Research Article

Jamila Abdulhamid Atata*, Taiwo Oluwaseun Ayoola, Abdullateef Abiodun Ajadi, Sani Adamu, Aishat O. Olatunji and Khalid Talha Biobaku

Anti-anaemic effect of ethanol leaf extract of *Cnidosculus aconitifolius* on cyclophosphomide-induced anaemia in rats

https://doi.org/10.1515/jcim-2020-0006
Received March 13, 2019; accepted February 10, 2020;
published online May 15, 2020

Abstract

Background: The folkloric claim that *Cnidoscolus aconitifolius* (Chaya) could ameliorate anaemia requires scientific revalidation in anaemic models.
Objective: The aim of this study is to investigate the anti-anaemic effect of *Cnidoscolus aconitifolius* on cyclophosphamide-induced anaemia in rats.
Methods: The leaves of *Cnidoscolus aconitifolius* were collected, dried and extracted with ethanol. Twenty five Wistar Albino rats weighing 120–180 kg were used. Anaemia was induced in groups 2, 3, 4 and 5 with a single injection of cyclophosphamide, while group 1 served as a negative control without anemia and was treated with 0.5 mL of normal saline, Group 2 rats were treated with 100 mg/kg body weight of *Cnidoscolus aconitifolius*. Group 3 rats were treated with 500 mg/kg body weight of *Cnidoscolus aconitifolius*. Group 4 served as the positive control rats and was treated with 0.5 mL of normal saline. Group 5 which served as the standard control rats and were treated with 5 mg/kg body weight of standard drug Chemiron. The treated lasted for two weeks during which blood samples were collected from each rat for haematological analysis.
Results: The result shows that hematological parameters were elevated in groups 2, 3 and 5 rats treated with 100 mg/kg and 500 mg/kg body weight of ethanol extract of *Cnidoscolus aconitifolius* and chemiron respectively.
Conclusions: Ethanol leaf extract of *Cnidoscolus aconitifolius* at a graded dose of 500 mg/kg body weight had higher ameliorative effect on the haematological parameters of cyclophosphamide-induced anemia in rats.

Keywords: anaemia; *Cnidoscolus aconitifolius*; cyclophosphamide; haematinic; rat.

Introduction

Anaemia is a common nutritional deficiency disorder and global public health problem in both developing and developed countries with major socio-economic consequences. Anemia has attained epidemic proportions worldwide with about two billion patients estimated to suffer from anaemia worldwide and it is reported to account for three quarters of one million deaths a year in Africa and South-East Asia [1]. Anaemia can be defined as the reduction in the number of circulating erythrocytes or haemoglobin concentration (Hb) or both below the normal values in the peripheral circulation [2]. The condition is usually accompanied by hypoxia due to a failure to meet tissue oxygen demand and could also result in increased susceptibility to infection. Anaemia impairs virtually all bodily function and this results in multiple organ disturbances including mental and physical fatigue [3].

Despite causing such high levels of disability, the public health community does not pay optimum attention to this condition. This lack of prioritization may be partly due to the conception that anaemia is a clinical sign that occurs sequel to other infectious and non-infectious diseases [1]. In developing countries, mild to moderate anaemia are often overlooked and ignored with the notion that once the pathological processes are addressed, the bone marrow takes care of the anaemia. However, this might not be true for severe anaemia which in most cases

*Corresponding author: Jamila Abdulhamid Atata, Department of Veterinary Pathology, University of Ilorin, Ilorin, Nigeria, Phone: +234 8061671252, E-mail: atata.aj@unilorin.edu.ng
Taiwo Oluwaseun Ayoola: Faculty of Veterinary Medicine and Surgery, University of Ilorin, Ilorin, Nigeria
Abdullateef Abiodun Ajadi: Department of Veterinary Pathology, University of Ilorin, Ilorin, Nigeria
Sani Adamu: Department of Veterinary Pathology, Ahmadu Bello University, Zaria, Nigeria
Aishat O. Olatunji and Khalid Talha Biobaku: Department of Veterinary Pharmacology and Toxicology, University of Ilorin, Ilorin, Nigeria

is life-threatening. In general, the medical treatment of anaemia is very expensive and may require the use of haematinic, blood transfusion, erythropoietin therapy or even bone marrow transplant depending on the severity of the anaemia. The potential role of medicinal plants as hematinic agents is supported by the ethno-medicinal surveys and traditional folkloric beliefs. In Nigeria, the use of herbal concoctions to treat anaemia is becoming increasingly popular because it is considered to be more readily accessible and economically viable compared to the use of orthodox medicines.

