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Article

# Investigation on effects of *Hibiscus sabdariffa* L. on hemoglobin level of mice with phenylhydrazine-induced anemia

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**Abstract:** With Sub-Saharan Africa having the highest prevalence of anemia globally and inequitable access to health care, traditional medicines are used by some of the population for treatment. *Hibiscus sabdariffa* L. (HS) is known to have medicinal applications, including its use in the treatment of anemia. The purpose of this study was to investigate the effect of HS on the hemoglobin level in anemic mice. A total of 15 mice were divided into three groups: Group A was the control group, while Groups B and C were the experimental groups, in which anemia was induced using phenylhydrazine. Only group C received oral administration of HS at 400 mg/kg. Blood samples were collected from the tail vein and analyzed using a HemoCue hemoglobin meter. The HS iron content was assessed using an atomic absorption spectrometer. The mean iron content for HS was 6.3 ± 0.6 mg/L. No significant difference ($p$ = 0.359) was observed in the mean hemoglobin levels between Groups A and B, which were 14.88 g/dl and 13.78 g/dl respectively. Higher hemoglobin levels were observed when Group C (17.46 g/dl) was compared with Group A ($p$ = 0.005) and Group B ($p$ = 0.001). These findings suggest the efficacy of HS in improving the hemoglobin level in anemic mice. Further investigations on doses and the mechanism are needed before recommendations for human consumption.

**Keywords:** *Hibiscus sabdariffa* L.; anemia; hemoglobin; phenylhydrazine-induced anemia; iron content

## 1. Introduction

Anemia is a public health problem that is widely spread and is associated with an increased risk of morbidity and mortality globally, especially in pregnant women and young children [1]. The World Health Organization (WHO) identified iron deficiency anemia as one of the 10 most serious risks for infant and adult mortality [2]. The global prevalence of anemia is 24.3% [3]. Anemia has the highest regional prevalence in Sub-Saharan Africa, with a slower decline over time than in other regions [4]. The contributing factors for this high burden include famine, poor diet, lack of access to good-quality healthcare systems, and infections, such as malaria, parasitic infestation, and HIV [5,6]. For instance, compared with those in developed countries, preschool-aged children from developing countries have a higher prevalence of anemia (10%–20% vs. 30%–80%, respectively) [5]. *Hibiscus sabdariffa* L., commonly grown in Sub-Saharan Africa, grows in sandy soils largely cultivated for additional income in rural areas [7]. It is reported to contain vitamin C, iron, phosphorus, calcium, manganese, aluminum, magnesium, sodium, potassium, anthocyanins, and other antioxidants [8]. *Hibiscus sabdariffa* L. is known to have medicinal applications for various diseases, including anemia, pyrexia, liver damage, high blood pressure, diabetes, and cancer. Its impact when consumed as tea was reported as a good remedy for soothing colds, clearing a blocked nose, promoting kidney function, and aiding

digestion [9–12]. The primary objective of this study was to investigate the effect of *Hibiscus sabdariffa* L. on the hemoglobin level of mice with phenylhydrazine-induced anemia.

## 2. Materials and methods

Fifteen white mice with initial hemoglobin levels of 12–18 g/dl at day 0 were used in the experiment. The mice were bought from local breeders in Zomba District, Malawi, and were kept in well-ventilated wooden boxes at an animal house at the University of Malawi, where they were fed a standard diet of breadcrumbs and small fish (Bonya) residues. The mice were divided into three equal groups of five mice each, labeled as Groups A, B, and C. Group A was the control group, in which anemia was not induced and only food and water were administered. The remaining two groups (Groups B and C) were the experimental groups, in which anemia was induced. Group B received only food and water and served as the control anemic group, while Group C received a daily administration of 400 mg/kg of the aqueous extract of *Hibiscus sabdariffa* L. calyces once a day for three consecutive weeks [9].

### 2.1. Methodology for iron test

Air-dried *Hibiscus sabdariffa* L. was powdered using a lab mill. A total of 2 g of the powdered *Hibiscus sabdariffa* L. was further dried using a drying cabinet maintained at 105 degrees Celsius for 2 hours and 30 minutes. The sample was then heated in a furnace for four hours to produce ash. A total of 1 g of the ash was dissolved in a mixture of 2ml sulfuric acid and 25ml ammonium nitrate. The mixture was boiled on a hotplate until a clear solution was obtained. The solution was then transferred to a 100ml flask and made to volume. The mixture was then analyzed in an atomic absorption spectrometer.

### 2.2. Preparation of extract

Dry *Hibiscus sabdariffa* L. calyces (500 g) were bought from the Mponda market in Mchinji District, Malawi. The calyces were boiled for 20 minutes in 1 L of water up to the point at which the color of water changed to rose red. The extract was then filtered using a filter paper so that only the aqueous part remained, and the residues were removed. This method of extraction was chosen according to the traditional use of the plant in Malawi. The concentration of the aqueous extract was measured by dividing the total mass of the calyces by the total volume of the extract. The extract was put in a bottle and stored in the refrigerator for preservation purposes. The extract was then administered to Group C at 400 mg/kg once a day for three weeks.

