Effect of Aqueous Extract of *Alchornea Laxiflora* (Benth) Leaf on Gastric pH and Disaccharidases in Iron Deficient Rats

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Abstract: The effects of administration of aqueous extract of *Alchornea laxiflora* leaf at the doses of 100, 200 and 300 mg/kg body weight on Gastric pH and Disaccharidases (maltase, lactase and sucrase) in iron deficient 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. The gastric pH and activities of the disaccharidases (maltase, lactase and sucrase) of rats of the iron deficient group were significantly reduced ($p<0.05$) compared with the rats fed on iron sufficient diets (control). The result revealed that the level of gastric pH of untreated iron deficient rats was 2.59 while the level of gastric pH of iron-deficient rats administered with aqueous extract of *Alchornea laxiflora* leaf at doses of 100, 200 and 300 mg/kg body weight increased to 3.26, 3.58 and 3.53 respectively. Furthermore, there was a significant ($P<0.05$) increase in the activities of disaccharidases of the iron-deficient rats at all doses of the extract. The results indicate that the administration of aqueous extract of *A. laxiflora* leaves has reversed anaemic conditions in the iron-deficient rats.
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1.0 Introduction

Iron plays a significant role in several metabolic processes and iron balance is very important because both iron excess and deficiency are deleterious. The small intestine is the main organ responsible for the homeostasis of iron by controlling its absorption in response to changes in the amount of iron in the body (Wayhs *et al.*, 2004). Iron deficiency is a major public health problem, especially in infants, children, and women of childbearing age in developing countries (UNICEF/UNU/WHO, 2001). It has been demonstrated that this type of deficiency affects growth, mental performance, muscle capacity, thermogenesis, and immunity (Oski, 1979; Dallman, 1982; Thibault *et al.*, 1993). As far as the digestive tract is concerned, iron deficiency anemia is known to be associated with stomatitis and glossitis (Jacobs, 1960; Baird *et al.*, 1961; Scott *et al.*, 1985; Ranasinghe *et al.*, 1987), with reduction of gastric acid secretion (Ghosh *et al.*, 1972), abnormal absorption (Guha *et al.*, 1968), decrease in intestinal disaccharidase (Prassad and Prassad, 1991), and changes in intestinal permeability (Beraut *et al.*, 1992).

Medicinal plants play a significant role in providing primary health care services to rural people and are used by about 80% of the marginal communities around the world (Prajapati and Prajapati, 2002; Latif *et al.*, 2003; Shinwari *et al.*, 2006). Each medicinal plant species has its own nutrient composition besides having pharmacologically important phytochemicals. These nutrients are essential for the physiological functions of human body. Such nutrients and biochemicals like carbohydrates, fats and proteins play an important role in satisfying human needs for energy and life processes (Novak and Haslberger, 2000). Plants are also known to have high amounts of essential nutrients, vitamins, minerals, fatty acids and fibre (Gafar and Itodo, 2011). *Alchornea laxiflora* (Benth) (Euphorbiaceae) 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 purpose of this work is to study the effect of aqueous extract of *A. laxiflora* leaf on the activities of disaccharidases (maltase, lactase and sucrase) and gastric pH level in rats fed with iron deficient diets (iron deficient 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 rats 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 rats (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
Fiber	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 $(CO_3)_2$ (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 two weeks (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 feed) designed as CDG (change of feed group).

B3- Iron deficient rats orally administered on daily basis for 14 days with reference iron supplement tablet (ferrous sulphate) 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-100mg.
B5- Iron deficient rats orally administered with *A. laxiflora leaf* extract (200 mg/kg/rat/day) for 14 days designed as IDA-200mg.
B6- Iron deficient rats orally administered with *A. laxiflora leaf* extract (300 mg/kg/rat/day) for 14 days designed as IDA-300mg.
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

The rats were placed under diethyl ether anaesthesia; the neck area was quickly 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 and about $3cm^3$ of blood was collected into EDTA sample bottle for the haematological assay (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 Gastric Juice pH

The stomach was ligated at both opening and 3ml of distilled water was injected to collect gastric juice. The pH of the gastric juice is then measured using pH meter (Moore, 1968).

2.9 Preparation of small intestine homogenate

The serosal surface was dried with tissue paper and the whole intestinal sample weighed (W_1). This was then spread on the dissecting board and cut open and the mucosa was rapidly scraped with a glass slide. The intestinal sample above was then cut at a specific length (uniform in all the animals) and 1g was weighed and mechanically homogenized with four parts (v/w) of cold normal saline using a Teflon glass homogenizer, the sample was chilled with crushed ice during homogenization. The remaining intestinal sample was weighed to calculate the wet mucosa mass (W_2). The homogenate was centrifuged using a bench centrifuge at 4000g for 10 minutes and the supernatant was decanted and used for enzymes (Lactase, Maltase and Sucrase) assay (Dahlqvist, 1968).

