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Ameliorative Effects of Some Natural Blood Boosters on Cyclophosphamide-induced Anemia in Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors CON and BA preconceived, designed the experiment and managed the analysis of the study, while author EA performed the data analysis. Author PA managed the literature searches and wrote the first draft of the manuscript. All authors approved the final manuscript.

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ABSTRACT

Aim: This study was carried out to investigate the hepatoprotective, cholesterol lowering, and renal effects of aqueous extracts of *Jatropha tanjorensis*, *Beta vulgaris* and *Solanum melongena* during cyclophosphamide-induced anemic state.
Methodology: Thirty male Wistar rats divided into six equal groups were treated for 14 days as follows; group 1 were fed normal rat chow and water, group 2-6 received a onetime intraperitoneal administration of 150 mg/kg body weight (bw) cyclosphosphamide, group 3, 4, and 5 received 100 mg/kg bw of *Jathropha tanjorensis*, *Beta vulgaris*, and *Solanum melongena* aqueous extract respectively, and group 6 were anemic rats treated daily with 100 mg/kg bw of mixture of *Jatropha tanjorensis* leaf, *Beta vulgaris* leaf, and *Solanum melongena* leaf aqueous extracts in equal volumes.

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Results: *Solanum melongena,* and the mixture of the three blood boosters showed complete cholesterol lowering effect after alterations by cyclophosphamide. The significant increase in LDH, ALT, and AST during anemic state was normalized by *Beta vulgaris*, and the mixture of blood boosters completely ameliorated the alterations of cyclophosphamide on ALP. Only *Solanum melongena* completely ameliorated the Total Protein levels while in addition to the mixture of blood boosters normalized the Total Bilirubin levels after anemia induction. The oral treatment with *Jatropha tanjorensis* proved most effective in restoring the altered urea, creatinine, Na, and K levels after cyclophosphamide induced anemia, while the mixture of blood boosters, and *Beta vulgaris* significantly normalized the serum Fe and Cl levels.

Conclusion: The study has established the required empirical pharmacological evidence to support the folklore claims that these blood boosters investigated, are antianemic agents.

Keywords: Cyclophosphamide; anaemia; blood boosters; cholesterol; pharmacological.

1. INTRODUCTION

Cyclophosphamide is an alkylating agent mostly used as an antineoplastic and immunosuppressive agent. It is widely used for the treatment of various cancers [1], multiple sclerosis and rheumatoid arthritis [2,3]. Notwithstanding its tumor selectivity, extensive usage of cyclophosphamide induces a wide range of toxic effects such as nephrotoxicity, hepatic toxicity, lowering of blood cell counts (anemia) [4,5,6,7] and cardiac decompensations [8]. Lespine et al. [9] have also reported the onset of hypertriglyceridemia and hypercholesterolemia in rabbits treated with cyclophosphamide. Apart from their utilization for culinary purposes, some plants have been found to exhibit various kinds of therapeutic potentials. *Jatropha tanjorensis* also known as “iyana ipaja”, catholic vegetable or “hospital-too-far” has found usage as an edible vegetable, and as a medicinal plant [10]. Commonly found in Southern Nigeria, it belongs to the family Euphorbiaceae, and mostly grow as roadside weeds, and weeds of bush regrowth [11]. Orhue et al. [11] also reported the modulatory effects of *J. tanjorensis* leaf powder on hematological indices, particularly on bone marrow. Numerous phytochemical compounds such as the saponins, alkaloids polyphenols, and tannins have been associated with *J. tanjorensis* [12]. *Beta vulgaris* (Beet root) also known as shamandar, is a vegetable mostly cultivated in Asia, America, and Europe [13]. It belongs to the family Amaranthaceae. Beetroot has been reported to possess rich phytochemical compounds, such as phenolic acids, ascorbic acid, flavonoids, and carotenoids [14]. Apart from being used as a natural colorant in many cuisines, it has been applied for the treatment of various diseases [13]. Beetroot has been proven effective for the treatment of kidney and liver diseases, boosting of the immune system, and other anticarcinogenic effects [15]. L.V´ali et al. [16] have reported the cardiovascular, hemostatic, and renal effects of beetroot. Further, the hepatoprotective [17] and antioxidant [18] potentials of Beet roots have been demonstrated. Eggplant (*Solanum melogena*), popularly called “Gauta” in Hausa, “afufa” in Igbo, and “igbagba” in Yoruba Nigeria, [19], belongs to the subgenius *Leptostemanum melogena* [20]. This plant have been reported to possess several medicinal and nutritional properties due to appreciable enrichment with nutrients and phytochemicals like polyphenols, monophenols, and flavonoids responsible for the cardiovascular and anticarcinogenic properties [21,22]. Among other health benefits, eggplants are used as effective remedies for haemorrhoids, coelic abnormalities [23], elevated blood cholesterol [19] and treatment of uterine discomfort [24].

