Effect of Aqueous Extract of *Khayagrandifoliola* C. DC. Stem Bark on some Disaccharidases Activity in Iron-Deficient Weanling Rats

ABSTRACT

This study was aimed at investigating the effect of the aqueous extracts of *Khayagrandifoliola* stem bark on some disaccharidases(lactase and sucrase) in diet-induced anemia in weanling rats. Weanling rats of 21 days old were maintained on iron-deficient diets for four weeks to induce anemia before treatment. A total of 35 weanling rats were used, grouped into five rats per group of iron-deficient diet/distilled water, iron-sufficient diet/distilled water, change of iron-deficient diet after four weeks to iron-sufficient diet, iron-deficient diet/ Standard drug, iron-deficient diet/25mg/kg body weight of aqueous plant extract, iron-deficient diet/50mg/kg body weight of aqueous plant extract, and iron-deficient diet/100mg/kg body weight of aqueous plant extract. Phytochemical screening of the extract revealed the presence of alkaloids, saponins, cardiac glycosides, tannins, anthraquinones and flavonoids. The extract administered orally produced significant increase in the activity of intestinal mucosa sucrase and lactase ($P<.05$). The change of diet from iron deficient diet to iron sufficient diet increased disaccharidases activities in the intestinal mucosa. However, the aqueous extract of *Khayagrandifoliola*showed a higher disaccharidases activity when compared to the group of rats that were fed iron-sufficient diet. This study revealed that plant extract administered increased disaccharidases activity in the intestinal mucosa in diet-induced anemic group of weanling rats and thus lends credence to *Khayagrandifoliola* use in folklore medicine in the management of anemia.

Keywords: *Khayagrandifoliola*,iron-deficient, Lactase, Sucrase

1. INTRODUCTION

Iron deficiency anemia (IDA) is the most common type of anemia that results from an inadequate iron supply to aid the production of healthy red blood cells [1]. Iron deficiency is usually a result of the depletion of the body's iron stores; hence, a restricted supply of iron to various tissues becomes apparent. This may result in a reduction in the synthesis of red blood cells and iron-dependent intra-cellular enzymes participating in many metabolic pathways [2]. Iron deficiency has been reported to reduce the activity of some disaccharidases [3,4].

Although there are various drugs used for the treatment of anemia [5,6,7], they are not affordable to many poor people in the developing countries such as Nigeria. Furthermore, the rural populations in various parts of the world do not have appreciable access to high-quality drugs for the treatment of anemia, hence, the reliance on herbal products for the treatment of diseases and anemia. Some of the plants with ethnobotanical and scientific claims being used for the treatment of anemia include *Waltheriaindica, Sorghum bicolor, Khayagrandifoliola* ,and*Mangiferaindica*[8,9,10,11,12].

*Khayagrandifoliola*is a medicinal plant commonly found in Nigeria and many parts of theplant are valuable to traditional medicine. Some sicknesses like gastric pain, lumbago, stomach ache, worm infestation, cough, fever, and rheumatism are treated with concoctions made from the bark of*Khayagrandifoliola*[13]. The stem bark of this plant has also been previously reported to have anti-malarial, anti-ulcer, anti-hypoglycaemic, anti-hypoproteinaemia, and anti-hypocholesterolaemia activity [14,15,16]. Several research studies have lent scientific credence to the use of various parts of *Khayagrandifoliola*in the treatment of several diseases, however, there is still no adequate information on the effect of the plant extract on lactase and sucrase activity as an indicator for anti-anemic potentials of *Khayagrandifoliola*. This study investigated the effect of aqueous extract of *Khayagrandifoliola*stem bark on lactase and sucrase activity in the intestinal mucosa of iron-deficient weanling rats.

2. Materials and Methods

2.1. Experimental Animals

Forty-five weanling albino rats of both sexes (*Rattusnorvegicus*) with a mean weight of 40.0 g ±3.0 g were obtained from the Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Nigeria. This study was carried out as approved by the Department of Biochemistry Ethical Committee on the use of laboratory animals at the University of Ilorinwith ethical number BCH/SCI/029.

2.2. Feed Components

Yellow maize (*Zea mays*) and locust bean (*Parkiabiglobosa*A. Jacq) seeds were obtained locally from Baboko Market, Ilorin, Nigeria, while the soybean oil used was a product of Grand Cereals and Oil Mills Limited, Bukuru, Jos, Nigeria. The vitamin mix was a product of BASF Aktiengesellschaft, Germany Pantex, Netherland. Component chemicals of the mineral mix used were products of Sigma-AldrichChemical Limited, London, UK.

