Anti-Anaemic Effect of *Cnidoscolus chayamansa* (*Mc Vaugh*) Leaf Extract on Phenyl Hydrazine-Treated Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author RJK designed the study and performed the statistical analysis. Author LLI wrote the protocol and the first draft of the manuscript. Author MKJ managed the analyses and the literature of the study. Author CEM managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Background: Anaemia is one of the world's threatening disease conditions of blood disorder that leads to the decrease in red blood cells which affects people of all ages.
Aim of the Study: This study aimed to evaluate the effects of *Cnidoscolus chayamansa* leaf extract on phenylhydrazine hydrochloride induced anaemia in albino rats.
Study Duration: This study was conducted on 30th June 2019 at the Department of Biochemistry, Faculty of Basic Medical Sciences, University of Jos, Nigeria.
Methodology: A total of 16 rats were split into four groups. Group A the baseline for negative control, group B positive control, group C pre-treatment and group D post-treatment. Induction was done by intraperitoneal with phenylhydrazine at a dose of 40 mg/kg. Blood was taken for each 2 days interval for PCV, RBC, WBC and Hb. Preliminary phytochemical screening was investigated by standard procedures.
Results: The results show a significant (P<0.05) increase in serum PCV, RBC, WBC and Hb concentration when compared with the untreated group. Decrease in serum alanine aminotransferase (15.46±4.443 & 7.80±0.429), aspartate aminotransferase (20.89±1.095 &

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11.66±0.898), total bilirubin (13.70±0.351 & 10.45±0.620), direct bilirubin (7.49±0.659 & 3.76±0.042) and alkaline phosphatase (254.25±2.287 & 192.00±1.474) and a significant (P<0.05) increase in serum protein and albumin when compared with untreated group. Iron binding shows a significantly (P<0.05) increase (153.49±0.530 & 166.09±1.334) and (85.16±0.824 &98.43±0.562) while there was a significant (p<0.05) decrease in serum Urea (2.39±0.027&4.70±0.047), Creatinine (68.38±0.404&75.01±0.891), and Uric acid (234.92±2.761&246.52±1.136) when compared with the baselines.

Conclusion: The positive effect of the vegetable may be attributed to its rich phytochemicals, nutrients which supports the use of the leaves for food and ethnomedicinal purposes in many parts of Nigeria.

1. INTRODUCTION

Anaemia is a medical condition characterized by lowered haemoglobin level. There are over 400 types of anaemia, with haemolytic anaemia being the most frequent [1]. More than half of the world's population experience some forms of anaemia in their life time. The incidence of anaemia is higher in the third world than in developed countries [2]. A study in a rural population of Nigeria reported that 19.7% of the children were anaemic [3]. Such prevalence has been attributed to various aggravating factors such as poor nutrition, high prevalence of blood parasites for example, plasmodium, trypanosome and helminthes infection [4]. Prolonged use of non-steroidal anti-inflammatory drugs as well as exposure to toxic chemicals such as phenyl hydrazine have also been implicated to cause the condition [5]. Anaemia causes blood disorder that leads to the decrease in red blood cells which affects people of all ages especially the elderly, both pregnant women and infant, it is known that women are susceptible to anaemia during pregnancy due to high demand from the developing foetus [6]. As a result of the fact that anaemia is very common and the incidence is likely to increase in future, there is a need to prevent it or seek for more cost-effective and better treatment strategies [7].

Cnidoscolus chayamansa Mc Vaugh (Euphorbiaceae) is commonly known as 'chaya' in Central America. In South East Mexico, because of its high nutritional values, is an important part of the diet of many indigenous communities. Chaya is also used as a traditional remedy for the treatment of diabetes, rheumatism, gastrointestinal disorders and inflammation-related diseases [8]. Cnidoscolus chayamansa medicinal properties, including antioxidant, antitumoral, antimutagenic, antidiabetic, hypocholesterolemic, hepato-protective, gastroprotective and cardioprotective. Cnidoscolus chayamansa is one of most used and valued medicinal plants, only few studies on documenting its pharmacological properties can be found [9].