Anemia is regarded as one of the challenges of public health, predominant in developing countries due to nutritional deficiencies, and the cost of treatment of anemia with synthetic drugs. The disease anemia is seen as wide spread because anemia frequently results from most diseases that causes blood shortage. Clinically, anemia is confirmed when blood hemoglobin is less than 13 g/dl and 12 g/dl for adult males and females respectively [25]. Holden [26] noted that all types of anemia share similar features of decreased circulating RBC and HB count.

Thus, with all the reported health benefits of these plants, it was imperative to evaluate their effectiveness against cyclophosphamide induced anemic conditions.

2. MATERIALS AND METHODS

2.1 Sample Collection

The leaves of *Jatropha tanjorensis*, *Beta vulgaris*, and *Solanum melogena* were purchased from Fruit Garden Market in Port Harcourt Rivers State, and identified at the Department of Plant Science and Biotechnology, University of Port Harcourt, Choba, Rivers State.

2.2 Sample Preparation

Five hundred grams (500 g) of each of the plant samples were obtained, dried and ground into powder and macerated in 500 ml of water for 48 hrs. The contents were sieved using Whatman No. 1 filter paper. The filtrate was placed on a rotary evaporator to concentrate the extract to a 20% yield, and thereafter refrigerated until usage.

2.3 Experimental Design

Thirty (30) male Wistar rats weighing 180-200 g were used for this study, and were randomly distributed equally into six groups.

The study was carried out after approval by the Animal Welfare Research Ethics Committee of the University of Port Harcourt Rivers state Nigeria. Animal experiments were conducted in accordance with the internationally accepted principle for laboratory animal use and care [27].

The induction of anemia was done by a onetime intraperitoneal injection of 150 mg/kg bw, and confirmed from the Hb levels of the animals less than 12 g/dl after 14 days. Daily oral treatments of the animals are given as follows;

- Group 1: Normal control rats fed *ad libitum* only normal feed and normal saline.
- Group 2: Anemic rats fed normal feed and normal saline.
- Group 3: Anemic rats treated with 100 mg/kg bw *Jatropha tanjorensis*.
- Group 4: Anemic rats treated with 100 mg/kg bw *Beta vulgaris*
- Group 5: Anemic rats treated with 100 mg/kg bw *Solanum melongena*
- Group 6: Anemic rats treated with 100 mg/kg bw *Jatropha tanjorensis*, *Beta vulgaris* and *Solanum melongena* (1:1:1).

After 14 days, the animals were sacrificed after subjecting to mild anasthecia using chloroform. Blood was collected by cardiac puncture and transferred in an EDTA bottle.

2.4 Lipid Profiling

Plasma total cholesterol (TC), triglycerides (TG), and high density lipoproteins (HDL) were determined enzymatically using commercially available kits (Randox kits). Low density lipoproteins (LDL) were determined using the formula of Friedewald et al. [28].

2.5 Liver Enzymes

Lactate dehydrogenase (LDH) activity was assayed following standard procedures as described in the assay kits by the manufacturers from Randox laboratories Ltd, Diamond Road, Crumlin, United Kingdom. Concentrations of aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) were obtained by kinetic methods with kits from Mindray test kits (Mindray Medical International Limited, China) using a double-beam spectrophotometer. Other reagents used were of analytical grade.

2.5.1 Determination of serum total protein (TP)

Biuret method was used to determine the level of total protein in the samples according to the method of Flack and Woollen, [29] and Tietz, [30].

2.5.2 Determination of total bilirubin (T-BIL) concentration

Jendrassik-Grof method [31] of Mindray test kit was used to determine the level of Total Bilirubin in the samples.

2.6 Renal Function Test

2.6.1 Determination of urea

Urease-glutamate Dehydrogenase -UV method according to Berthelot's method [32] was used to determine the level of Urea in the samples. Mindray test kits (Mindray Medical International Limited, China) was used for the analysis.

