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Article

Impact of the use of *Persea Americana* (avocado) leaves extract to treat anaemia: A clinical trial in mice

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Abstract: Anaemia remains a global disease burden with Sub-Saharan Africa having the highest prevalence. In recent years, African traditional medicines are being researched for their potential to increase universal health coverage. This study assessed the effectiveness of avocado leaves extract on haemoglobin levels in phenylhydrazine induced anaemic mice. Anaemia was induced in 15 mice using intraperitoneal injection of 2.5% phenylhydrazine at a dose of 40 mg/kg. Mice were divided into three groups, group A was the control which received no treatment, group B received iron supplementation and group C received avocado leaves extract. Blood samples were analysed using a hemocue. Analysis of data was done using ANOVA and t-test. Avocado leaves increased haemoglobin levels by 2.5 ± 1.6 g/dl, ($P = 0.031$) and iron supplementation increased haemoglobin by 2.9 ± 1.9 g/dl ($P < 0.001$), compared to the control. Compared to avocado leaves, iron tablets increased haemoglobin levels by 0.3 ± 1.1 g/dl, ($P = 0.951$). A mean 2.9 ± 0.02 mg/L of iron was found in the avocado leaves used in this study. Phytochemical analysis detected the presence of flavonoids and alkaloids in the avocado leaves. The study reveals the potential of avocado leaves extracts in reversing anaemia. Further studies are recommended to validate adequate dose of the avocado leaves extract in treating anaemia.

Keywords: anaemia; heamoglobin; traditional medicine; avocado leaves; iron supplementation

1. Introduction

Avocado leaves (*Persea Americana)* are a traditional Malawian medicine used for the treatment of anaemia [1]. Avocados have the highest iron content (1.02 mg/100 g) of any fresh fruit [2]. When crushed into powder, *P.Americana* leaves comprise of calcium (56.13 g/100 g), iron (14.61 g/100 g), flavonoid (8.11 g/100 g) and alkaloid (0.51 g/100 g) [3]. Iron plays an important role in erythropoiesis in living organisms and flavonoids greatly affect iron status by regulating expression and activity of proteins involved in the systemic regulation of iron metabolism and iron absorption [4]. A review on the impact of avocados on metabolic syndrome reported the use of avocado leaf extract in treating diabetes and hypertension, protecting against cardiovasular diseases, lowering plasma lipids and is high in bioactive compunds which show antioxidant activity [5]. Phytochemicals such as orhamnetin, luteolin, rutin, quercetin and apigenin have been isolated from avocado leaves which can help prevent progress of various diseases related to oxidative stress; cardiovascular and neurological conditions [6].

A recent systematic review of national and subnational survey data estimated a 24.3% global prevalence of anaemia in all ages. The bulk of the global anaemia burden was driven by under 5 children and women [7]. Sub-Saharan Africa had the highest

reported prevalence of anaemia which was attributed to poor nutrition, poor medical services, HIV/AIDS, infectious diseases and elevated levels of blood-sucking parasitic agents like plasmodium, trypanosomes and helminthes [7]. A 2017 Malawian cross-sectional survey reported a 8.2%, 6.7% and 1.2% prevalence of mild, moderate and severe anaemia respectively in rural men and in 19.4%, 12.0% and 7.4% in rural women aged 18 and above [8]. Among men, prevalence of both mild and moderate/severe anaemia was lowest in the 18–24-year age group and increased with age, particularly after 55 years of age. Among women, anaemia prevalence peaked in the 35–44-year age group [8]. While iron supplementation is the acceptable treatment for different forms of anaemia in Malawi, most people use water from boiled avocado leaves as a remedy [1]. This knowledge has been passed along for generations but there is a lack of scientific evidence to back its efficacy. The aim of this study was to assess the effectiveness of *P. americana* (avocado) leaves extract on haemoglobin levels in phenylhydrazine (PHZ) induced anaemic mice.

