±0.06^{b}
IDG	59.54±9.10^{c}	5.52±0.95^{c}	50.95±4.10^{a}	2.04±0.68^{b}
CDG	95.61±15.45^{b}	9.68±1.52^{bc}	80.69±5.64^{a}	13.39±6.33^{ab}
RDG	82.48±4.40^{bc}	9.06±0.81^{bc}	70.48±2.64^{a}	26.17±13.85^{ab}
IDA-100mg/kg b.wt	75.90±5.30^{bc}	9.24±1.77^{bc}	149.63±77.98^{a}	17.95±7.58^{ab}
IDA-200mg/kg b.wt	79.31±10.06^{bc}	20.26±4.81^{a}	50.53±4.00^{a}	37.36±11.96^{a}
IDA-300mg/kg b.wt	129.01±8.43^{a}	16.45±3.92^{ab}	54.76±5.29^{a}	27.00±13.17^{ab}

Values are expressed as Mean ± SEM (n = 4). Values in each column with different superscript are significantly different (P<0.05). IDG: Iron Deficient Group; ISG: Iron Sufficient Group; CDG: Change of Diet Group; RDG: Reference Drug Group; IDA-100 mg: 100 mg/kg b.wt of *Alchornea laxiflora* leaf group; IDA-200 mg: 200 mg/kg b.wt of *Alchornea laxiflora* leaf group; IDA-300 mg: 300 mg/kg b.wt of *Alchornea laxiflora* leaf extract group

4.0 Discussion

Iron deficiency anemia continues to be the most common specific nutritional deficiency in the world. Despite the advances in infant feeding during the last decades, it failed to eliminate iron deficiency as a public health problem (John, 1995). If not treated, iron deficiency anemia may cause stunted growth, impaired mental development, poor school performance, reduced productivity, increased morbidity and mortality, and lower self-esteemed. Iron is essential for all eukaryotes and most prokaryotes, where it is used in the synthesis of

heme, iron-sulfur (FeS), and other cofactors. Fe-S proteins are involved in catalysis, redox reactions, respiration, DNA replication, and transcription. Iron homeostasis is tightly regulated to avoid iron toxicity or iron deficiency in normal condition. In human systemic iron metabolism, iron uptake, trafficking, export and fortification are highly regulated. (Tolentino and Friedman, 2007; King *et al.,* 2008; Hattangadi and Lodish, 2004; Ye and Rouault, 2010).

The most reliable indication of iron deficiency anaemia is haemoglobin. This is because it is the iron-containing protein found in red blood cells that allows the red blood cells to function as the oxygen transport system to the tissues of the body. Next to haemoglobin in this regard is the haematocrit (Ht) or packed cell volume (PCV) which is a measure of the portion of the blood volume made up by red blood cells (Oladiji *et al.,* 2005). Result from table 1 showed the establishment of iron deficiency anaemia in the first five weeks of this study. The significant decrease in the haematological parameters of the iron deficient group when compared with iron sufficient group is sufficient to conclude that the feed induced intended condition of this study i.e. iron deficiency anaemia.

The causes of increased oxidative stress and decreased antioxidant defense in iron deficiency anaemia have not been completely explained, although a significant increase in lipid peroxidation has been found (Kumerova *et al.,* 1998; Garg *et al.,* 2005). In addition to decreased antioxidant defence system activity and increased lipid peroxidation in erythrocytes of patients with iron deficiency anemia, Kumerova *et al* (1998) found that pentose phosphate pathway activity was also reduced in iron deficiency anemia. Bartal *et al.* (1993) reported that iron deficiency anemia erythrocytes were more susceptible to oxidation but had good capacity for recovery. Some researchers observed increased SOD activity in patients with iron deficiency anemia (Acharya *et al*., 1997; Jansson *et al*., 1985). Jansson *et al.* (1985) suggested that increased SOD formation was a compensatory factor for increased oxidant stress. Other research (Kumerova *et al.,* 1998; Madhikarmi and Murthy, 2011) showed decreased activities of antioxidant enzymes, such as SOD, and catalase, in patients with iron deficiency anemia

In the present study, Superoxide dismutase (SOD) and catalase (CAT) activity in iron deficient rats was lower than that of control group which might be caused by insufficient nutrition and oxidative stress under hypoxic condition. Also, the level of reduced glutathione (GSH) was reduced in iron deficient rats while the level of malondialdehyde (MDA) increases in iron deficient rats. Cellular non-enzymatic antioxidants are also known as free radical scavengers that protect a cell against toxic free radicals. Reduced GSH is the chief constituent of the thiol pool and a vital intracellular scavenger of free radicals (Madhikarmi and Murthy, 2011). Therefore, decreased GSH levels may reflect a depletion of non-enzymatic antioxidant reserves. On the other hand, GSH plays a prominent role in the antioxidant defense system and in the reactions of catalysis, regulation, electron transportation and in preserving the correct structure of proteins (Madhikarmi and Murthy, 2011). Decreased levels of total GSH have been reported in various pathologies, including anaemia (Tolentino and Friedman, 2007; Fusco *et al.,* 2007). The significant increase in the level of MDA could be attributed to the increased formation or inadequate clearance of free radicals by the cellular antioxidants. The present observations are in agreement with other reports on IDA (Fusco *et al.,* 2007; Gadjeva *et al.,* 2005)**.** It is well known that reactive oxygen species especially hydrogen peroxide, inhibit SOD activity (Hodgson and Fridovich, 1976). Furthermore, decreased SOD activity may contribute to free radical production. The free radicals are produced in different metabolic process of the body and they can damage a wide range of biomolecules such as proteins, DNA and amino acids in the body (Maestri *et al.,* 2006; Ashwell *et al.,* 2010; Edwin *et al.,* 2008; Djeridane *et al.,* 1997; Katalinic *et al.,* 2006).

In the present study, superoxide dismutase (SOD), catalase (CAT) activity significantly increased in iron deficient groups after treatment with iron sufficient diet, reference iron drug and aqueous extract of *Alchornea laxiflora* leaf at doses of 100, 200 and 300 mg/kg body weight. The level of reduced glutathione (GSH) increase and the level of malondialdehyde (MDA) decrease after treatment with iron sufficient feed, standard iron drug and aqueous extract of *Alchornea laxiflora* leaf at dose of 100, 200 and 300 mg/kg body weight. Recently, many researchers have been reported that many medicinal plants possesses more potential antioxidant activity and their phytochemical constituents (Phenolic acids, flavonoids and tannins, etc.) have potential biological activities (Srikanth *et al.,* 2013). Antioxidants protect cells against the damaging effects of reactive oxygen species otherwise called free radicals such as singlet oxygen, super oxide, peroxyl radicals, hydroxyl radicals and peroxynite which results in oxidative stress leading to cellular damage (Mattson and Cheng, 2006). These antioxidants exert their activity by scavenging the 'free-oxygen radicals' thereby giving rise to a fairly 'stable radical' (Doughari, 2012). The present studies have shown that the extracts of *Alchornea laxiflora* have free radicals scavenging ability.

5.0 Conclusion

Iron deficiency causes increased lipid peroxidation and decreased antioxidant status in iron deficient rats. Oral administration of aqueous extract of *Alchornea laxiflora* leaf increased the activity of antioxidant enzymes and reduced lipid peroxidation in iron deficient rats. Hence, the antioxidant potential of the plant extract might have been brought about by the phytochemicals present in the plant.

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