The leaf is a commonly consumed vegetable in many parts of Nigeria. It is also popular as a natural remedy against anaemia in this region [10]. Phytochemical screening of C. chayamansa leaf revealed that it contains bioactive principles such as alkaloids, flavonoids, tannins, cardiac glycosides, anthraquinones, and saponins [11]. Although plant based natural medicines are popularly acclaimed to be safe, the need for adequate toxicity testing have been emphasized [12]. This present study aimed to evaluate the anti-anaemic and phytochemical analysis of *Cnidoscolus chayamansa* leaf.

2. MATERIALS AND METHODS

2.1 Chemical and Reagents

Phenylhydrazine used was products of Sigma, St. Louis, USA. All other chemicals were of analytical grades and prepared in glass apparatus using distilled water.

2.2 Plant Material

The Fresh leaves of *Cnidoscolus chayamansa* were collected from a residential area in hill station Jos North L.G.A, Plateau state, Nigerian and deposited in a clean polythene bag after been washed with clean water.

2.2.1 Plant identification and authentication

The plant, *Cnidoscolus chayamansa* was identified and authenticated by O.E Agyeno from

the Department of Plant Science and Biotechnology, University of Jos, Nigeria.

2.3 Preparation Aqueous Plant Extract

The method described [13] was adopted for the preparation of the aqueous extract of *Cnidoscolus chayamansa* leaves. 100 g of fresh leaves of *Cnidoscolus chayamansa* was blended with a blender mixer and macerated into 560 ml of distilled for 48 hours the extract was filtered using Whatman filter paper and a suction pump to extract the liquid and the residue. The resulting residue was transferred to hot air oven where it was dried to a weight of 25 g at $40^{o}C$ after which it was mix with their feeds. The liquid extract was stored in the refrigerator at the department of biochemistry throughout the experiment.

2.4 Preparation and Induction of Anaemia with Phenylhydrazine Hydrochloride

Anaemia was induced by intra-peritoneal induction using 40 mg/kg per body weight with Phenylhyrazine hydrochloride which was prepared by 2.5% neutralize concentration as described [14] with slight modified.

2.4.1 Calculations per body weight

To prepare 40 mg/kg body weight 2.5% neutralized phenylhydrazine hydrochloride (PHZ) and maintenance dose of 15 mg/kg body weight 2.5% neutralized phenylhydrazine hydrochloride.

Note:

Dose = 40 mg/kg b. wt.

Concentration = 2.5% = 2.5 g/dL = 2.5 g/100 ml = 2500 mg/100 ml = 25 mg/ml

Remember that, 1000 mg = 1 g

Using the expression (derived from first principle);

Volume of PHZ = Dose (mg/kg) / Concentration (mg/ml) X Body weight (kg)

For a rat weighing 200 g = 0.2 kg,

Volume of PHZ = 40/25 X 0.2 = 0.32 ml

From the concentration of PHZ, 25 mg/ml,
If 1 ml of PHZ contains 25 mg of PHZ

Then 0.32 ml contains 25/1 x 0.32 = 8 mg = 0.008 g of PHZ, which can be weighed with a weighing balance.

The above calculation is done for all the animals and then the total weights of PHZ and distilled water (solvent) can be determined. After dissolving the PHZ in distilled water, the PH was determined to check the acidic content, if the solution is acidic. It must be neutralized with an alkaline solution because phenylhydrazine hydrochloride in acidic form can become toxic and may lead to the death of the organism.

The calculation using a maintenance dose of PHZ is done similarly,

Volume of PHZ = 15 mg/kg/25 mg/ml x 0.25 kg = 0.15 ml (If the weight of rat is 250 g)

1 ml contains 25 mg of PHZ

0.15 ml will contain 25/1 x 0.15 = 3.75 mg PHZ = 0.00375 g of PHZ.

2.4.2 Treatment with *Cnidoscolus chayamansa*

Sixteen healthy rats of both sexes were grouped into four groups each consisting of four rats as follows respectively;

Group A: Serves as Positive control, anaemia was induced without treatment and was given normal feed and water.

Group B: Serves as the Negative control, no inducement of anaemia and was given normal feed and water.

Group C: Serves as the post-treated group, anaemia was induced and confirmed after two days and treated orally with 5 ml of the plant extract for each of the four rats with the mixture of the plant residue with feeds of equal amount for fifteen (15) days.

Group D: Serves as the pre-treated group, treatment with 5 ml of plant extract and an equal amount of plant residues mix with the feed for seven (7) days before inducing anaemia and treated for another eight days respectively.

Both group A and B serve as the baseline for comparison of Group C and D.