Cnidoscolus aconitifolius also known as chaya is a fast growing, drought-resistance and heavy rain tolerant perennial shrub which is mostly used worldwide because of it high nutritive and numerous medicinal values. It belongs to the family Euphorbiaceae, subfamily Crotonoidea and tribe Manhotea [4, 5], it is cultivated year-round due to its ease of propagation and its resistance to adverse weather conditions [6]. The leaves of *Cnidoscolus aconitifolius* (Figure 1) are commonly crushed using water and taken to cure anaemia. Many of the locals swear by its potency in increasing haematological parameters (especially packed cell volume) few days after the use of this decoction.

Various agents has been used to induce anaemia in experimental models, the most common agents includes phenylhydrazine and cyclophosphamide. Cyclophosphamide (CP) is an alkylating agent used as an immunosuppressive or cytotoxic drug in cancer chemotherapy either alone or in combination with other drugs [7, 8]. The major adverse effects of cyclophosphamide therapy include haematological disorders, infertility, haemorrhagic cystitis, secondary malignancies and gastrointestinal disorders [9]. On average, over one third of patients become fatigued and anemic after three cycles of chemotherapy [10].



Figure 1: *Cnidoscolus aconitifolius* leaves (alchetron.com).

Although existing studies on *C. aconitifolius* leaves suggest that the plant has anti-anaemic effects [11–13], but studies on the potency of this plant as a remedy to cyclophosphamide induced anaemia have not been reported and hence this recent work. The aim of the study is to investigate the anti-anaemic effect of ethanol leaf extract of *Cnidoscolus aconitifolius* on cyclophosphamide-induced anaemia in rats.

Materials and methods

Chemicals and reagents

All chemicals and reagents used were of analytical grade. Cyclophosphamide was purchased from CELON®, India.

Ethical approval

Approval for this study was sought and obtained from the ethical committee for animal use, Faculty of Veterinary medicine, University of Ilorin, Ilorin.

Plant collection and identification

The fresh leaves of the plant were harvested in August from Tanke in Ilorin south local government area of Kwara state. The plant was authenticated at the Herbarium of the Department of Plant biology, Obafemi Awolowo University, Ile-Ife, Osun state, Nigeria. Air-dried powder (1 kg) of *C. aconitifolius* leaves was extracted and the powdered sample stored for further use.

Experimental animals

A total of fifty (25) wistar albino male and female rats weighing between 120 and 180 g were obtained and kept in well aerated laboratory cages in the Animal house, Veterinary teaching hospital, University of Ilorin, Kwara state, Nigeria. The animals were allowed to acclimatize to the laboratory environment for a period of two weeks before the commencement of the experiment. They were fed with water and grower mash from Vital Feed *ad libitum*.

Preparation of ethanol leaves extract

The preparation of the ethanol plant extract was carried out as previously described [14]. 70% ethanol was prepared and one L of the ethanol was used to dissolve 100 g of the leave powder. The prepared solutions were allowed to stand for 48 h. Each extracted solution was filtered off using a Whatman® filtered paper (Cat no. 1001 125) of pore size 125 mm. The extracted solution was concentrated by distilling off the solvent followed by evaporation to dryness on a water bath set at 45 °C. The extract obtained without the solvent was weighed. The percentage yield was calculated in terms of air dried weight of the plant material as: Percentage yield = (Amount of extract obtained/ Amount of initial sample) x 100.

Induction of anaemia

Rats were injected once intravenously with 10 mg/kg of cyclophosphamide (CP). Anaemia was allowed to establish in 24 h. Packed Cell volume < 35 % was considered as an index for anaemia.

Experimental design

The experimental rats were randomly divided into five (5) groups, with four rats per group and treated for a period of two weeks. Except for group 1, all other groups were anaemia-induced.

a. (Negative control rats without cyclophosphamide induced anemia): The rats were treated with 0.5 mL of normal saline].
b. (Rats with cyclophosphamide-induced anemia): The rats were treated with 100 mg/kg b.w. of ethanol extract of *Cnidoscolus aconitifolius*.
c. (Rats with cyclophosphamide-induced anemia): The rats were treated with 500 mg/kg b.w. of ethanol extract of *Cnidoscolus aconitifolius*.
d. (Positive control rats with cyclophosphamide-induced anemia): The rats were treated with 0.5 ml of normal saline.
e. (Standard control rats with cyclophosphamide-induced anemia): The rats were treated with 5 mg/kg body weight of standard drug Chemiron®.