2.10 Biochemical Assay: Determination of disaccharidases activity

Diluted enzyme solution (0.1 ml) was mixed with 0.1 ml of the appropriate substrate solution (20 mg/ml of lactose, sucrose or maltose) in a test tube to which a drop of toluene was added as preservative. The tube was incubated in a constant temperature water bath at 37^0C for 60 minutes. After 60 minutes of incubation, 0.8 ml distilled water was added and the enzymatic reaction was immediately interrupted by immersion of the tube in boiling water for 2 minutes after which the tube was cooled with tap water. A blank with the same composition 0.1ml each of the diluted enzyme solution and 0.1ml distilled water (substrate) was also prepared and immersed in boiling water immediately after mixing. The absorbance of the sample was immediately read at 415nm using Spectronic 21 spectrophotometer (Bausch and Lomb, NY). Enzymes activity was expressed as U/g (Dahlqvist, 1968).

2.11 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 feed (IDF) decrease significantly ($p<0.05$) when compared with rats fed on iron sufficient feed (ISF) (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 activities of disaccharidases (maltase, lactase and sucrase) in the small intestine of iron-deficient rats. This was compared with the iron sufficient group (ISG) which is the control group and other treated groups. The result shows that there was a significant ($p<0.05$) decrease in the activities of the disaccharidases of the iron deficient rats. The oral administration of aqueous extract of *Alchornea laxiflora* leaf significantly increase the disaccharidase activity when compared with the control, this was showed to be dose dependent with 300mg/kg bwt of the extract having the highest values of disaccharidases activities. Reference iron drug and change of diet also

significantly ($p<0.05$) increase these disaccharidases activities of iron deficient group (Table 3). Maltase has the highest activity in the treated groups when compared with the activity of lactase and sucrose except in the group of rats administered with 100 mg/kg body weight of the extract which has lower activity when compared to the activity of lactase of the same group (Table 3).

Table 4 shows the effect of aqueous extract of *Alchornea laxiflora* leaf on the gastric pH level of iron-deficient rats. This was compared with the iron sufficient group (ISG) which is the control group and other treated groups. The result revealed that the level of gastric pH of untreated iron deficient rats was 2.59 while the level of gastric pH of iron-deficient rats administered with aqueous extract of *Alchornea laxiflora* leaf at doses of 100, 200 and 300 mg/kg body weight increased to 3.26, 3.58 and 3.53 respectively. The gastric pH level of rats in the change of diet group (CDG), reference iron Drug Group (RDG), and extract administration groups (100, 200 and 300mg/kg body weight) shows no significant ($p<0.05$) difference when compared with the iron sufficient group (Control).

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

Haematological parameters	Iron Sufficient Group (ISG)	Iron Deficient Group (IDG)
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; WBC: White Blood Cell

Table 3: Activity of Maltase, Lactase and Sucrase in iron deficient rats administered with aqueous extract of *Alchornea laxiflora* leaf

Groups	Maltase ($\times10^{-3}$ U/g)	Lactase ($\times10^{-3}$ U/g)	Sucrase ($\times10^{-3}$U/g)
ISG (Control)	6.22 ± 1.04^{b}	$3.62\pm.18^{c}$	5.98 ± 1.15^{a}
IDG	1.73 ± 0.11^{c}	$1.75\pm.09^{d}$	1.45 ± 0.10^{c}
CDG	5.76 ± 0.18^{b}	$3.57\pm.18^{c}$	6.05 ± 1.40^{a}
RDG	5.93 ± 0.36^{b}	$6.59\pm.14^{a}$	4.94 ± 0.28^{ab}
IDA-100mg/kg bwt	6.48 ± 0.07^{b}	$5.79\pm.41^{b}$	2.57 ± 0.17^{bc}
IDA-200mg/kg bwt	7.83 ± 0.12^{a}	$6.66\pm.16^{a}$	4.82 ± 1.36^{ab}
IDA-300mg/kg bwt	8.49 ± 0.15^{a}	$7.19\pm.01^{a}$	6.89 ± 0.73^{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-100mg: 100mg/kg bwt of *Alchornea laxiflora* leaf group; IDA-200mg: 200mg/kg bwt of *Alchornea laxiflora* leaf group; IDA-300mg: 300mg/kg bwt of *Alchornea laxiflora* leaf extract group

Table 4: Gastric pH of iron deficient rats administered with aqueous extract of *Alchornea laxiflora* leaf