2.5 Collection of Blood Sample

The methods described [15] was used for blood collection. The different animal groups received their respective doses orally, once daily (10am), for 14 days. About 1 ml of the blood sample was collected through ear puncture with the capillary tube and deposited in the EDTA container for PCV, RBC, Hb and WBC respectively. 1 ml of the blood sample was first collected within 2 days interval before inducing anaemia and after inducing anaemia follow with treatment to check the level of their various blood parameters during the experiment with intervals of day 0, 3, 6, 9 and 12. After which the animals were sacrificed on day 15 and 4 ml of blood samples were collected in EDTA bottles for further analysis.

2.6 Determination of Biochemical, Haematological and Immunological Parameters

According to the method described [16] was used to evaluates the biochemical, haematological and immunological parameters.

2.7 Phytochemical Screening (Qualitative)

The presence of alkaloids, flavonoids, tannins, terpenes, steroids, phenolics, cardiac glycosides, resins, balsam and saponins were determined by the methods described [17].

2.8 Statistical Analysis

The data were expressed as Mean ± Standard Error of Mean. Statistical analysis was performed using analysis of variance (ANOVA) and Duncan multiple range test at 5% level of confidence ($p < 0.05$).

3. RESULTS

3.1 Phytochemical Analysis

Various phytochemical tests performed on extracts of *cnidoscolus chayamansa* shows positive results for alkaloids, flavonoid and phenolic contents. Table 1 illustrates the presence of various phytoconstituents.

Cnidoscolus chayamansa extracts has a significant effect on some biochemical parameters and hematological assays as indicated from the tables below:

Table 1. Phytochemical analysis of *Cnidoscolus chayamansa*

Test	*Cnidoscolus chayamansa*
Alkaloid	+
Flavonoids	+
Tanins	+
Saponins	+
Terpenes and steroids	+
Cardiac glycosides	-
Balsam	+
Phenol	+
Resin	-

The phytochemical analysis conducted on *C. chayamansa* showed the presence of alkaloid, flavonoids, tanins, saponins, terpenes, steroids, balsam and phenol with the absence of cardiac glycosides and resin.

Table 2. Base line for hematological parameters

Groups	Positive Control	Negative Control
PCV	18.25±0.853	43.00±1.080
HAEMOGLOBIN	10.23±0.019	14.36±0.378
WBC	3375.00±193.110	7350.00±64.555
RBC	6.40±0.111	10.03±0.329

Values are expressed as mean ± SEM, n=4. PCV =Parked cell volume; WBC=White blood cell; RBC= Red blood cell

Table 3. Effect of *Cnidoscolus chayamansa* on hematological parameters for pre-treated group

Group	DAY 0	DAY 3	DAY 6	DAY 9	DAY 12	DAY 15
PCV	16.00 ± 0.408^{bd}	19.00 ± 0.912^{ad}	23.50 ± 1.708^{ad}	25.00 ± 1.581^{ad}	33.50 ± 2.901^{ad}	38.75 ± 1.493^{ad}
Hemoglobin	9.60 ± 0.195^{bd}	10.12 ± 0.042^{bd}	10.43 ± 0.093^{ad}	10.93 ± 0.092^{ad}	11.13 ± 0.119^{ad}	12.37 ± 0.547^{ad}
WBC	3126.50 ± 58.898^{bd}	3337.50 ± 102.820^{ad}	3720.00 ± 64.420^{ad}	4775.00 ± 201.56^{ad}	5950.00 ± 225.46^{ad}	7825.00 ± 125.00^{ac}
RBC	4.93 ± 1.047^{bd}	4.79 ± 0.075^{bd}	5.18 ± 0.017^{bd}	5.89 ± 0.203^{bd}	7.69 ± 0.186^{ad}	9.60 ± 0.398^{ad}

Values are expressed as Mean ± SEM (n = 4); Values in each column with different superscript (ad-bd) are significantly different (p<0.05); PCV =Parked cell volume; WBC =White blood cell; RBC = Red blood cell

Table 4. Effect of *Cnidoscolus chayamansa* on hematological parameters on post-treat group