Qualitative phytochemical screening

This chemical test was carried out on the extract using a standard procedure for identification of phytochemical constituents as described by [14–16]. Extract solution was prepared by dissolving 10 g of extract in distilled water to make up 100 ml solution. The extract solution was then used for phytochemical screening.

Table 1: Results of phytochemical analysis of ethanol extract of *C. aconitifolius* leaves.

Parameters	Type of test	Observation	Test result
Alkaloid	Dragendorffs	Brownish-red colour	+
Glycosides	Salkowski's	Reddish-brown colour	+
Tannins	Ferric chloride	Deep red colour	+
Saponin	Frothing	Persistence foam	+
Flavonoid	Pew's	Red colour	+
Phenols	Buchard	Violet colour	+
Anthraquinones	Bornirager's	Pink-red	–

+ = present – = not detected.

Blood collection for haematological analyses

Blood sample (2 mL) was collected from each of the experimental rats by cardiac puncture using 10 mL hypodermic syringe and needle. The blood was dispensed into a tube coated with ethylene diamine tetra-acetic acid as anti-coagulant and used for haematological evaluations.

Haematological analyses

Packed cell volume (PCV), Hb, red blood cell count and total and differential white blood cell counts were determined using established laboratory protocols [17]. Mean corpuscular volume (MCV) and mean corpuscular haemoglobin [MCH] concentration (MCHC) were calculated from values of red blood cell count, PCV and Hb concentration as described [2].

Statistical analysis

Statistical package "Graph pad prism(Graph pad, La Jolla, CA, USA) version 5.2" was used to express the data as mean ± standard error of mean. The data obtained were subjected to one-way analysis of variance. Turkey's post hoc test was employed to compare the various groups. Significance was accepted for values of $p < 0.05$ with a 95% confidence interval.

Results

The percentage yield of the extract was 68% W/W, and colour was dark to black.

Ethanolic leaf extract of *Cnidoscolus aconitifolius* revealed the absence of anthraquinones, and the presence of cardiac glycosides, saponins, steroids, tannins and alkaloids (Table 1).

The effect of ethanolic extracts of *Cnidoscolus aconitifolius* leaves on Packed Cell Volume (PCV) of cyclophosphamide (CP)-induced anaemic rats after two week of administration of 100 mg/kg and 500 mg/kg of the extract is summarized on Table 2.

Table 2: Effects of ethanol leaf extracts of *C. aconitifolius* on packed cell volume of cyclophosphamide-induced Anaemic Rats.

Group	Pre-induction PCV (%) (Day 0)	Post-induction PCV (%) (Day 1)	PCV (%) on Day 7	PCV (%) on Day 15	Net increment in PCV (%).	Mean increment in PCV (%)
1	42.5 ± 7.8	42.5 ± 2.6	43.5 ± 1.2	43.8 ± 2.0	+ 1.3	+ 0.65
2	42.5 ± 0.7	28.4 ± 2.9	30.0 ± 3.0	40.0 ± 8.1	+ 11.6	+ 5.8
3	39.5 ± 2.1	24.8 ± 9.3	38.25 ± 0.9	45.6 ± 6.2	+ 24.7	+ 12.4
4	43.5 ± 4.9	25.7 ± 3.8	19.0 ± 4.2*	20.5 ± 3.5*	– 5.1	– 2.5
5	44.8 ± 3.5	32.5 ± 5.2	24.75 ± 2.9	35.7 ± 5.0	+ 3.2	+ 1.6

Values are means ± SD. * = significantly significant ($p < 0.05$) difference.

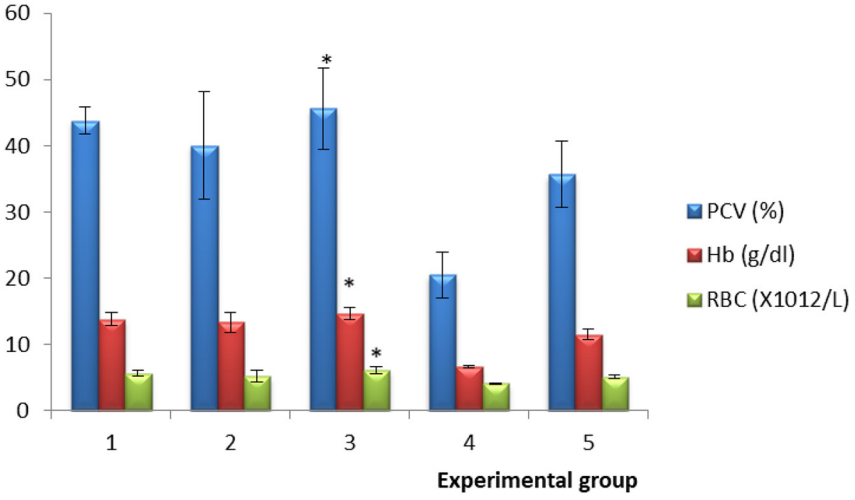


Figure 2: Mean packed cell volume, Hb and total red blood cell count of the experimental rats.