### 2.3. Induction of anemia in mice

The mice were weighed, and their weight ranged between 13–15 g. Anemia was induced in the mice by an intraperitoneal injection of 2.5% phenylhydrazine at 40 mg/kg for two days [13]. Each mouse received a different dose according to the weight of its body.

## 2.4. Blood collection and hematological analysis

Blood samples were collected from the tail vein using a syringe. The volume of blood samples collected ranged from 0.27 to 0.45 ml [13]. On day 0 of the experiment (before any induction of anemia or administration of the extract), samples of blood were collected from all three groups and analyzed using a HemoCue hemoglobin meter. Then, phenylhydrazine was injected into Groups B and C to induce anemia. On day 3 of the experiment, blood samples were collected from all three groups to determine if anemia was successfully induced. The blood samples were analyzed using the HemoCue hemoglobin meter. After three weeks of administration of the *Hibiscus sabdariffa* L. extract, blood samples of the three groups were collected and analyzed using the HemoCue hemoglobin meter.

## 2.5. Statistical analysis

The statistical analysis was conducted using R Studio statistical software. Descriptive statistics via mean ± standard deviation were used to define the data. The student *t*-test and one-way analysis of variance were used to compare the differences among the groups. A *p*-value of 0.05 was set to determine statistical significance.

# 3. Results

The mean iron content of *Hibiscus sabdariffa* L. was found to be 6.3 ± 0.6 mg/L. The hemoglobin level of Group A did not significantly change from day 0 to day 21, with a mean of 14.8 ± 0.08 g/dl, as shown in **Figure 1**. This was expected, for Group A served as the control. In Group B, while there was a 1.1 g/dl increase in hemoglobin after anemia was induced, this difference was not statistically significant ($p = 0.314$). In Group C, a 6.44 g/dl increase in hemoglobin was observed, which was statistically significant ($p = 0.001$), indicating an effect of *Hibiscus sabdariffa* L. on the hemoglobin level. When compared with that of Group A, Group B had a 1.1 g/dl lower hemoglobin level on day 21 ($p = 0.359$), while Group C had a 2.58 g/dl higher hemoglobin level ($p = 0.005$) on day 21. Group C also had a 3.68 g/dl higher hemoglobin level when compared with that of Group B ($p = 0001$) on day 21.

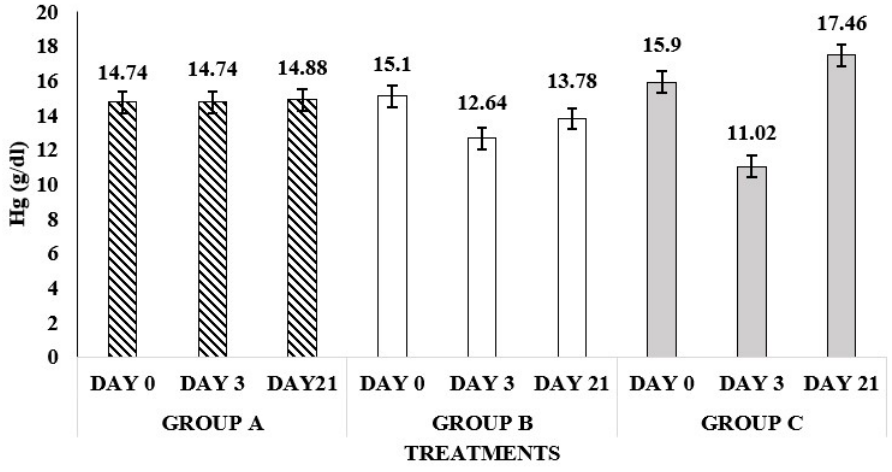


**Figure 1.** Comparison of mean hemoglobin content in mice before and after phenylhydrazine injection and after *Hibiscus sabdariffa* L. administration.

## 4. Discussion

The recommendation for daily iron intake is 10 mg/day for healthy men and postmenopausal women, 11 mg/day for children, 18 mg/day for women of reproductive age, and 27–30 mg/day for pregnant women [14]. *Hibiscus sabdariffa* L. (HS) is a good source of iron in the human diet, as indicated in our study, which showed 6.3 mg/L of iron content. It is worth noting that the iron content in plants is affected by a number of factors, including the type of soil and the location where the plant is grown [15]. A study in Nigeria reported 9.7 ± 0.3 mg/100 g of iron content in HS [16]. Iron bioavailability in plant-based sources is low, especially when compared with those of animal sources, and hence a higher quantity needs to be consumed to make a large impact [17].

In this study, the result indicates a better improvement from anemia in mice treated using the HS extract compared with the result of untreated mice, as well as a higher hemoglobin level compared with those of healthy mice receiving a standard diet. A similar study conducted by Ahmed et al. [9] investigated the effect of the aqueous extract of HS seeds on the hematological parameters of anemic rats. The study reported a significantly improved hemoglobin level in anemic rats receiving HS seed extract compared with that of the control. Together with our study, there is evidence indicating the efficacy of both the calyces and seeds of HS in treating anemia. There is still a lack of data on the impact of HS on anemia, even though most cultures in Sub-Saharan Africa use HS as a remedy. It is therefore important that more research studies are conducted to provide evidence for the use and safety of HS, as well as its appropriate doses.