Groups	Gastric pH
ISG (Control)	3.83 ± 0.02^{a}
IDG	2.59 ± 0.14^{b}
CDG	3.40 ± 0.20^{a}
RDG	3.49 ± 0.27^{a}
IDA-100mg/kg body weight	3.26 ± 0.21^{a}
IDA-200mg/kg body weight	3.58 ± 0.19^{a}
IDA-300mg/kg body weight	3.53 ± 0.09^{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-100mg: 100mg/kg bwt of *Alchornea laxiflora* leaf group; IDA-200mg: 200mg/kg bwt of *Alchornea laxiflora* leaf group; IDA-300mg: 300mg/kg bwt 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). The reduction in body weight of iron deficient rats has reportedly been linked to both Protein Energy Malnutrition and reduced disaccharidases (enzymes that catalyse the last stage of carbohydrate digestion) activities (Gudmand-Hoyer and Skovbjerg 1996; Vieira *et al.,* 2000).

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 anemia.

Carbohydrates are a vitally important dietary component and the disaccharidases are an essential subset of digestive enzymes required for the terminal step of carbohydrate digestion (Gudmand-Hoyer and Skovbjerg 1996). The enzymatic activity of each individual disaccharidase is influenced primarily by the dietary level of their corresponding substrate (Goda *et al.,* 1999) and under normal conditions this level of activity is controlled by the abundance of the mRNA encoding the proteins (Traber *et al.,* 1992; Goda *et al.,* 1999).

The activities of the disaccharidases (maltase, lactase and sucrase) were significantly ($p<0.05$) reduced when compared with the iron sufficient group (control) and other anaemia treated groups. This is in agreement with the works of Hoffbrand and Broitman (1969); Sriratanaban and Thayer (1971); Lanzykowsky *et al*. (1981) and Vieira *et al*. (2000) which all showed that feeding animals with iron deficient feed for a period of four weeks or more will not only lead to anaemia in the rats but also decrease the activity of disaccharidases in the animals and that this decrease is reversible by iron supplementation (Fernandes *et al.,* 1997; Lanzykowsky *et al*., 1981; Vieira *et al*., 2000). The significant decrease observed in the activity of the diasaccharidases in iron deficient rats may be due to an alteration of the expression of the genes encoding the enzymes (maltase, lactase and sucrase); either by impairment to enterocyte differentiation or by iron-sensitive mechanisms that regulate the mRNA levels in enterocyte. Since lactase and sucrase mRNA expression and the presence of enzymatically active protein are considered to be markers for differentiated enterocytes (Freeman, 1995; Trugnan *et al.,* 1987), it is possible that iron status alters the normal pattern of differentiation as the cells migrate to the villus tip, or that a specific regulatory mechanism sensitive to the effects of iron deficiency controls the abundance of the disaccharidase mRNA in each individual differentiated enterocyte (Adrian and Phillip, 2005). Also, the expression of pancreatic duodenal homebox 1 protein, PDX-1, a repressor of sucrase and lactase promoter, was 4.5 folds higher in iron deficiency, or by iron-sensitive mechanisms that regulate mRNA levels in enterocyte (Adrian and Phillip, 2005). Buts *et al.* (1986) suggested that iron deficiency causes changes in the secretory components of enterocytes with no alteration of erythrocyte membrane morphology i.e. the ability of enterocytes to synthesize these enzymes may be reduced.

In patients with iron-deficiency anaemia, acid secretion was found to be reduced and was associated with chronic gastritis (Floch and Meroney, 1962; Bock *et al.*, 1963; Dagg *et al*., 1964; Ikkala and Siurala, 1964; Cowan *et al.,* 1966). In this study, the gastric pH of iron deficient rats was significantly ($p>0.05$) reduced when compared to that of iron sufficient group and other treated groups (Table 4). The reduced gastric pH in iron deficient rats may be due to gastric mucosal abnormalities (Davidson and Markson, 1955), anaemia (Floch and Meroney, 1962) and/or deficiency of an iron-containing enzyme system (Delamore and Shearman, 1965).

In the present study, the gastric pH and activities of disaccharidases significantly increased in the iron deficient group after treatment with iron sufficient diet, standard iron drug and aqueous extract of *Alchornea laxiflora* leaf at doses of 100, 200 and 300 mg/kg body weight. This may be due to the presence of vitamin and mineral contents of the leaves of *A. laxiflora*. These constituents are well known haemopoietic factors that have direct influence on the production of blood in the bone marrow.

5.0 Conclusion

Iron deficiency causes decreased gastric pH and activities of disaccharidases in iron deficient rats. Oral administration of aqueous extract of *Alchornea laxiflora* leaf at all doses increased the activities of the disaccharidases and gastric pH in all iron deficient rats. Hence, this may be made possible by

the extract's ability to make readily available iron which is needed in the restoration of the deficiency state.

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