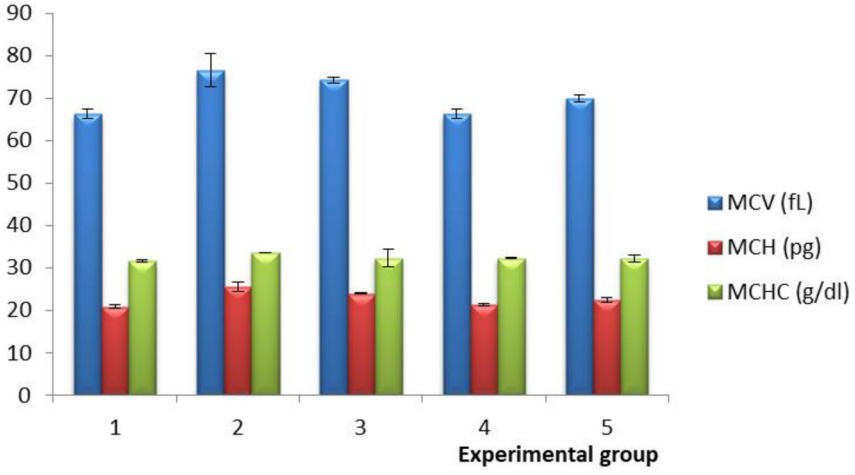


Figure 3: Mean erythrocytic indices of the experimental rats.

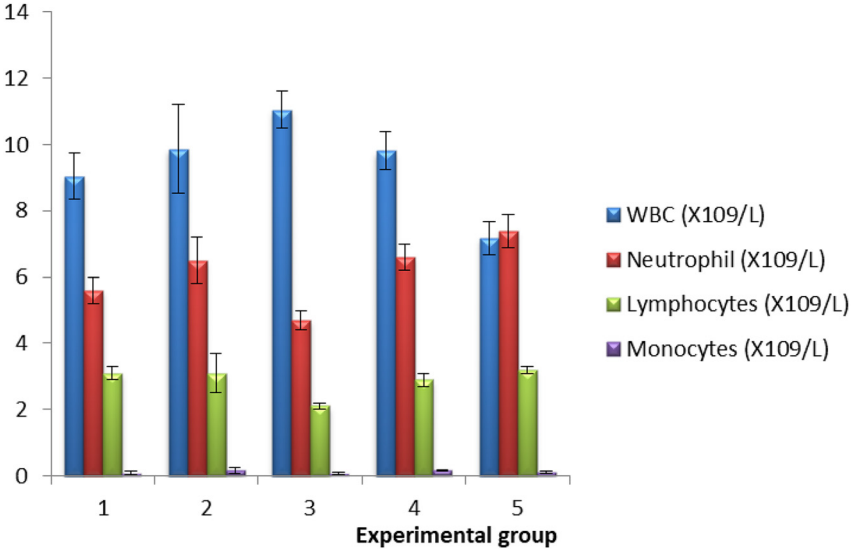


Figure 4: Mean total and differential leucocyte counts of the experimental rats.

The effect of ethanolic extracts of *Cnidoscolus aconitifolius* leaves on haematological parameters of cyclophosphamide (CP)-induced anaemic rats after two week of administration of 100 mg/kg and 500 mg/kg of the extract is as shown in Figures 2–4.

Discussion

This experiment was carried out to determine the anti-anaemic potential of the ethanolic leave extracts of *Cnidoscolus aconitifolius*. The drop in PCV values of CP-induced experimental groups on day 1 compared with PCV values of day 0 and with PCV values of the normal control group, confirmed induction of anaemia with a single intravenous injection of cyclophosphamide and this agrees with previous finding [9]. Anaemia caused by cyclophosphamide is usually due to alkylation of the nucleic acid of the erythrocytes resulting in formation of covalent bond with subsequent cell distortion and erythrophagocytosis of the deformed erythrocytes [18]. Statistically significant increase in PCV value of rats in 500 mg/kg treated group on day 4 indicates anti-anaemic potential of the extracts and this could be linked with the phytochemical constituents of *Cnidoscolus aconitifolius* leaf [11–20].

