

Original Research Article

The effect of aqueous extract of *Hibiscus sabdariffa* seeds on hematological parameters of anemic rats

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Abstract

The current study was conducted to investigate the effect of the aqueous extract of *H. sabdariffa* seeds on anemic rats. Anemia was induced in rats by two models, the first model is feeding rats with iron deficient diets for the induction of nutritional anemia while the hemorrhagic anemia model was induced in rats by bleeding off 30 % their blood volume. 400 mg of the extract/kg body weights of rats administered to anemic rats has increased their hematological parameters. This increase was significant in the hemoglobin, PCV and RBC count of hemorrhagic anemic rats and the extract caused significant increase in the hemoglobin level of the nutritionally iron-deficient rats.

Keywords: Roselle, medicinal plants, hematology, nutrient supplements

Introduction

Anemia is a public health problem that affects populations in both rich and poor countries. Its primary cause is iron deficient dietary intake, but a number of other conditions, such as malaria, parasitic infection, other nutritional deficiencies, and hemoglobinopathies are also responsible, often in combination. According to the WHO the global prevalence of anemia for the general population is 24.8% and it is estimated that 1620 million people are affected by anemia [1]

Hibiscus sabdariffa (Roselle) is a herbal shrub plant native to tropical Africa and grow in warm countries. The seeds of *Hibiscus sabdariffa* are used for edible oil production and the bi products of this process are used for poultry feeding [2]. Also, the seeds of this plant were reported to have various biological activities such as anti-oxidant [3] and anti- bacterial activities [4]

Many medicinal plants were used traditionally for the treatment of various types of anemias. One of these plants is the *Hibiscus sabdariffa* seeds that are used by natives in Western Sudan for the treatment of anemia.

Material and Methods

Seeds of *Hibiscus sabdariffa* were collected from the farm of Medicinal and Aromatic Plants research institute (MAPRI) at Khartoum State where the plant was cultivated and then authenticated by the taxonomists in MAPRI.

Preparation of the extract

Seeds of *Hibiscus sabdariffa* were dried in shades and coarsely ground. Then they were soaked overnight in distilled water. The concentration of the extract was 50 % w/v. Then it was minced, filtered and kept at 4°C for daily administration. This method of extraction was chosen according to the traditional use of the plant in Western Sudan. The dose used in this study was 400 mg/kg body weight.

Animals

Fifteen Wistar albino rats weighing between 100 g and 125 g were used in the hemorrhagic anemia model. While other fifteen male Wistar rats newly weaned, weighing between 50g and 90 gm were used in the nutritional anemia model. All of the rats were obtained from the animal house of MAPRI. The animals were acclimatized for 7 days in the department of Pharmacology and Toxicology of MAPRI before experimentation. They were fed *ad. Libitum* on a standard diet prepared in the animal house of the institute.

Diets

Two types of rats' diets were used in these 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 only so that iron would be of a lower bioavailability to rats.

Determination of iron content in the diet

1 gm of the ground powder of the rats' diet was weighed in a crucible and inserted in an oven of 500 °C for 3 hours. 50 ml of 20 % HCl were added and then filtered in a volumetric flask washed with distilled water and then the volume was completed to 50 ml. All the organic matter is then converted to inorganic ions [5]. The iron content in the diets was then determined by using an atomic absorption, Perkin- Elmer, 2380.

Hematological studies

The effect of the aqueous extract of *Hibiscus sabdariffa* seeds was investigated on two rat- model types of anemia, the nutritional anemia model (iron-deficiency anemia) and the hemorrhagic anemia model.

In the hemorrhagic model, rats were made anemic by bleeding off 30 % of their blood volume through the orbital plexus which is 5-7 ml in each rat [6]. 24 hours after bleeding hematological parameters of the bled rats were determined and thus considered as the baseline of the hematology values [7]. Hemorrhagic anemic rats were divided into three groups of 5 rats in each group and labeled A, B and C. The first group A was the normal un-bled group. They received distilled water only and served as the control un-bled group. Groups B and C were bled 30 % off their total blood volume. The second group B was administered with distilled water and served as control bled group. While the third group C received a daily dose of 400mg/kg body weight of the aqueous extract of *Hibiscus sabdariffa* seeds. The administration of the extract continued for consecutive three weeks. Hematological parameters

were measured twice, before and after treatment at the end of the experiment.

In the iron-deficient model is a modified model of [8]. The newly weaned rats were made anemic by feeding them with experimentally iron-deficient diet for 28 consecutive days and throughout the period of treatment.

After determining the basal hematological values of the nutritionally anemic rats, the treatment with the extract commenced. Hematological parameters were determined before treatment and three weeks later. Multiple comparisons were made between the treated group, the control with iron deficient diet and another control group that was fed with a normal iron-content diet.

Hematological parameters studied in both experiments were: Hb level, PCV, MCV, MCH, MCHC levels and RBC counts.

Results

Base line hematological parameters of the bled rats were recorded in table 1. It was found that administration of 400mg/kg from *Hibiscus sabdariffa* extract to hemorrhagic anemic rats for three weeks has caused significant changes in some hematological parameters of rats. These changes were compared statistically to changes made by control groups, bled and unbled rats.

The increase in the hemoglobin level of the bled treated rats was found to be statistically significant at $P < 0.05$ when compared with the hemoglobin values of the control groups. On the other hand, a remarkable increase (at $P < 0.05$) was observed in the PCV levels and RBC counts of the bled treated rats.

However, the extract caused a non -significant increase in the MCV level and no significant change on MCH and MCHC levels of the hemorrhagic rats, table 2.

