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Optimizing dose of aqueous extract of *Mangifera indica* L stem bark for treating anaemia and its effect on some disaccharidases activity in iron deficient weanling rats

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ABSTRACT

Iron deficiency, the main cause of anaemia, has been linked with decreased disaccharidases activity. The highest prevalence of iron deficiency is recorded in Africa where plants, including *Mangifera indica*, with ethnobotanical claims of being used for the treatment of iron deficiency anaemia are 'housed'. Although some scientific findings have been reported on the anti-anaemic potential of *M. indica*, none is yet to give a clearer picture of this ethnobotanical claim. This work investigates the effects of aqueous extract of *M. indica* stem bark on iron deficiency anaemia and disaccharidases' activities in iron deficient rat. The aqueous extract formulated into three doses, 25, 50 and 75 mg/kg body weight were administered to weanling albino rats induced with iron deficiency through diet. After four weeks of feeding the rats, the Packed Cell Volume, Haemoglobin concentration and Red Blood Cell count of the iron deficient rats were significantly reduced ($P < 0.05$) compared to those of healthy rats fed with iron sufficient feed. These iron status indicators were significantly increased ($P < 0.05$) in rats treated with the extract when compared with untreated rats. The extract also revert decreased sucrase and lactase activity in treated iron deficient rats when compared with untreated rats. The efficacy of the extract may be due to its components including iron, saponin and cardiac glycosides. This work proposed 25 mg/kg body weight as the likely non-lethal effective dose of the extract for the treatment of anaemia, though, further toxicological studies are still required to ascertain this claim.

1. Introduction

Iron is essential for a wide variety of metabolic processes in living systems. Hence iron deficiency is manifested as a complex systemic disease [1]. One of the major manifestations of iron deficiency is iron deficiency anaemia, which gives indication of the importance of iron in haemoglobin synthesis [2]. Iron deficiency anaemia is a worldwide nutritional deficiency. It occurs in about one-eighth of the world population. The latest world estimate has 42% of pregnant women, 30% of non-pregnant women, 47% of preschool children, and 12.7% of men older being anaemic [3].

Aside iron deficiency anaemia, iron deficiency has been reported to reduce disaccharidases activities [1,4,5]. These enzymes catalyse the last stage of carbohydrate digestion [6]; hence it is not a surprise that weight loss is one of the symptoms of iron deficiency [7]. Different animal models including man have been used to link iron deficiency with reduced activity of disaccharidases, yet an explicit explanation of their relationship is yet to be elucidated.

High prevalent and economic loss due to iron deficiency is more in developing countries [3]. Poverty has been reported to be the likely cause of this high prevalence [8,9]. Paradoxically, African countries falling in developing countries in the world [10] are rich in plants traditionally used for the treatment of anaemia [11,12]. Of these plants reported, only a few have been scientifically verified for their efficacies in the treatment of iron deficiency anaemia. Among the few verified are *Sorghum bicolor, Jatropha tanjorensi* and *Mangifera indica* L among others [13–15].

A previous study by Nvvinuka et al. [15] showed that crude extract of *Mangifera indica* L stem back has anti-anaemic activity. However, a major drawback was the lack of optimized quantification of the extract that elicits this effect, a problem associated with

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arbitrary use of herbs. In addition ethanolic extract of *Mangifera indica* L stem bark has been reported to also have anti-anaemic potential [16]. However, ethnobotanical survey reports showed that aqueous extract of *Mangifera indica* L stem bark is used in the treatment of anaemia. Moreover, most of these studies on anti-anaemic potential of *M. indica* were carried out on normal rats. Hence, there is a need to verify this claim using an anaemic model, and quantitate the minimum effective dose of aqueous extract of *Mangifera indica* L stem bark that will elicit anti-anaemic properties. Aside anaemic condition, reduced disaccharidases activities has been reported in iron deficient rats [1,17,18]. This work aims to investigate minimum effective dose of the extract and its effect on some disaccharidases activity.