2.6.2 Determination of creatinine

A Modified method according to Bartels and Bolmer [33] was used to determine the level of Creatinine in the samples. Mindray test kits (Mindray Medical International Limited, China) was used for the analysis.

2.6.3 Determination of serum electrolyte

Na and K were determined by flame photometery using Jenway P7 Flame photometer. Chloride ion levels were determined according to the instructions on their diagnostic kit (Randox Laboratories UK). Bicarbonate was determined using Forrester et al. [34] method. Serum Fe levels was determined by the spectrophotometric method of Sanchez et al. [35].

2.7 Statistical Analysis

Data was expressed as mean ± SD of triplicate determinations. The data were analyzed by using one way analysis of variance (ANOVA) using the least standard deviations (LSD). p values < 0.05 were considered as significant.

3. RESULTS AND DISCUSSION

Figs. 1-4 shows the HDL, LDL, TC, and TG levels of levels of normal rats, untreated anemic rats, and anemic rats administered with *J. tanjorensis, B. vulgaris,* and *S. melongena*. Administration of cyclophosphamide significantly lowered the HDL levels (Fig. 1), but elevated other lipid profile indices, relative to the control group. 100 mg/kg bw of both *J. tanjorensis* and *B. vulgaris* significantly elevated the HDL levels after alterations by cyclophosphamide, however, only animals in group 5 and 6 showed complete restoration of the HDL levels due to treatment using *Solanum melogena*, and a mixture of the three blood boosters. Also, the results revealed that both exclusive administration of *J. tanjorensis*, and *B. vulgaris* significantly reduced the LDL (Fig. 2) and TG (Fig. 4) levels without complete restoration, while only *S. melongena* extract and the mixture of the three blood boosters provided complete restoration of the levels of LDL and TC. The TC levels (Fig. 3) were comparable to the control levels after treatment using each of the blood boosters. However, no significant difference was found between cyclophosphamide induced anemic rats, and those anemic rats treated with *J. tanjorensis*, *and B. vulgaris*. Bristow-Craig et al. [36] reported relationships between serum lipid and lipoprotein concentrations, and iron levels in animal models. Venkateshwarlu et al. [37] similarly found decreased HDL levels in iron deficiency-induced anemic subjects. The findings of this study on cyclophosphamide induced alterations of HDL were also in agreement with the reports of Mythili et al. [8]. In agreement with the findings of this study, the cholesterol lowering properties of *J. tanjorensis*, *B. vulgaris*, and *S. melongena* have been demonstrated by Oyewole and Akingbala [38], Mohammed et al. [13], and Praca et al. [39] respectively. The findings of this study indicates that *S. melongena*, and the mixture of the three samples, are more effective at managing lipoproteins dysfunction than *J. tanjorensis* and *B. vulgaris*. This provides the scientific basis behind the use of this plant for the treatment and management of heart diseases especially in Africa which might be related to the fiber contents of *S. melongena.* LDL, together with TC and TG levels, is seen as indicators of cardiovascular diseases [40], while HDL facilitates the removal of bad cholesterol deposited on the artery and transports them to the liver for re-utilization or excretion.

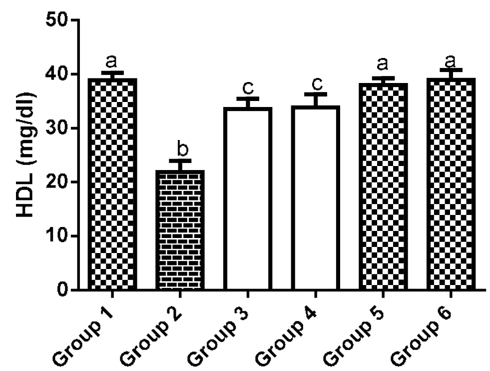

Fig. 1. HDL levels of normal rats, untreated anemic rats, and anemic rats administered with natural blood boosters. Bars bearing similar superscript letters (a-c) are not significantly different (p>0.05)

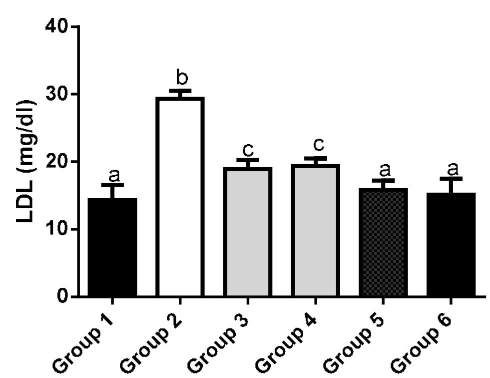

Fig. 2. LDL levels of normal rats, untreated anemic rats, and anemic rats administered with natural blood boosters. Bars bearing similar superscript letters (a-c) are not significantly different (p>0.05)

