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Potential capability of *Azanza garckeana* fruits aqueous extract on enhancement of iron absorption in Wistar albino rats

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Abstract

The current study has evaluated the effect of the aqueous extract of the fruits of *Azanza garckeana* on iron deficiency anemia by using iron deficient rats and determination of iron absorption capability of the plant in everted gut sacs of Wistar albino rats. Administration of 2g/kg body weight of *Azanza garckeana* aqueous extract to iron deficient rats for 3 weeks caused slight alterations on hematological parameters of the nutritionally iron deficient rats except on red blood cells counts of these animals. *Azanza garckeana* enhanced iron absorption in everted sacs of rats. Phytochemical screening of the plant extract revealed the presence of moderate contents of iron element and various phytochemical components like triterpenes, flavonoides and saponins.

Keywords: Plant, iron deficiency, anemia.

1. Introduction

Anemia is a public health problem that affects people from both developed and un developing countries. It is defined as a condition in which the number of red blood cells (RBC) or the amount of hemoglobin (Hb) which carries oxygen in them is low. Iron deficiency anemia is the most prevalent type of anemia worldwide and approximately 30% of the world population is affected by iron deficiency anemia (Staubli Asobayire, 2005). Anemia in the developing world is most commonly caused by an iron deficiency, which not only impairs the production of red cells in the blood, but also affects general cell growth and proliferation in tissues like the nervous system and the gastrointestinal tract (Isselbacher, 1994). Inadequate dietary iron, iron absorption and intense exercise, along with blood loss and parasitic infestations, are some etiologies of iron deficiency anemia. Iron is a vital element for the living organisms for its many functions as it is attributed to oxygen transport, DNA synthesis, and electron transport. Absorption of iron occurs primarily in the duodenum and mostly in the upper jejunum. Iron deficiency occurs when iron needs of the body exceeds its absorption from the diet. So, normal iron balance can be maintained by regulation of iron absorption (Maurice, *et al* 1999). Medicinal plants are used since ancient times by man for the treatment of various ailments; they have been used widely as food supplements in cases of poor nutrition and to counteract blood loss. *Azanza garckeana* fruits (locally known as Jakjak in Sudan) are widely consumed in Western parts of Sudan as a nutritive plant and in cases of iron deficiency anemia.

The current study aimed to assess the putative assumption for using this plant by natives in Sudan for the treatment of anemia. This assumption is evaluated by using different in vivo and in vitro pharmacological methods.

2. Materials and Methods

2.1. Plant extraction:

The aqueous extract of the plant was prepared by taking 500 gm of fruit pulp, ground and sifted. Boiling water was added and it was agitated for 90 minutes. It was steeped for 4 hours at 4◦C and then filtered with a vacuum pump. The extract was evaporated and freeze-dried.

2.2. Phytochemical screening

General phytochemical screening for the active constituents was carried out for all extracts using the methods described by Sofowora (1993), and Harborne (1984) with few modifications.

2.3. Determination of iron content of the plant:

Determination of iron content of *Azanza garckeana* fruits was carried out by using atomic absorption spectrophotometer as stated by Stewart (1989).

2.4. Effect of *A. garckeana* extract on iron deficient rats:

Fifteen male Wistar rats of 21 days of age were used in the nutritional anemia experimental model. All of the rats were obtained from the animal house of Medicinal and Aromatic Pants Research Institute-National Center for Research-Sudan (MAPRI). Two types of diets were used in the above experiments. The first diet (A) has contained 39.15 mg Fe/kg diet. The source of iron in this diet was from animal protein. While diet (B), the iron deficient diet has contained 26.7 mg Fe/kg diet and the source of iron was from plant origin (wheat only). Ten rats were fed with iron deficient diet (26.7 mg Fe/kg diet) for 28 consecutive days before experimentation and this diet continued for another 3 weeks after the beginning of the experiment. These iron deficient rats were then divided into two groups: one of them was treated with *A. garckeana* extract at a dose of 2g/kg body weight. The other group was served as control iron deficient group. The third group of rats was fed with a normal iron-content diet (39.15 mg Fe/kg diet) and served as a control group. Blood was withdrawn at the before and after the treatment period from orbital plexus of rats. Hematological parameters measured were: Hb, PCV, RBC count, MCV levels, MCH levels and MCHC levels by using an automatic hematology analyzer. Data were analyzed by using SPSS program version 16. Results are considered significant at $P \leq 0.05$.