2. Materials and methods

2.1. Experimental animals and anaemia induction

A total of 15 mice sourced from local markets were used for this study; the sample size was calculated using the formula by Arifin et al. [9] which showed 15 to be the minimum required sample for one repeated measurement in mice that would not be sacrificed. Inclusion criteria included mice aged one month old with normal haemoglobin levels of 11–18 g/dl and a mean weight of 12 g. The mice were divided into 3 treatment groups of 5 each, each group had 2 females and 3 males using block randomisation. The control group (A) received no treatment upon induction of anemia. Group B was treated with iron supplement at a dose of 1 dissolved tablet per day for 21 days upon inducing anaemia. Group C was treated with boiled avocado leaves extract at a dose of 2 g/kg once a day, this was an unblinded study. The mice were housed in the experimental animal house of the Department of Biological Sciences and Biomedical Health Sciences at the University of Malawi, they were acclimatised to the environment for 2 weeks before the study commenced and were properly monitored. They were all fed the same diet of bread crumbs, rice, nsima and small fish (locally known as bonya) at 2 h intervals and given plenty of water. Anaemia was induced by intraperitoneal injection of 2.5% phenylhydrazine (PHZ) at 40 mg/kg for 2 days [10]. Mice were considered anaemic 2 days after PHZ administration (haemoglobin concentration drop below 11 g/dl). Blood samples were collected from the tail vein on day 0 before PHZ administration and on days 3 and 21 after PHZ injection for analysis using the hemocue machine. Blood was collected on day 3 to detect signs of anaemia after phenylhydrazine administration.

2.2. Avocado extract preparation

Fresh avocado leaves (500 g) were plucked from local avocado trees in Zomba catchment area. These were washed under running tap water and boiled in water for 1 h in order to extract the fluid. A total of 2.5 L of fluid extract was collected and kept in 5 tightly sealed containers at volumes of 500 mL each for use.

2.3. Phytochemical analysis

Clean avocado leaves were air dried in the laboratory for 3 days and the leaves were crushed into powder and stored in airtight Ziploc plastic bags. For the flavonoid test, 0.5 g of avocado leaves powder was added to a test tube of 10 mL distilled water, mixed well then filtered. 5 mL of dilute ammonia followed by 1 mL of concentrated sulphuric acid (H_2SO_4) were added to the filtrate. The presence of a yellow color would indicate presence of flavonoids [11]. To test for alkaloids, 0.2 g of avocado leaves powder was mixed with 3 mL of hexane in a test tube then filtered. 2% hydrochloric acid was poured into the filtrate then heated. After filtering, a few drops of picric acid were added to the filtrate. Formation of a yellow colored precipitate indicated presence of alkaloids [11].

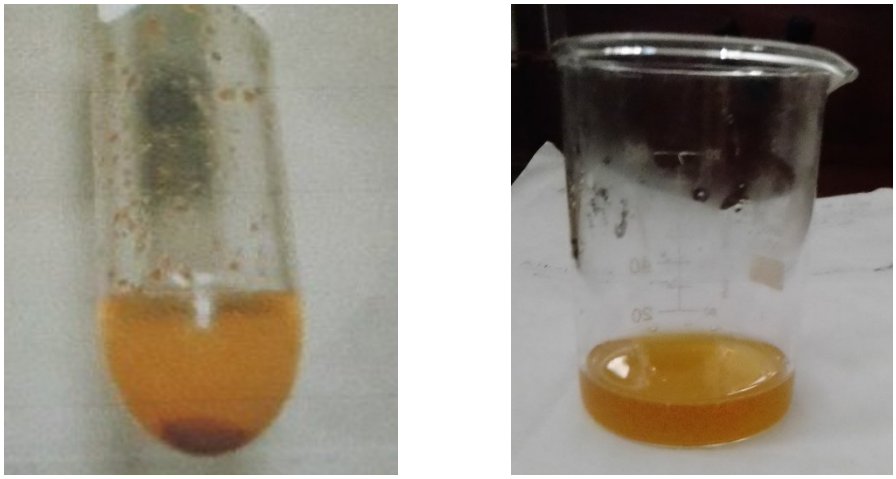
To estimate iron content, avocado leaves were chopped and washed, then air dried in the laboratory. Air dried samples were powdered using a lab mill fitted with a sieve of 2 mm diameter. 2 g of the powdered samples were further dried in an oven maintained at 105 ℃ for 2 h and 30 min [12]. The powdered samples were then subjected to ashing (an analytical procedure where a sample is heated in a furnace to produce ash residue to determine mineral content) in order to dissolve iron. The methodology described by Fairweather-Tait et al [13], was adopted for iron estimation in avocado leaves sample. Briefly, 1 g of oven dried avocado powder was dissolved in a 25 mL of ammonia nitrate and 2 mL sulphuric acid mixture, which was then boiled on a hotplate until a clear solution was obtained. The solution was then transferred to a 100 mL volumetric flask and made to volume, this sample was then analyzed in a calibrated atomic absorption spectrometer.