Flavonoid is a known cyto-protective antioxidant which might have mopped up the cytotoxic reactive oxygen and nitrogen species that could have been generated consequent to administration of the cyclophosphamide in the Wistar rats and thereby improving erythrocyte cell membrane stability [20]. This is in agreement with previous finding [21]. Tannins are astringents which act to minimize the absorption of ingested xenobiotics and hence provide cell protection [20]. Although elemental and other nutritional analyses were not carried out in this study, there were reports that *Cnidoscolus aconitifolius* is a good source of protein, vitamins, calcium, and iron; and is also a rich source of antioxidants [22, 23], although elemental and other nutritional analyses were not analysed, previous reports suggest that the presence of these phytonutrients could be responsible for the anti-anaemic effect of the extract [12–23]. Heaematinic property of the leaf extract has also been reported by Azeez et al., [24] and Onuoha et al., [25] who reported increase in PCV and Hb following the administration of *Cnidoscolus aconitifolius* leaf extract which is also consistent with the findings of this study.

The mean values of erythrocyte indices (i e, Mean corpuscular volume, [MCV], MCH concentration [MCHC] and MCH) in the anaemic and non-anaemic group were within normal range and this is a pointer to the fact that anaemia of cyclophosphamide toxicity is normocytic normochromic, which is the type that characterizes bone marrow dyserythropoiesis [2]. The absence of significant differences among the mean values of total and differential leukocyte, and platelet counts of rats of the different experimental groups, as observed in this study, suggests that the possible haematopoietic effect of the leaf extract of *Cnidoscolus aconitifolius* was exerted most probably on only the erythrocytes and cells of bone marrow erythroid series.

Conclusion

The 70% ethanol leaf extract of *Cnidoscolus aconitifolius* had a stimulatory effect on haematopoesis especially on the erythroid series of the bone marrow precursor cells as evidence by the elevation of certain haematological parameters. The extract is therefore relatively safe for the treatment of haemolytic anaemia at the doses administered and this has substantiated its folkloric use in the treatment of anemia and other diseases in Nigeria.

Acknowledgments: The authors are grateful to the Mr. Moshood Bolaji of the Clinical Pathology Laboratory at the Faculty of Veterinary Medicine, University of Ilorin, Kwara state, Nigeria, for his technical support.
Research funding: None declared.
Author contributions: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.
Competing interests: The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.
Employment or leadership: None declared.
Honorarium: None declared.

References

1. WHO. Worldwide prevalence of anaemia 1993–2005: WHO global database on anaemia. 2008.
2. Esievo KAN. Veterinary clinical pathology, 1st edn. Spectrum Books, Ibadan, 2017; pp. 143–44.
3. Lopez A, Cacoub P, Macdougall IC, Peyrin-Biroulet L. Iron deficiency anaemia. Lancet 2016;387:907–16.
4. Ross-Ibarra J, Molina-Cruz A. The ethnobotany of *Chaya* (*Cnidoscolus aconitifolius* ssp*aconitifolius* Breckon): A nutritious Maya vegetable. Econ Bot 2002;56:350–65. https://doi.org/10.1663/0013-0001(2002)056[0350:teocca]2.0.co;2.
5. Ugwoke CEC, Anze SPG, Chukwube VO, Nweze AE, Diovu EO. Pharmacognostic Studies of the leaf of *Cnidoscolus aconitifolius*. Indo Am J Pharm Sci 2017;4:496–506.