Group	DAY 0	DAY 3	DAY 6	DAY 9	DAY 12	DAY 15
PCV	39.5 ± 1.041^{ad}	40.5 ± 0.866^{ad}	41.5 ± 0.645^{ad}	42.75 ± 0.479^{ad}	45.5 ± 0.957^{ac}	51.5 ± 1.323^{ac}
Hemoglobin	11.89 ± 0.258^{ad}	13.33 ± 0.562^{ad}	13.52 ± 0.542^{ad}	14.14 ± 0.545^{ad}	14.64 ± 0.457^{ac}	14.97 ± 0.369^{ac}
WBC	5575.00 ± 149.30^{ac}	6380.00 ± 214.63^{ac}	22614.00 ± 15514^{ac}	7425.00 ± 193.11^{ac}	8400.00 ± 195.79^{ac}	8755.00 ± 159.24^{ac}
RBC	7.63 ± 0.227^{ad}	8.39 ± 0.173^{ad}	8.91 ± 0.163^{ad}	9.18 ± 0.176^{ad}	9.77 ± 0.042^{ad}	12.25 ± 0.042^{ac}

Values are expressed as Mean ± SEM (n = 4); Values in each column with different superscript (ad-bd) are significantly different (p<0.05); PCV = Parked cell volume; WBC = White blood cell; RBC = Red blood cell

Table 5. Effect of Cnidoscolus chayamansa on liver

Group	Albumin	Protein	DB	TB	ALT	AST	ALP
Positive control	29.98±1.301	61.29±1.118	10.30±0.200	19.86±0.489	17.95±0.641	25.36±0.931	337.25±6.074
Negative control	36.30±0.238a	71.31±0.842a	4.04±0.049b	18.24±0.679b	13.91±0.161b	16.63±0.265b	280.75±2.056b
Pre-treatment	36.44±1.047ac	73.08±0.613ac	7.49±0.659bc	13.70±0.351bd	15.46±4.443bc	20.89±1.095bc	254.25±2.287bd
Post-treatment	39.80±1.202ac	76.94±1.571ac	3.76±0.042bd	10.45±0.620bd	7.80±0.429bd	11.66±0.898bd	192.00±1.474bd

Values are expressed as Mean ± SEM (n = 4); Values in each column with different superscript (a-b) are significantly different (p<0.05); DB = direct bilirubin; TB = total bilirubin; ALT = alanine amino transaminase; AST = aspartate amino transaminase; ALP = alkaline phosphatase

Table 6. Effect of *Cnidoscolus chayamansa* on Iron binding capacity

Groups	Total Iron (µmol/L)	TIBC (µmol/L)	Urea (mmol/L)	Creatinine (µmol/L)	Uric Acid (µmol/L)
Positive control	126.07±1.532	69.94±1.645	10.23±0.266	146.28±3.201	372.15±1.863
Negative control	148.44±1.283[a]	79.51±1.292[a]	3.40±0.086[b]	74.69±1.205[a]	206.43±3.117[b]
Pre-treatment	153.49±0.530[ac]	85.16±0.824[ac]	2.39±0.027[bd]	68.38±0.404[bd]	234.92±2.761[bc]
Post-treatment	166.09±1.334[ac]	98.43±0.562[ac]	4.70±0.047[bc]	75.01±0.891[bc]	246.52±1.136[bc]

Values are expressed as Mean ± SEM (n = 4); Values in each column with different superscript (a-b) are significantly different (p<0.05); TIBC = Total iron-binding capacity

4. DISCUSSION

This study was aimed at evaluating the effects of *C. chayamansa* leaf extract on phenylhydrazine hydrochloride induced anaemia in albino rats. It has been demonstrated previously that intraperitoneal administration of phenylhydrazine decreased haemoglobin concentration, red blood cells number, white blood cells and packed cell volume, which is also a known model for hepatic injury. A decrease in packed cell volume, red blood cells, white blood cells and haemoglobin concentration in the positive control group and pre-treatment group indicates the presence of anaemia induced with phenylhydrazine hydrochloride when compelling to the negative control group. However, treatments with leaf extracts of *C. chayamansa* restore the lost haematological parameters in the pre-treatment group. This effect was also reported [18] of the hematinic property of *C. chayamansa* which increase PCV and haemoglobin concentration which is also consistent with the findings of this study. The post-treatment group that received treatment of leaf extract of *C. chayamansa* one week earlier before inducing phenylhydrazine hydrochloride shows no anaemic effect as the blood parameters increase progressively when compelling to the pre-treatment, positive group and negative group as recorded.