2.4. Data and statistical analysis

Microsoft Excel data spreadsheets were used for data entry and some mathematical computations, and in graphical presentation of data. Differences between the means of groups A and B, B and C, and A and C were analysed using *t*-test in R Studio statistical package. Results were considered statistically significant at $P < 0.05$. ANOVA was used to determine the differences in haemoglobin levels in the control and treatment groups.

3. Results

The mean weight of mice at baseline was 13.6 ± 0.8 g, 13.9 ± 0.9 g and 13.4 ± 0.7 g in groups A, B and C respectively; the difference of which was not statitistically significant, $p > 0.005$. The mean iron content of avocado leaves was 2.9 ± 0.02 mg/L. The phytochemical analysis detected the presence of alkaloids and flavonoids evidenced by yellow precipitate, **Figure 1**. The baseline haemoglobin levels were similar across the three groups both on day 1 and day 3 after inducing anaemia, $p > 0.05$, **Table 1**. Iron supplementation significantly increased haemoglobin levels by 2.9 ± 1.9 g/dl ($P = 0.001$) compared to the control group, **Table 1**. and **Figure 2**. Avocado leaves extract increased haemoglobin levels by 2.5 ± 1.6 g/dl ($P = 0.031$) compared to the control group. A 0.3 ± 1.1 g/dl higher heamoglobin level was observed in the iron supplementation group compared to the avocado leaves extract group, $P = 0.951$.



(a) **(b)**

Figure 1. Tests for phytochemicals in *p.americana.* **(a)** results of for alkaloids test; **(b)** results for flavonoids test.

Table 1. Mean changes in haemogloblin levels on days 1, 3 and 21 of the study.

Group	Day 1 (Mean(CI[1]))	Day 3 (Mean(CI[1]))	Day 21 (Mean(CI[1]))
Control	14.9 (12.0,17.9)	10.6 (9.5,11.7)	12.1 (10.8,13.3)
Iron supplementation	14.8 (12.8,16.9)	10.5 (9.0,11.9)	15.0 (13.2,16.7)
Avocado extract	15.1 (14.3,16.0)	10.7 (7.3,14.2)	14.6 (13.5,15.8)

[1]CI = confidence interval.