2.3. Reagents

The reagents used were of analytical grade and were prepared in all glass-distilled water. The reagents were stored in reagent bottles [17].

2.4. Plant Identification and Preparation of Extract

The stem barks of the plant were obtained from Ikaro in the Ose Local Government Area of Ondo State in the South West region of Nigeria. It was authenticated in the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria, where a voucher specimen was deposited in the herbarium with voucher number UILH/003/1066.

The method described by Oladiji et al. [9] was used in the preparation of the plant extract. The stem barks of the plant were air-dried until a constant weight was obtained. The dried pieces were then pulverized using an electric blender, and the powder obtained was stocked in a plastic container. A known weight (15 g) of the powder was poured into 100 ml of distilled water and immediately boiled for 25 min. The resulting solution was left to cool and filtered with Whatman filter paper. The filtrate was concentrated on a water bath (kept at $45^{o}C$) until a constant weight

was obtained to give 2.32 g ± 0.03 of the residue (brownish-black slurry), which corresponded to percentage yield of 15.47%. The residues were pooled together and reconstituted in distilled water to give the doses of 25, 50, and 100mg/kg body weight.

The reconstituted aqueous extract was administered orally using an oropharyngeal cannula to all the animals in different groups.

2.5. Diet Formulation

The composition of iron-deficient and iron-sufficient diets per kg diet is described in Table 1. The components of the diets were thoroughly mixed and made into pellets to ensure homogeneity and proper handling by the animals. It was produced weekly and packed into air-tight polythene bags to prevent rancidity, auto-oxidation of the oil, and microbial contamination. The proximate analysis of the compounded feeds was also carried out.

Table 1:

Feed components of iron sufficient and iron-deficient diets

Feed components	Iron-sufficient (g/kg)	Iron-deficient (g/kg)
Locust bean seed	500	500
Maize flour	315	315
Soybean oil	40	40
Sucrose	100	100
Methionine	5	5
Mineral mix[b]	30	30
Vitamin mix[a]	10	10
$FeSO_4 \cdot 7H_2O$	157.36mg/Kg	38.216 mg/Kg

Soybean oil: polyunsaturated fatty acids (58%), monounsaturated fatty acids (29%), saturated fatty acids (13%).

[a]Vitamin mix (per kg of diet): vitamin A, 100,000 lU; vitamin D3, 10,000 lU; vitamin E, 100 mg; vitamin B1, 20 mg; vitamin B2, 40 mg; Lysine, 10g; d-calcium pantothenate, 100 mg; vitamin B6, 15 mg; vitamin C, 250 mg; vitamin K3, 15 mg; folic acid, 5000 mcg; nicotinic acid, 200 mg; biotin, 150 mcg; inositol, 80 mg.

[b]Mineral mix (g/kg): $CoCl_2 \cdot 6H_2O$ (0.001), $CuSO_4 \cdot 5H_2O$ (0.079), $MnSO_4 \cdot 7H_2O$ (0.178), KI (0.032), NaCl (3.573), $ZnCO_3$ (1.60), $CaSO_4$ (11.610), $MgSO_4 \cdot 7H_2O$ (2.292), K_2HPO_4 (10.559). Control diet contained 1.078 g $FeSO_4 \cdot 7H_2O$.

2.6. Animal Grouping and Extract Administration

The animals were kept in well-ventilated house conditions (temperature: 22±3^{o}C; photoperiod: 12h/12h light/dark cycle; relative humidity: 45-50 %). They were allowed free access to normal rat chow and distilled. The acclimatization was done for seven days, and they were then fasted for 24 hours (without food but water) before the commencement of the experiment. The animal grouping consisted of an initial two groups:

A – 10 weanling rats maintained on iron-sufficient diet designated as ISF.

B – 35 weanling rats maintained on an iron-deficient diet designated as IDF.

Animals in groups A and B were maintained on their respective diets for four weeks. At the end of the four weeks feeding period, five rats each from ISF and IDF groups were sacrificed. The remaining weanling rats in groups B were further grouped into six with five rats in each group as follows:

B1- Iron deficient weanling rats fed with iron-deficient feed for two weeks (iron deficient feed all through) designated as IDF

B2- Iron deficient weanling rats fed with iron sufficient feed for two weeks (change of feed) designated as COF

B3- Iron deficient weanling rats orally administered daily for two weeks with iron supplement syrup (a standard iron supplement) designated as SD

B4- Iron deficient weanling rats orally administered daily for two weeks with 25 mg/kg body weight of aqueous extract of *Khayagrandifoliola*stem bark designated as KG 25mg.