6. Mordi J, Uzuegbu U, Nwagwa E, Okunima A. Effect of the dry aqueous leaf extract of *Cnidoscolus aconitifolius* on blood alcohol clearance in rabbits. J Nat Sci Res 2013;3:245–51.
7. Lee HW, Kim H, Ryuk JA, Kil KJ, Ko BS. Hemopoietic effect of extracts from constituent herbal medicines of Samul-tang on phenylhydrazine-induced hemolytic anemia in rats. Int J Clin Exp Pathol 2014;7:6179–84.
8. Bell SJ, Thompson AJ. Cyclophosphamide-associated enteritis: A rare association with severe enteritis. World J Gastroenterol 2016; 22:8844–8. https://doi.org/10.3748/wjg.v22.i39.8844.
9. Wang X, Ren J, Zhang J, Yan Y, Jiang N, Yu J, et al. Prospective study of cyclophosphamide, thiotepa, carboplatin combined with adoptive Dc-CIK followed by metronomic cyclophosphamide therapy as salvage treatment for triple negative metastatic breast cancers patients (aged < 45). Clin Trans Oncol 2016;18:82–7. https://doi.org/10.1007/s12094-015-1339-2.
10. Glaspy J, Degos L, Dicato M. Comparable efficacy of epoetinalfa for anemic cancer patients receiving platinum- and nonplatinum-based chemotherapy: a retrospective subanalysis of two large, community-based trials. Oncologist 2002;7:126–35. https://doi.org/10.1634/theoncologist.7-2-126.
11. Achikanu CE, Eze-Steven PE, Ude CM, Ugwuokolie OC. Determination of the vitamin and mineral composition of common leafy vegetables in south eastern Nigeria. Inter J Curr Microbiol Appl Sci 2013;2:347–53.
12. Obichi EA, Monago CC, Belonwu DC. Effect of *Cnidoscolus aconitifolius* (Family Euphorbiaceae) aqueous leaf extract on some antioxidant enzymes and haematological parameters of high fat diet and Streptozotocin induced diabetic wistar albino rats. JASEM 2015;19:201–9. https://doi.org/10.4314/jasem.v19i2.5.
13. Chukwu EC, Osuocha KU, Uhegbu FO. Nutrient Composition and Selected Biochemical Effects of *Cnidoscolus aconitifolius* Leaf Extracts in Male Albino Rats. J Forensic Res 2018;10:409–17. https://doi.org/10.4172/2157-7145.1000409.
14. Sofowora EA. Medicinal plant and traditional use in African. Spectrum books Ltd, Ibadan Nigeria 2006; pp. 195–248.
15. Ejikeme CM, Ezeonu CS, Eboatu AN. Determination of physical and phytochemical constituents of some tropical timbers indigenous to Niger Delta Area of Nigeria. Eur Sci J 2014;10: 247–270. https://doi.org/10.2118/136997-ms.
16. Hikino H, Kiso Y, Wagner H, Fiebig M. Antihepatotoxic actions of flavonolignans from Silybum marianum fruits. Planta Med 1984; 50:248–250.
17. Coles EH. Veterinary clinical pathology. W.B. Saunders, London 1980; pp 156–193.
18. Shiraishi A, Sakumi K, Sekiguchi M. Increased susceptibility to chemotherapeutic alkylating agents of mice deficient in DNA repair methyltransferase. Carcinogenesis. 2000;21:1879–83. https://doi.org/10.1093/carcin/21.10.1879.
19. Kuti JO, Konuru, HB. Antioxidant capacity and phenolic content in leaf extracts of tree spinach (Cnidoscolus spp). J Agr Food Chem 2004;52:117–121. https://doi.org/10.1021/jf030246y.
20. Son SM. Reactive oxygen and nitrogen species in pathogenesis of vascular complications of diabetes. Diabetes & metabolism 2012; 36:190–8. https://doi.org/10.4093/dmj.2012.36.3.190.
21. Oyagbemi AA, Odetola AA. Hepatoprotective effects of ethanol extract of *Cnidoscolus aconitifolius* on paracetamol-induced hepatic damage in rats. Pak J Biol Sci 2010;13:164–9. https://doi.org/10.3923/pjbs.2010.164.169.
22. Awoyinka AO, Balogun IO, ogunnowo AA. Phytochemicals screening and in vitro bioactivity of Cnidoscolus aconitifolius (*Euphorbiaceae*). J Med Plant Res 2007;191:063–5.
23. Akachukwu D, Okafor PN, Ibegbulem CO. Phytochemical content of Cnidoscolus aconitifolius and toxicological effect of its aqueous leaf extract in Wistar rats. J Invest Biochem 2014;30: 100–9. https://doi.org/10.5455/jib.20140504023102.
24. Azeez OI, Oyagbemi AA, Oyeyemi MO, Odetola AA. Ameliorative effects of Cnidoscolus aconitifolius on alloxan toxicity in Wistar rats. Afr Health Sci 2010;10:131–47. https://doi.org/10.7176/jnsr/9-4-02.
25. Onuoha NO, Okafor AM, Eme PE, Odo EC. Haematinic Effect of Raw and Boiled Leaf Juice of Cnidoscolus aconitifolius using Cyclophosphamide-Treated Adult Male Albino Rats. ECNU 2017;7: 87–194.