2.5. Effect of Azanza garckeana fruits extract on iron absorption by everted gut sac method:

12 Wistar rats were sacrificed by cervical dislocation and 3 cm length pieces of duodenum were immediately extracted, stripped of adhering tissue and cleaned with a Ringer solution (9%) in mmol/L: NaCl 0.154, KCl 0.0034, HCO_3Na 0.0024 and $CaCl_2$ 0.021. The everted gut sacs were hanged in an incubatory medium containing 200Mmol/L of $FeSO_4$. In the first preparation, there were the physiological solution and the $FeSO_4$ only and it was kept as a control preparation and in the second preparation there was 20mg/ml of *A. garckeana* extract, the physiological solution and the $FeSO_4$. The two preparations were maintained at 37◦C and bubbled with O_2. Iron concentration was measured by using PERKIN- ELMER- 2380 atomic absorption spectrophotometer after 1, 5 and 15 minutes from addition of the extracts and the $FeSO_4$ solution. Each experiment was repeated six times for each group. And means of iron concentration for each group was recorded (Khemiss, *et al*. 2006).

3. Results

Phytochemical screening conducted on *Azanza garckeana* aqueous extract revealed the presence of high amounts of Triterpenes and moderate amounts of saponins in addition to other chemical constituents as presented in Table [1].

Table [1] Results of phytochemical screening. Key: + Trace, ++ Moderat, +++ High, - Negative

Test	Observation	Result
Alkaloids	Turbidity	+
Sterols	Green-blue colour	-
Triterpenes	Pink-purple colour	+++
Flavonoids	Yellow colour	++
Saponins	Foam	++
Cumarins	UV florescence	++
Tannins	Green-blue colour	++
Anthraquenones	No observation	-
Cyanogenic	No observation	-

The amount of iron content of *Azanza garckeana* fruits was found to be 6.8mg/ 100 g pulp. Studying the effect of *Azanza garckeana* on iron- deficient rats, revealed that the *A. garckeana* extract did not show any obvious alterations in the hematological parameters of the nutritionally iron deficient rats (Table 2, Figures 1 and 2). However, the extract caused a slight increase in the red blood cell count of the iron deficient rats (3%) expressed in the third week of treatment (Table 3).

Table [2] Effect of *A.garckeana* aqueous extract on Hb level (mg/dl) of iron-deficient rats

	Normal diet group	**Deficient diet group**	***A.garckeana* group**
Week 0	13.72 ± 0.19	12.18 ± 0.59	12.42 ± 0.48
Week 1	13.32 ± 0.44	12.2± 12.2	12.1 ± 1.1
Week 2	12.72 ± 0.31	11.72 ± 0.68	10.525 ± 0.79
Week 3	13.9 ± 0.52	12.04 ± 1.13	11.76 ± 1.83

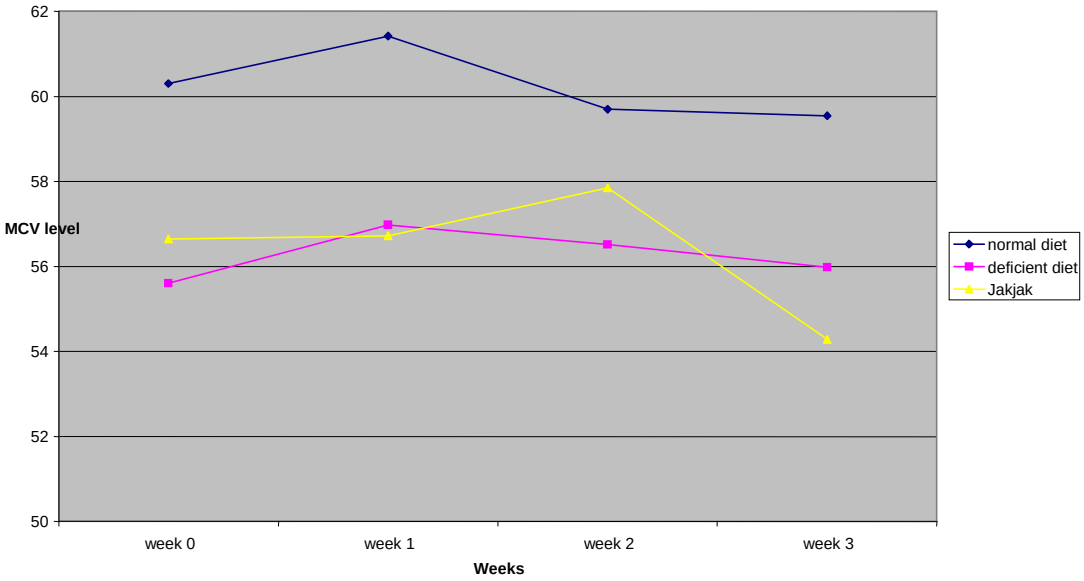

Figure [1] Effect of *A.garckeana* aqueous extract on MCV level of iron-deficient rats:

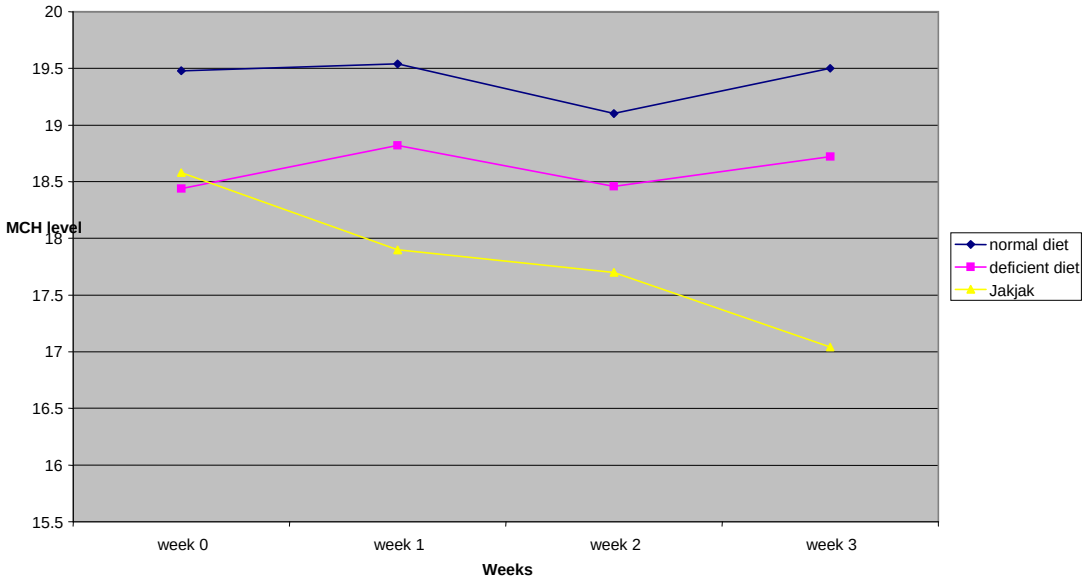

Figure [2] Effect of *A.garckeana* aqueous extract on MCH level of iron-deficient rats:

Table [3] Effect of *A.garckeana* aqueous extract on RBC count of iron-deficient rats

	Normal diet group	Deficient diet group	*A.garckeana* group
Week 0	7.062 ± 0.36	6.61 ± 0.3	6.698 ± 0.45
Week 1	6.822 ± 0.27	6.492 ± 0.5	6.498 ± 0.58
Week 2	6.66 ± 0.22	6.358 ± 0.52	5.9725 ± 0.51*
Week 3	7.128 ± 0.22	6.446 ± 0.68	6.886 ±0.7*