Table 1: Hematological Parameters after 24 hours from bleeding (Base line hematological Parameters)

Groups	Hb (g/dl)	PCV (%)	RBC ($10^6/\mu$)	MCV (fl)	MCH (Pg)	MCHC (g/dl)
Control unbled rats	13.45±0.4	44.4±3.64	7.57±0.7	58.7±0.88	17.87±1.34	30.42±1.88
Control bled rats	11.05±1.77	36.9±6.33	6.24±1.12	59.25±1.57	17.77±0.7	30±0.66
<i>H.sabdariffa</i> group	9.92 ±1	30.94±2.5	5.04±0.47	61.44±1.1	19.68±0.46	32±0.67

Table 2: Effect of *Hibiscus sabdariffa* aqueous extract on hemorrhagic anemic rats

Group	Hb (g/dl)	PCV (%)	RBC ($10^6 \mu$ L)	MCV(fl)	MCH(Pg)	MCHC(g/dl)
Control bled group	12 ± 0.19	35.9 ± 0.68	6 ± 0.25	59.67 ± 2	19.92 ± 0.7	33.43 ± 0.62
Control un bled group	12.25 ± 0.72	37 ± 2.4	6.2 ± 0.35	59.35 ± 1.7	19.6 ± 0.62	33 ± 0.38
<i>H. sabdariffa</i> group	13.76 ± 0.82*	43 ± 2.13*	6.9 ± 0.26*	61.88 ± 1.9	19.78 ± 0.82	32 ± 0.74

Data are expressed as Mean ± standard deviation

*: Significant increase at $P < 0.05$

Administrations of *Hibiscus sabdariffa* aqueous extract among the nutritionally anemic rats have shown significant increases in the hemoglobin level at $P < 0.05$. These results were compared with hematological values of control rats fed with iron- deficient diets. However, the extract did not cause specific change on the MCV, MCHC and RBC values, table [4].



Table 3: Effect of *Hibiscus sabdariffa* aqueous extract on iron-deficient rats (before treatment):

Groups	Hb (g/dl)	PCV (%)	RBC ($10^6\mu\text{L}$)	MCV(fl)	MCH(Pg)	MCHC(g/dl)
Deficient diet group	12.8 \pm 0.59	36.76 \pm 2	6.61 \pm 0.3	55.6 \pm 0.86	18.44 \pm 0.36	33.16 \pm 0.76
Normal diet group	13.64 \pm 0.23	43.04 \pm 1.29	7.08 \pm 0.37	60.86 \pm 2.24	19.32 \pm 0.93	31.68 \pm 0.67
<i>H.sabdariffa</i> group	12.52 \pm 0.86	38.46 \pm 2.6	6.69 \pm 0.41	57.48 \pm 1.5	18.72 \pm 0.46	32.54 \pm 0.6

Table 4: Effect of *Hibiscus sabdariffa* aqueous extract on iron-deficient rats (after treatment):

Groups	Hb (g/dl)	PCV (%)	RBC ($10^6\mu\text{L}$)	MCV(fl)	MCH(Pg)	MCHC(g/dl)
Deficient diet group	11.72 \pm 0.77	41.62 \pm 2.1	7.05 \pm 0.43	59.1 \pm 1.7	16.55 \pm 0.42	28.15 \pm 0.61
Normal diet group	12.72 \pm 0.71	45.32 \pm 2	7.33 \pm 0.37	61.85 \pm 1.38	17.38 \pm 0.61	28.07 \pm 1
<i>H.sabdariffa</i> group	12.7 \pm 0.65 *	39.24 \pm 2.48	7 \pm 0.48	56.12 \pm 1.32	18.24 \pm 0.56	32.48 \pm 0.5

Data are expressed as Mean \pm standard deviation. *: Significant increase at P<0.05

Discussion

Since ancient times, *Hibiscus* species (Malvaceae) have been used as a folk remedy for the treatment of skin diseases, as an antifertility agent, antiseptic, and carminative. The fat of the seed is a good source of essential fatty acids (arachidonic, linoleic and linolenic acids) which are required to prevent fatty acid deficiency diseases such as skin lesions and low growth rate [9].

An important fact about this plant is that it is used in Sudan as a meat substitute when cooked and fermented for 9 days as described by [10]. Thus, it was important to take advantage of these characteristics and to evaluate the efficiency of this plant in the treatment of anemia. In the current study, iron-deficiency and hemorrhagic types of anemia were induced experimentally in rats and the effect of aqueous extract of *H. sabdariffa* seeds was evaluated. This plant caused beneficial effects on some hematological parameters of both groups of anemic rats with special emphasis on Hb level, PCV and RBC count. However, further studies are needed with different doses and various treatment periods to investigate the possible potentials of this plant in the treatment of anemia and to isolate the active compounds responsible of these effects.

In conclusion, *H. sabdariffa* seeds can be used with caution for repletion in cases of iron deficient anemia and these cautions are

attributed to the fact of the presence of several antinutrients and the highly acidic taste of the seed [11]. However, these factors may be encountered by methods of processing that will reduce their effects without significant loss of nutrients.

Authors Contribution

Dr. Reem Hassan Ahmed has carried out the experiments and prepared the manuscript.

Prof. Abd Wahab Hassan Mohammed has revised the manuscript

Prof. Hashim Mohammed El Hadi has revised the manuscript before submission

Dr. Mofida Yousif El khalifa has aided in interpretation of results

This research article is part of the joined research project "Study of plants used for the treatment of anemia" which is a joined research project between Medicinal and Aromatic Plants Research Institute and Traditional Medicine Research Institute- National Center For Research- Khartoum- Sudan

Acknowledgments

Special thanks are made to Mr. Osman Abakar herbal practitioner who provided us with the traditional knowledge of the plant.



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