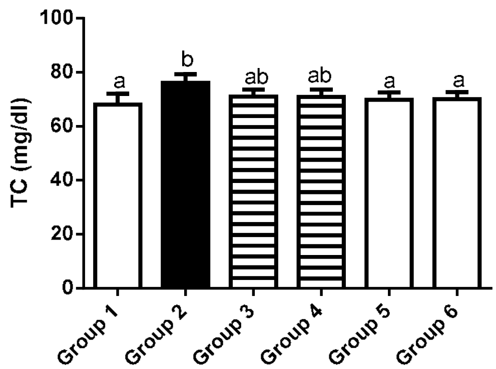

Fig. 3. TC levels of normal rats, untreated anemic rats, and anemic rats administered with natural blood boosters. Bars bearing similar superscript letters (a, b) are not significantly different (p>0.05)

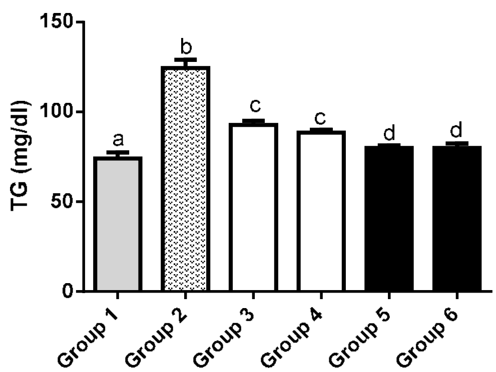

Fig. 4. TG levels of normal rats, untreated anemic rats, and anemic rats administered with natural blood boosters. Bars bearing similar superscript letters (a-d) are not significantly different (p>0.05)

The effect of *J. tanjorensis, B. vulgaris, S. melogena,* and a mixture of these blood boosters on liver function markers of cyclophosphamide induced anemia were shown in Figs. 5-10.

The alterations of the liver function markers, after the administration of cyclophosphamide, might be suggestive of hepatotoxicity (Figs. 5-10). This result is in agreement with the reports of Abdalla et al. [41] and Senthilkumar et al. [42] who reported elevated levels of serum LDH, AST, ALT, and ALP in animals intraperitoneally injected with cyclophosphamide. Drent et al. [43] reported that the elevation of LDH activity may be related to oxidative stress due to possible disruption during the process of lipid peroxidation. The results showed that the LDH (Fig. 5) and ALP (Fig. 8) contents were totally restored by only *B. vulgaris, S. melogena,* and the mixture of the three blood boosters, after cyclophosphamide induced anemia. Further, the results for the ALT levels in Fig. 6 proved that *B. vulgaris* and the mixture of the three blood booster were most effective in completely reversing the alterations observed during anemic condition, while Fig. 7 showed that all the plant extracts used for this study were effective in normalizing the levels of AST after cyclophosphamide induced anemia. *Jatropha tanjorensis* on average had the least hepatomodulatory effect. This finding is in line with the result of Oluwole et al. [44] who showed a possible leakage of hepatic enzymes on administration of *J. tanjorensis*. AST is localized both in the cytoplasm and mitochondria of most organs like heart, liver, kidney, and brain, [45] while ALT is a more specific marker of hepatotoxicity [46]. An elevated level of ALP is suggestive of hepatobiliary injury and cholestasis [47,17]. The total protein concentration shows the functional status of the liver [48]. Other researchers suggested that liver plays a central role in the synthesis of serum proteins and thus elevated levels of serum proteins can indicate liver damage [10,49]. No significant change (p>0.05) was observed between the Total Protein of the untreated anemic rats and the anemic rats treated with *J. tanjorensis*, while relative to the control group, only treatment using *S. melongena* proved completely restorative (Fig. 9). From the data presented in Fig. 10, Total Bilirubin was also elevated in the serum of rats administered with cyclophosphamide, but all the extracts proved effective in ameliorating the anemia induced alterations, and only the Total Bilirubin levels of rats in group 5 and 6, were not significantly different from the control. Total bilirubin levels are commonly used to monitor hepatic biliary obstruction and hepatocellular damage proving effective as a marker for liver diseases [50,51].