B5- Iron deficient weanling rats orally administered daily for two weeks with 50 mg/kg body weight of aqueous extract of *Khayagrandifoliola*stem bark designated as KG 50mg.

B6- Iron deficient rats orally administered daily for two weeks with 100 mg/kg body weight of aqueous extract of *Khayagrandifoliola*stem bark designated as KG 100mg.

The rest of the animals in group A were still fed with iron sufficient feed for two weeks (iron sufficient all through) designated as ISF.

The extracts and the distilled water were administered to the various groups using the oro-pharyngeal cannula.

2.7. Determination of lactase and sucrase activity

Method described by Dahlqvist[18]was used and the result of the intestinal mucosa disaccharidases was reported as units (U).

2.8. Statistical Analysis

Results were expressed as the mean ± SEM of five determinations. The data were analyzed using Duncan Multiple Range Test and complemented with Student's t-test. The differences were considered statistically significant at $P<.05$. All the analyses were done using SPSS version 16.0 software (SPSS Inc., Chicago, IL, USA).

3. Results

Phytochemical screening of the aqueous stem barks of *K. grandifoliola* revealed the presence of alkaloids, flavonoids, tannins and saponins, cardiac glycosides, and anthraquinones in its aqueous extracts.

The proximate composition of both iron-deficient (ID) and iron sufficient (IS) formulated feeds are shown in Table 2. Proximate analysis of the diets showed that the components in the iron-deficient formulated feed (ID) were substantially similar to those in the iron-sufficient formulated feed.

Table 2:

Proximate composition of iron-deficient and iron-sufficient diets

Components	ID (%)	IS (%)
Crude protein	20.78	21.44
Carbohydrate	60.00	59.65
Lipid	5.03	5.15
Crude fiber	2.97	3.02
Total ash	4.20	4.35
Moisture	7.02	6.39

ID, iron-deficient; IS, iron-sufficient; crude protein was obtained using the expression nitrogen×6.25

Effect of *Khayagrandifoliola* on intestinal mucosa sucrase activity in iron-deficient weanling rats

From Table 3, there was significant decrease in the intestinal mucosa sucrase activity in IDF group when compared to that of ISF group. The activity of sucrase in the SD group and ISF group showed a significant increase when compared IDF group. Also, there was also a significant increase in sucrase activity in COF and SD groups when compared to IDF group. The result also showed a significant increase in the activity of sucrase in COF group when compared with both SD and ISF groups. Administration of the aqueous extracts of *K. grandifoliola* stem bark led to significant ($P<.05$) increase in the activity of the intestinal mucosa Sucrase (Table 3)

at all dose levels investigated compared to the other groups. However, the dosage at 50mg/kg body weight of aqueous extract of *K. grandifoliola* stems bark has the highest significant increase ($P<.05$) in the activity of intestinal mucosa Sucrase.

Table 3:

Effect of *Khayagrandifoliola* on intestinal mucosa sucrase in iron deficient weanling Rats

Group	Sucrase Specific Activity(U/g)
ISF	$9.00\pm1.7^{a,b}$
IDF	5.27 ± 0.2^{a}
COF	$9.13\pm1.4^{a,b}$
SD	$6.93\pm0.3^{a,b}$
25mg/kg b. wt.	$8.93\pm0.2^{a,b}$
50mg/kg b. wt.	16.20 ± 1.6^{c}
100mg/kg b. wt.	9.67 ± 1.7^{b}

Values are expressed as mean of five replicates ± S.E.M. and those with different superscripts along a column are statistically different ($P<.05$). The extract was administered for 14days; IDF: Iron-deficient diet fed rats; ISF: Iron-sufficient diet fed rats; COF:Change of feed to iron-sufficient diet; SD: Group treated with Standard Drug; ; Kg b. wt.: Kilogramme Body weight

Effect of *Khayagrandifoliola* on intestinal mucosa lactase in iron deficient weanling rats

From Table 4, lactase activity showed a significant decrease in IDF group when compared with ISF group. SD group has significant increase in the intestinal mucosa lactase activity when compared with both ISF and COF groups. In this study, the SD group showed a significant increase in lactase activity when compared with ISF group. This result also showed a significant increase in lactase activity in COF group when compared IDF group. Administration of the aqueous extract of *K. grandifoliola* stem bark led to significant increase in the activity of

intestinal mucosa lactase (Table 4) at all doses investigated compared to other groups ($P<.05$). However, the dosage at 50mg/kg body weight of aqueous extract of *K. grandifoliola* stems bark has the highest significant increase in the activity of intestinal mucosa lactase ($P<.05$).