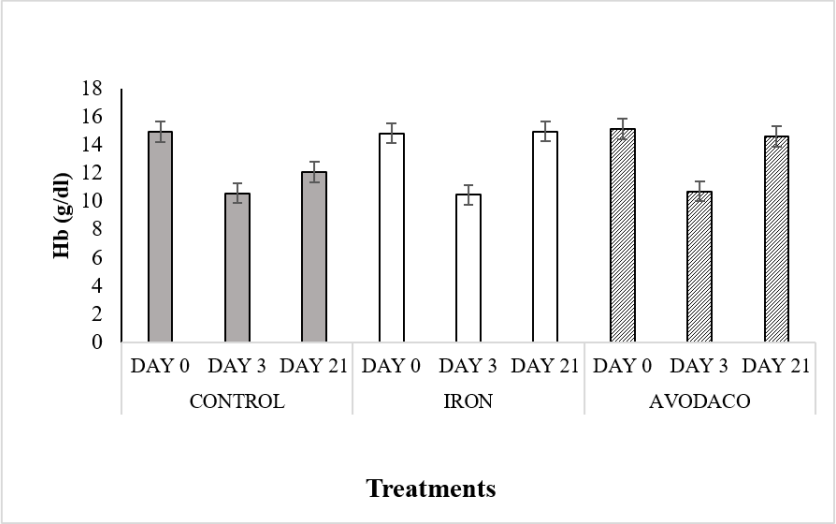


Figure 2. The effects of avocado extracts and iron supplements on haemoglobin levels.

4. Discussion

Since the dawn of time, herbal remedies have been relied upon as a solution for different diseases and used as an active ingredient in pharmacology [10]. Due to economic considerations, personal convictions or the difficulty of obtaining pharmaceutical drugs, many people throughout the world still turn to alternative

solutions such as Chinese medicine and African herbal medicine for treatment [14]. A large number of natural products are widely used in folk medicine to prevent and alleviate anaemia, these include herbs and products such as beetroot [15] and hibiscus sabdariffa decoctions [16]. This analysis has shown that supplementation of avocado leaves extract in phenylhydrazine induced anaemic mice increases haemoglobin levels in similar levels to iron tablet treatment and higher than the control (untreated mice). Avocado leaves have been shown to contain the phytochemicals alkaloids and flavonoids, and iron.

The presence of iron (2.9 ± 0.02 mg/L) and phytochemicals (alkaloids and flavonoids) could account for the anti-anaemic properties of avocado leaves observed in this study. Alkaloids and flavonoids protect cells by acting as powerful antioxidants which prevent or repair damage done to red cells by free radicals or highly reactive oxygen species [17]. Flavonoids have been reported to reverse hemolysis in hydrogen peroxide induced oxidative and increase anti-oxidative enzyme activities [4]. In another study, the authors concluded that flavonoids might protect erythrocytes from oxidative damage through attenuating oxidative stress, protecting anti-oxidative enzyme activities, and preserving integrity of erythrocyte structure [18]. Furthermore, flavonoids possess iron chelating properties that increase iron absorption, reduce iron excretion and increase the deposition of excess iron in tissues hence can be a possible treatment of iron deficiency anaemia [19].

A 1997 study on determination on iron content in herbs used in anaemia treatment by Omolo et al, revealed how leaves of blue sweet berry (*Bridelia cathartica*) have high amounts of iron (7.49 mg/100 g) hence effective in treating anaemia [20]. Other investigators also reported that the extract of African mahogany (*khaya senegalensis)* stem bark has anti-anaemic activity in phenylhydrazine-induced anaemic rats [21]. Another report indicates that oral administration of teak (*Tectona grandis)* extract to rats previously treated with phenylhydrazine, increased the concentration of haemoglobin, red blood cell number, hematocrit, and reticulocytes counts [17]. In these studies, PHZ caused oxidative damage to RBC by increasing formation of reactive oxygen species. The phytochemical screening in the extracts used in these past studies revealed the presence of saponins, flavonoids and alkaloids. Thus it appeared that the presence of these antioxidants in the plant extracts reversed the damaging effect of PHZ [22].

5. Conclusion

Our study indicates a similar effect of avocado leaves extract and iron supplementation on haemoglobin levels in anaemic mice. While the pathway for this observation is not well understood, the presence of iron, flavonoids and alkalods in the avocado leaves could explain the anti-anaemic properties reported. With the high rates of poor access to health care by most people in Sub-Saharan Africa, avocado leaves provide an easily accessible and cheaper potential remedy for aneamia; it is therefore important that further research is conducted to provide the necessary evidence for its efficacy.

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