The renal effects of the extracts on experimental animals were shown in Figs. 11-17. The kidney performs the major function of excreting drugs and metabolites from the system, regulation of extracellular pH, and electrolyte contents, hence, plays a central role in homeostasis [52]. The urea and creatinine, and electrolyte levels are vital for the evaluation of the functionality of the kidney [53]. *Jatropha tanjorensis* was most effective in restoring the urea (Fig. 11), creatinine (Fig. 12) and Na levels (Fig. 13). Urea is the major nitrogen-containing metabolic waste product of protein catabolism in mammals. It is a cell breakdown product of endogenous or exogenous proteins of the system [54]. The result indicates that the chemical properties of *J. tanjorensis* are more effective in modulating the kidney function and protein catabolism potentials. Creatinine results from creatine and creatine phosphate. Creatinine directly relates to the muscle mass, and its serum level is used to evaluate the glomerular filteration rate because it is readily filtered and not subjected to any significant tubular reabsorption. In this study, administration of cyclophosphamide increased the creatinine levels (Fig. 12) which means impairment of urine excretion, but was reversed by administration of *J. tanjorensis*, and the mixture of the three blood boosters. According to Ogunka-Nnoka, and Horsfall, [55] high elemental sodium content of a sample might indicate elevated serum sodium levels. With this, the restoration of the sodium levels by *J. tanjorensis* after cyclophosphamide depletion (Fig. 13) suggests high elemental sodium content. All the samples administered except *S. melongena* restored the levels of K (Fig. 14), while except for *J. tanjorensis* all the samples proved effective in restoring the Fe levels, comparable to the control (Fig. 15). The results of Fig. 16 showed that only the group 4 and 6 produced Cl levels similar to the control, while all the samples administered, effectively reversed the depletion of bicarbonate levels induced by cyclophosphamide administration (Fig. 17). The results have shown the distinct potentials of the blood boosters to restore the electrolyte reabsorption capacity, secretion and diuretic effect in the kidney tubules.

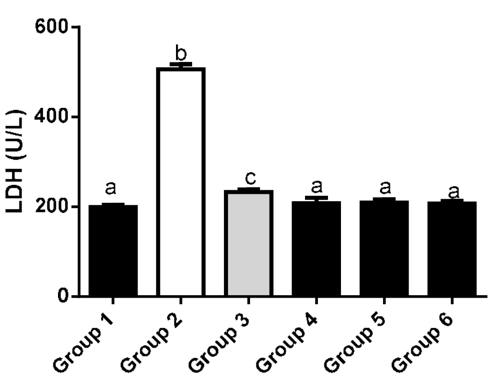

Fig. 5. LDH levels of normal rats, untreated anemic rats, and anemic rats administered with natural blood boosters. Bars bearing similar superscript letters (a-c) are not significantly different (p>0.05)

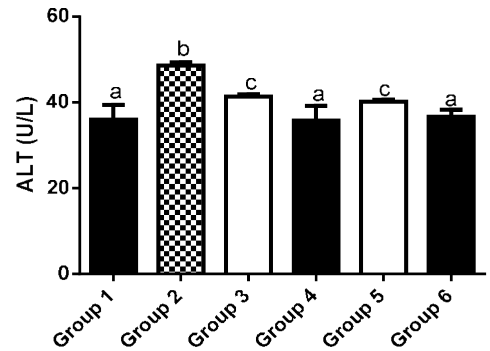

Fig. 6. ALT levels of normal rats, untreated anemic rats, and anemic rats administered with natural blood boosters. Bars bearing similar superscript letters (a-c) are not significantly different (p>0.05)

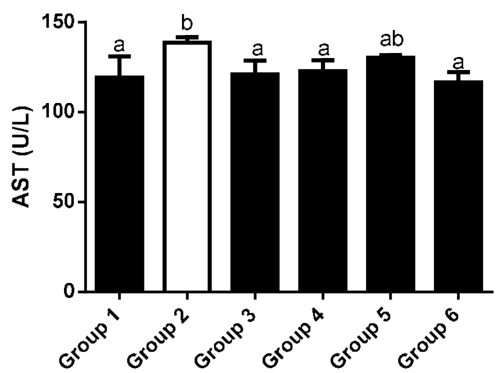

Fig. 7. AST levels of normal rats, untreated anemic rats, and anemic rats administered with natural blood boosters. Bars bearing similar superscript letters (a, b) are not significantly different (p>0.05)