(Data are expressed in Mean SD)
* significant at P≤ 0.05

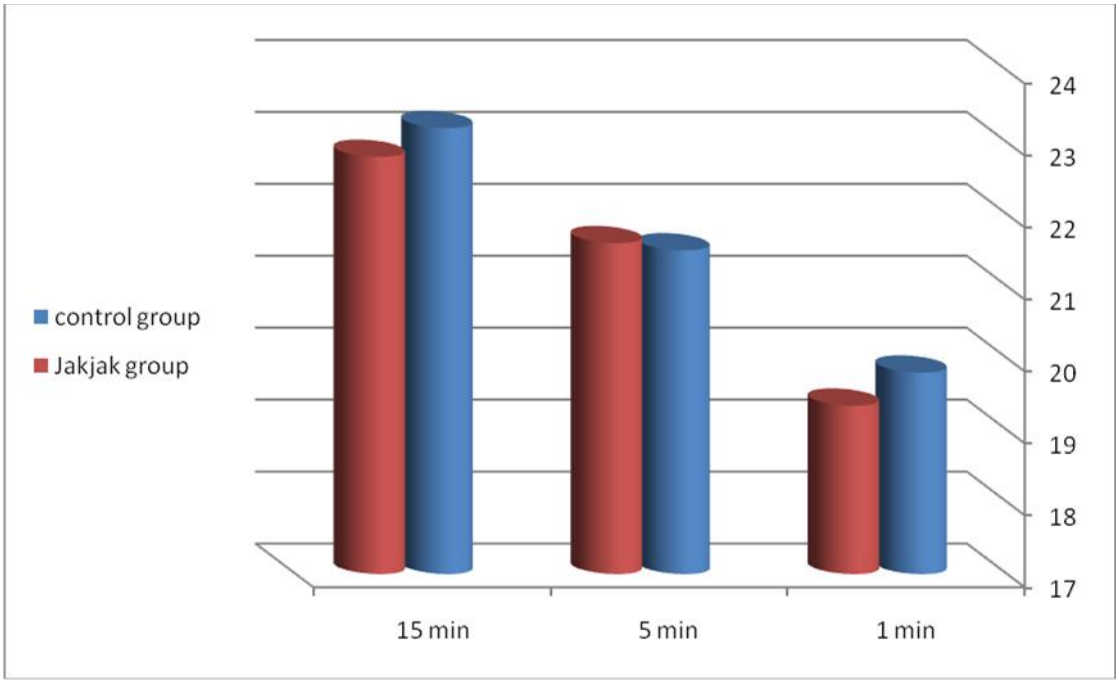

Figure [3] Effect of Azanza garckeana on iron absorption by using everted gut sacs.

Studying the effect of *Azanza garckeana* on iron absorption by using the everted gut sac method, revealed that iron concentration in the solution of the control group was higher than in the treated groups. It was estimated by 19.8 Mmol/L, 21.5 Mmol/L and 23.2 Mmol/L at incubation times of 1, 5 and 15 minutes respectively after the addition of $FeSO_4$ solution while in the treated group, it was found to be 19.3 Mmol/L, 21.6 Mmol/L and 22.8 Mmol/L at the same periods of time. These results show the potential effect of *Azanza garckeana* in enhancing iron absorption in the fifteenth minute after addition of Fe SO_4 solution, when compared with control group (Figure 3).

4. Discussion

This study has aimed to evaluate the potential effect of *A. garckeana* aqueous extract in enhancing iron absorption by using the everted gut sac method and thus its efficiency in treating anemia as assumed in its traditional use by natives in Western Sudan.

Anemia is a very common condition in Sudan and many medicinal plants have been used traditionally for the treatment of iron deficiency anemia and some of these plants have shown high levels of iron content (Yagi *et al*, 2013). *Azanza garckeana* is a valuable edible fruit tree species that is widely distributed in east and southern Africa and it is reported to grow in Sudan either Mbuya (1994). Fruits of this species are edible and are eaten while slightly green or when ripe Palgrave, (1988) and Mojeremane *et al* (2004). Phytochemical studies of this plant revealed that the aqueous and organic fractions from *A. garckeana* showed that this plant had a weak anti-malarial activity Connelly *et al* (1996). Whereas Roy, *et al* (1992) stated that the solvent fractions of the heart

wood of *Azanza garckeana* had yielded six naphthoquinones).

In the current study, the iron content of *Azanza garckeana* fruits showed a moderate level of iron content when compared to other plant species in the same family (Yagi *et al*, 2013). However, evaluation of its aqueous extract on iron absorption at the duodenal level, revealed its beneficial effect on enhancing iron absorption compared to the control group. This effect may justify its use for treatment of iron deficiency anemia as this plant contributes to enhancement of iron deficiency rather than providing the body with rich iron source. Thus, *A. garckeana* extract was found to have stimulating iron absorption properties when used on in- vitro iron absorption model. However, these stimulating effects were not found in in-vivo iron- deficient anemia model, except of a minor increment showed in the red blood cell count of the treated group. As it has been proven previously, saponins contents of plants has the ability to trigger hemolysis of red blood cells (Rosi *et al*, 2104). Thus this effect of *A. garckeana* extract may be attributed to its saponins contents causing an increase in production of red blood cells and hence increasing their numbers. These slight alterations in the effect of the plant upon iron absorption and treatment of nutritional can be further elucidated by using various models of induced iron deficiency anemia.

Conclusion

Fruits of *A. garckeana* that used traditionally in Sudanese folk medicine for the treatment of anemia, has a potential effect in increasing iron absorption rather being a rich source of iron and different doses of the extract can be used to establish its stability in inducing favorable erythropoietic properties.

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