Table 4
Effect of *Khayagrandifoliola* on intestinal mucosa lactase in Iron-deficient weanling rats

Group	Lactase Specific Activity(U/g)
ISF	$4.13\pm1.0^{a,b}$
IDF	1.73 ± 0.9^{a}
COF	$3.27\pm2.0^{a,b}$
SD	$5.73\pm1.8^{a,b}$
25mg/kg b. wt.	$4.93\pm0.7^{a,b}$
50mg/kg b. wt.	$7.13\pm1.3^{a,b}$
100mg/kg b. wt.	$5.87\pm0.8^{a,b}$

Values are expressed as mean of four replicates ± S.E.M. and those with different superscripts along a column are statistically different ($P<.05$). The extract was administered for 14days; IDF: Iron-deficient diet fed rats ;ISF: Iron-sufficient diet fed rats ; COF:Change of feed to iron-sufficient diet; SD: Group treated with Standard Drug; ; Kg b.wt.: Kilogramme Body weight

4. Discussion

Secondary metabolites screening carried out on the aqueous stem barks of *K. grandifoliola* indicated that, *K. grandifoliola* stem barks contain alkaloids, flavonoids, tannins and saponins, and anthraquinones in its aqueous extracts[13]. These phytochemicals are known to perform several general and specific roles in plants, and may exhibit different biochemical and pharmacological actions in different species of animals when consumed.

In this study, the effect of aqueous extract of *Khayagrandifoliola* on some disaccharidases activity in iron-deficient weanling rats was investigated. It has been reported previously that disaccharidases have reduced activity in iron-deficient anemia [3, 4].

From tables (3&4), the results showed that, there was significant decrease in the activities of the disaccharidases in weanling rats fed iron- deficient diet in the study. Similar reports have shown that dietary iron deficiency can cause a significant decrease in disaccharidase activity [3, 19, 20, 21, 22, 23, 24].

Iron-deficient diet fed rats treated with the reference drug and the group of iron deficient weanling rats administered aqueous extract of *K. grandifoliola* stem bark showed significant increase in the activity of disaccharidases in the intestinal mucosa. This result is in agreement with claim that, decrease in disaccharidases activity is reversible by iron supplementation [3, 21, 24].

The change of diet from iron deficient diet to iron sufficient was also able to increase the activities of intestinal mucosa sucrase and lactase, however, the plant aqueous extract of *K. grandifoliola* showed higher increase in the activity of the disaccharidases.

The significant increase in the activities of the disaccharidases in the iron-deficient weanling rats from administered aqueous extract of *K. grandifoliola* stem bark in this study may be as a result of the chemical constituent(s) of the of the aqueous extract of *K. grandifoliola* stem bark. It is

possible that the extracts mechanism(s) of action for the significant increase in the activities of the disaccharidases may be due to an increase in the gene expression of both sucrase and lactase. Since the extract contains saponins and they are membrane active agents. It is possible that saponins stimulate the release of glucocorticoids that further initiate various biochemical reactions, hence, resulting in the induction of the disaccharidases in the intestinal cells.

The administered doses of aqueous extract of *Khayagrandifoliola* in this study were within the tolerable limit and produced no toxic effect. Tolerable limit of aqueous extract of *Khayagrandifoliola*, as investigated and reported by Njikam and Njikam [16], showed that the LD_{50} of the plant extract was 5.5g/kg bodyweight. The LD_{50} is over a thousand times more than the 50mg/kg bodyweight shown to be the most efficacious of the three doses administered.

5. Conclusion

In this study, the administration of the aqueous extract of *Khayagrandifoliola* stem bark increased the activity of both sucrase and lactase in the intestinal mucosa of iron-deficient weanling rats and 50mg/kg.bt wt. dose showed to be the most efficacious of all the dose levels. This investigation furthers strengthen the claim of the usage of the aqueousextracts of *Khayagrandifoliola* stem bark in the management of iron-deficient anemia.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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