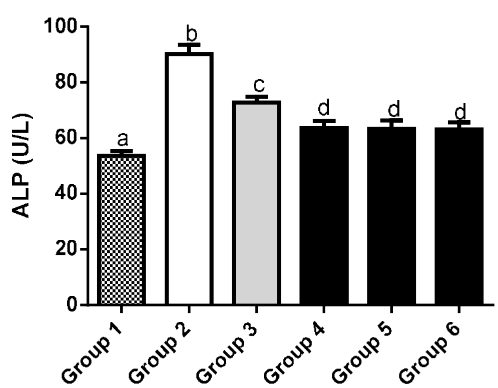

Fig. 8. ALP levels of normal rats, untreated anemic rats, and anemic rats administered with natural blood boosters. Bars bearing similar superscript letters (a-d) are not significantly different (p>0.05)

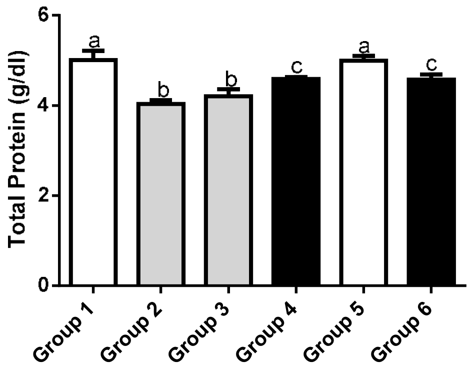

Fig. 9. Total Protein levels of normal rats, untreated anemic rats, and anemic rats administered with natural blood boosters. Bars bearing similar superscript letters (a-c) are not significantly different (p>0.05)

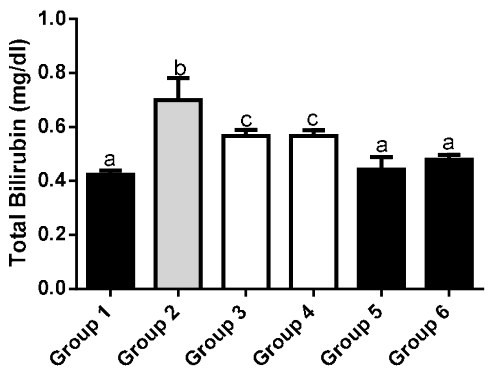

Fig. 10. Total Bilirubin levels of normal rats, untreated anemic rats, and anemic rats administered with natural blood boosters. Bars bearing similar superscript letters (a-c) are not significantly different (p>0.05)

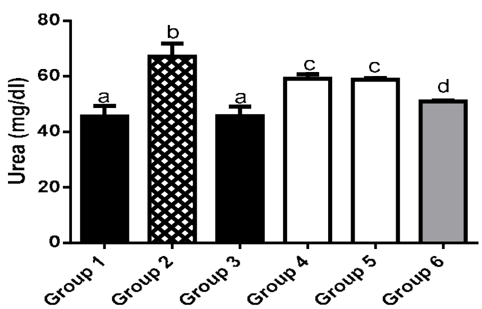

Fig. 11. Urea levels of normal rats, untreated anemic rats, and anemic rats administered with natural blood boosters. Bars bearing similar superscript letters (a-d) are not significant different (p>0.05)

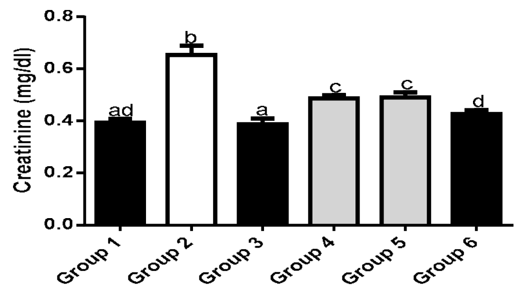

Fig. 12. Creatinine levels of normal rats, untreated anemic rats, and anemic rats administered with natural blood boosters. Bars bearing similar superscript letters (a-d) are not significant different (p>0.05)