2. Materials and methods

2.1. Laboratory animals

Thirty six [36] weanling albino rats of both sexes with mean weight of 40.0 g ± 3.0 g were obtained from the small animal holding unit of the Department of Biochemistry, University of Ilorin, Nigeria. This study was approved by the Departmental Ethical Committee on the Use of Laboratory Animals.

2.2. Feed components

Yellow maize (Zea may) and locust bean [Parkia biglobosa (A.) Jacq] seeds were obtained locally from Oke-Oyi Market, Ilorin, Nigeria while soybean oil and vitamin mix were product of Grand Cereals and Oil Mills Ltd, Bukuru, Jos, Nigeria and Globe Vet Ltd, Bristol, England respectively. Components of the mineral mix were products of Sigma–Aldrich, 3050 Spruce St., St. Louis, MO 63103, USA.

2.3. Reagents

All other reagents used were of analytical grade and were prepared in all glass-distilled water. The reagents were stored in reagent bottles except biuret reagent which was stored in plastic container [19].

2.4. Plant authentication and extract preparation

Fresh stem bark and leaves of *Mangifera indica* L obtained from Faculty of Agriculture, University of Ilorin was authenticated in the Department of Plant Biology, University of Ilorin where voucher specimen was deposited with voucher number UIH 1080.

The aqueous extract of the plant stem bark was prepared using the method described by Oladiji et al. [13]. The dark brownish slurry obtained from the extraction process was reconstituted with distilled water to 25 mg/kg body weight, 50 mg/kg body weight and 75 mg/kg body.

2.5. Diet formulation

The method of Oladiji et al. [13] was used for diet formulation with slight modification. The locust bean was soaked in clean water for 24hr and sundried until a constant weight was obtained. It was ground and sieved to remove the seed peel. The yellow maize obtained from the market was ground to powdery form. All the components of the diets as shown in Table 1 were thoroughly mixed and made into pellets to ensure optimum feeding of the animals. The feeds were produced on a weekly basis and packed into air-tight polythene bags to prevent rancidity, auto-oxidation of the oil and microbial contamination.

Table 1
Composition of formulated diets (g/kg).

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

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

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, 10 g; Methionine, 15 g; 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.

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. Proximate analysis of the formulated diet

The iron sufficient and deficient diets were analysed for ash and organic minerals [20], fat and crude fibre [21], crude protein, carbohydrate and moisture content [22].

2.7. Animal grouping and extract administration

The rats were assigned into 2 groups (A and B). Group A (the control group) consisted of eight [8] rats while group B (the study group) consist twenty eight [28] rats. The rats in each group were housed together in metabolic cages under standard conditions (12-h light:12-h dark, 28 °C ± 3 °C and 40–55% humidity). The animals were fed with rat pellet and allowed free access to water and libitum. The acclimatization period was seven days. They were fasted for 24 h without food but given free access to water before feeding them of experimental diets.

The animals were fed with iron deficient diet to induce iron deficiency state. Rats in group A were maintained on iron sufficient (IS) diet while those in group B were maintained on iron deficient diet for a period of four weeks after which four rats from each groups were sacrificed and their haematological indices determined. The remaining rats in group A were maintained on Iron sufficient diet while those remaining in group B were randomly assigned into 6 groups (4 rats per group) as follows:

B_1- Rats maintained on iron deficient feed for the next two weeks (ID).

B_2- Rats maintained on iron sufficient feed for the next two weeks (change of feed) (CF).

B_3- Iron deficient rats orally administered with a reference iron supplement for the next two weeks on daily basis (SD).

Animals in groups B_4, B_5 and B_6 were orally administered 25, 50 and 75 mg/kg body weight of the aqueous extract of *Mangifera indica* L stem bark on daily basis the next two weeks and were designated MI25, MI50 and MI75 respectively. These doses were selected based on previously observed effect of 100 mg aqueous extract of *Mangifera indica* L stem bark on albino rats [23].

The extracts and the reference drug were administered to the corresponding groups via oropharyngeal cannula.