## 5. Conclusion

*Hibiscus sabdariffa* L. was found to contain a high amount of iron, making it a good addition to the usual diet. It was also found to improve the hemoglobin level in mice with phenylhydrazine-induced anemia, providing evidence for the efficacy of *Hibiscus sabdariffa* L. in reversing anemia and why communities continue to use it as a remedy. However, there is not enough evidence to lead to a recommendation for its use in anemic patients, and so controlled clinical trials in humans should be conducted to investigate this health claim.

**Author contributions:** Conceptualization, IP and YK; methodology, IP and YK; investigation, IP; writing—original draft preparation, IP; writing—review and editing, YK; supervision, YK; project administration, YK. All authors have read and agreed to the published version of the manuscript.

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**Conflict of interest:** The authors declare no conflict of interest.

## References

1. Kraemer K. In: Nutritional anemia. Sight and Life Press; 2007.

2. Adamson P, MI/UNICEF. Vitamins & mineral deficiency: A global report. Available online: https://peteradamsonwriting.com/pla/vitamin-and-mineral-deficiency-a-global-report/ (accessed 22 June 2024).
3. Gardner WM, Razo C, McHugh TA, et al. Prevalence, years lived with disability, and trends in anaemia burden by severity and cause, 1990–2021: findings from the Global Burden of Disease Study 2021. The Lancet Haematology. 2023; 10(9): e713–e734. doi: 10.1016/S2352-3026(23)00160-6
4. Adamu AL, Crampin A, Kayuni N, et al. Prevalence and risk factors for anemia severity and type in Malawian men and women: urban and rural differences. Population Health Metrics. 2017; 15(1): 12. doi: 10.1186/s12963-017-0128-2
5. Osungbade KO, Oladunjoye AO. Anaemia in Developing Countries: Burden and Prospects of Prevention and Control. In: Anemia, Silverberg D (editor). IntechOpen; 2012.
6. MacDonald C, Mildon A, Neequaye M, et al. Anemia—can its widespread prevalence among women in developing countries be impacted? Available online: https://www.wvi.org/sites/default/files/Anaemia-Ghana-Malawi-Chapter.pdf (accessed on 9 June 2024).
7. Yaye KGD, Djibril S, Mansor N. Direct genetic transformation of Hibiscus sabdariffa L. African Journal of Biotechnology. 2004; 3(4): 226–228. doi: 10.5897/ajb2004.000-2041
8. Mahadevan N, Shivali, Pradeep K. Hibiscus sabdariffa Linn.—An overview. Available online: http://nopr.niscpr.res.in/handle/123456789/3769 (accessed 14 June 2024).
9. Ahmed R, Hassan AA, Hadi HME, Khalifa MYE. The effect of aqueous extract of Hibiscus sabdariffa seeds on hematological parameters of anemic rats. International Journal of Phytomedicine. 2013; 5(3): 278–281.
10. Liu H, Liang ZM, Li R, Yu YG. Advances in the mechanisms of Hibiscus sabdariffa L. on hypertension. E3S Web of Conferences. 2020; 145: 01039. doi: 10.1051/e3sconf/202014501039
11. Mohamed BB, Sulaiman AA, Dahab AA. Roselle (*Hibiscus sabdariffa* L.) in Sudan, Cultivation and Their Uses. Bulletin of Environment, Pharmacology and Life Sciences. 2012; 1(6): 48—54.
12. Odigie IP, Ettarh RR, Adigun SA. Chronic administration of aqueous extract of Hibiscus sabdariffa attenuates hypertension and reverses cardiac hypertrophy in 2K-1C hypertensive rats. Journal of Ethnopharmacology. 2003; 86(2–3): 181–185. doi: 10.1016/s0378-8741(03)00078-3
13. Diallo A, Gbeassor M, Vovor A, et al. Effect of Tectona grandis on phenylhydrazine-induced anaemia in rats. Fitoterapia. 2008; 79(5): 332–336. doi: 10.1016/j.fitote.2008.02.005
14. Clarke L, Dodds AJ. Iron deficiency anaemia: Causes, symptoms and treatment. Medicine Today. 2014; 15(11): 36–42.
15. Gassama-Dia YK, Sané D, Ndoye M. Direct genetic transformation of Hibiscus sabdariffa L. African Journal of Biotechnology. 2004; 3(4): 226–228. doi: 10.5897/ajb2004.000-2041
16. Oyewole AO, Diosady LL. Evaluating the potential of Hibiscus sabdariffa beverage to address the prevalence of iron deficiency in sub-Saharan Africa. LWT. 2023; 188: 115433. doi: 10.1016/j.lwt.2023.115433
17. Platel K, Srinivasan K. Bioavailability of Micronutrients from Plant Foods: An Update. Critical Reviews in Food Science and Nutrition. 2016; 56(10): 1608–1619. doi: 10.1080/10408398.2013.781011