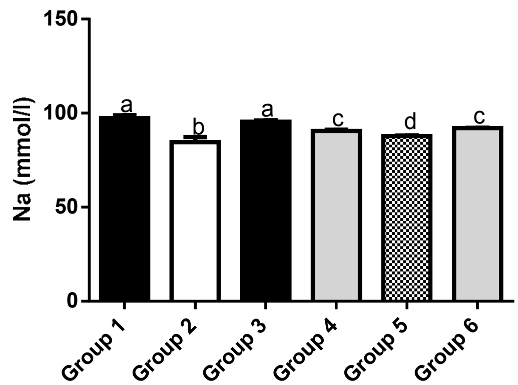

Fig. 13. Sodium levels of normal rats, untreated anemic rats, and anemic rats administered with natural blood boosters. Bars bearing similar superscript letters (a-d) are not significant different (p>0.05)

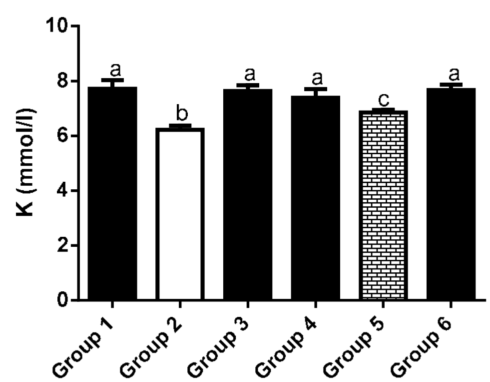

Fig. 14. Potassium levels of normal rats, untreated anemic rats, and anemic rats administered with natural blood boosters. Bars bearing similar superscript letters (a-c) are not significant different (p>0.05)

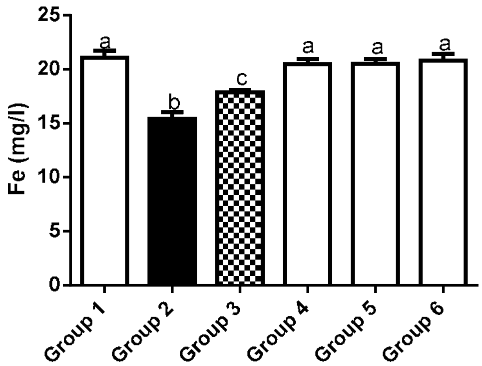

Fig. 15. Iron levels of normal rats, untreated anemic rats, and anemic rats administered with natural blood boosters. Bars bearing similar superscript letters (a-c) are not significant different (p>0.05)

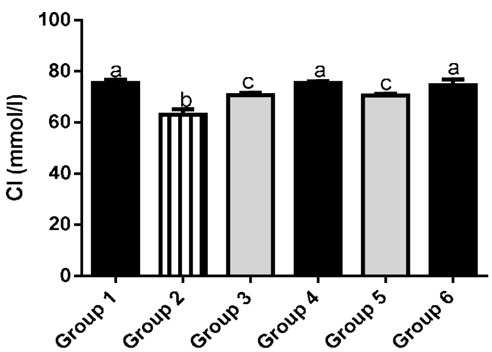

Fig. 16. Chloride levels of normal rats, untreated anemic rats, and anemic rats administered with natural blood boosters. Bars bearing similar superscript letters (a-c) are not significant different (p>0.05)

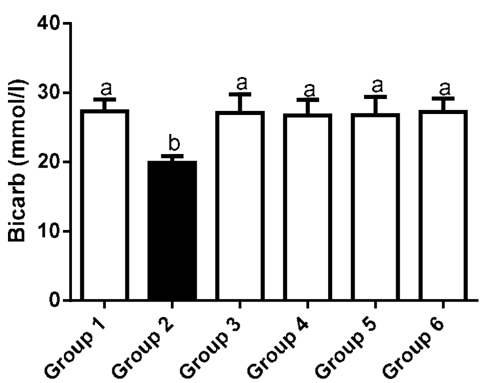

Fig. 17. Bicarbonate levels of normal rats, untreated anemic rats, and anemic rats administered with natural blood boosters. Bars bearing similar superscript letters (a, b) are not significant different (p>0.05)

4. CONCLUSION AND RECOMMENDATION

The study has shown the effectiveness of the blood boosters in ameliorating the alterations induced by cyclophosphamide. The mixture of the three blood boosters is recommended for the management of anemia-induced hypecholesterolema and hepatotoxity, while *Jatropha tanjorensis* can be recommended for the treatment of anemia-induced renal toxicities. Notwithstanding the effectiveness of these extracts shown in this study, it is ideal to extensively study and further confirm their effects on other biochemical parameters, and those parameters evaluated in this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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