In the assessment of liver damage by phenylhydrazine, the determination of enzyme marker levels such as ALT, ALP, TB, DB and AST is often used. In necrosis or membrane damage, the enzymes are released into circulation and it can be measured in serum as markers of hepatic damage [19] suggested that high levels of AST indicate liver damage due to viral hepatitis as well as cardiac infarction and muscle injury as observe in the positive control and pre-treatment group. The ALT in the liver catalyzed the conversion of alanine to pyruvate and glutamate and is released similarly; therefore, ALT is more specific to the liver and is thus a better parameter for detecting liver injury [20] as also observed in this study. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in the liver [21]. Similarly, serum ALP and bilirubin level on the other hand are related to the function of the hepatic cell. Increase in serum level of ALP is

due to increased synthesis; in the presence of increasing biliary pressure has been reported [22].

Present results using the model of phenylhydrazine induced hepatotoxicity in the rats demonstrated that *C. chayamansa* at the same doses caused a decrease in ALT, ALP, DB, TB and AST levels in both treatment groups. Increase in serum level of ALP is due to increased synthesis, in the presence of increasing biliary pressure [23]. The abnormally high level of serum ALT, AST, ALP and bilirubin observed in this study are the consequences of phenylhydrazine induced anaemia resulting in liver dysfunction and denoted the damage to the hepatic cells. However, treatment with *Cnidoscolus chayamansa* both the pretreatment group and post-treatment reduced the level of serum ALT, AST, ALP DB and TB, which seem to offer the protection and maintain the functional integrity of liver cells. An increase in Total Serum Protein (TSP) and albumin for both treatment group observed in the rats may be associated with the decrease in the number of hepatocytes which in turn may result into increased hepatic capacity to synthesize protein. Treatment with *Cnidoscolus chayamansa* decreased the serum levels of ALT, AST and ALP towards near normal values which are an indication of stabilization of plasma membrane as well as repair of hepatic tissue.

Evaluations in total iron and total iron-binding capacity concentration by pretreated and post-treated rats with *Cnidoscolus chayamansa* leaf extract indicates the serum content of the elements, increased iron content enhance the transport and regulation of oxygen in the blood by the haemoglobin and a significant ($p<0.05$) decrease in creatinine, uric acid and urea in the pretreated and post-treated group compare with the positive control indicates that the treatment with aqueous extract of *Cnidoscolus chayamansa* maintain the level of urea, uric acid and creatinine in the blood as high content of urea and uric acid indicates hyperuricemia.

The phytochemical analysis conducted on *Cnidoscolus chayamansa* showed the presence of alkaloid, flavonoids, tannins, saponins, terpenes and steroids, balsam and phenol which aligns with the bioactive constituents of medicinal plants reported by Singh and Bismita

[24] and therefore suggests their use as medicinal plants.

In humans and animals, alkaloids and flavonoids have been observed to possess antidiuretic, anti-inflammatory and analgesic effects [25]. The analgesic, anti-inflammatory, anti-bacterial and other properties of the phytochemicals present in these plants hence explains their use in preventing anaemia crisis, inflammations, bacterial infections, stroke and other symptoms of sickle cell anaemia.

5. CONCLUSION

Today, a wide range of people are found to be anaemic, especially, young children and thus, they go for allopathic iron supplements, which comes up with a large number of adverse effects like constipation, irritation, stomach upset, pain, diarrhoea, nausea, and vomiting. So, this plant extract may prove helpful in an anaemic patient, as it contains inorganic substance like iron, anti sicking amino acids and phytochemicals were most likely the active nutrients responsible for the haematological activities and hepatic resolution of the plant. The elevation in the PCV, RBC, WBC and Hb with the iron concentration in serum, decrease in enzymes of the liver and increase protein and albumin strongly indicate the haematinic property and therefore, suggest that *Cnidoscolus chayamansa* dietary supplementation has a potential haematinic property and could be of immense benefit as a dietary supplement to alleviate anaemia.

However, the nutritional approaches to the management of sickle cell disease remain the current and the most promising approach in the management of sickle cell disease. There is no doubt that *Cnidoscolus chayamansa* prove worthwhile as frontline nutrients in the management of sickle cell disease.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the

research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

This is not applicable.

ETHICAL APPROVAL

The experimental design was conducted in accordance with the guidelines approved by the institutional animal ethical committee of University of Jos, Nigeria.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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