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Research paper

Effect of carissa edulis (VAHL) apocynaceae stem bark on hematological parameters in ccl¬4 induced albino rats

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Abstract

Aim/Objectives: There are claims that *Carissa edulis* has positive effects on hematological parameters. The present study was conducted to evaluate the safety and claimed hematological effect of *Carissa edulis* on rats intoxicated with carbon tetrachloride.
Method: Thirty six male albino rats were randomly allotted to six groups of six rats per group. The animals were treated with (250, 500 and 1000mg/kg of the stem bark extract of *Carissa edulis*. The extract was administered daily by gavage to the animals for 28 consecutive days. The 50%v/v Carbon tetrachloride (CCl_4) and olive oil was gavaged twice a week. The animals of the normal control group were given 5ml/kg of distilled water while those of the CCl_4 group were administered CCl_4 only twice a week.
Result: Results of the study showed that the CCl_4 group caused a significant decrease in most of the hematological parameters while the extract treated groups showed a significant increase in these parameters.
Discussion / Conclusion: This suggests that the ethanolic stem bark extract has erythropoietic, thrombopoietic and positive effect on the immune system.

Keywords: *Carissa edulis; Thrombopoietin; Erythropoietin; Hematological Parameters; CCl_4 and Gavage.*

1. Introduction

1.1. Herbal medicine

The utilization of herbal medicines by practitioners for treatment of diseases remains the basis of healthcare system and is gaining increasing popularity particularly among the rural populace in the developing countries. Its rising popularity could be ascribed to its advantage of being efficacious and also a cheap source of medical care. There is a growing disillusion with modern medicine coupled with the misunderstanding that herbal products being natural may be without adverse and toxic effects associated with conventional medicines (Ogbonnia *et al.,* 2010).

1.2. Hematological investigations and health status

Various workers have shown that hematological investigation could be employed to assess the physiological status of an animal (Kakade *et al.,* 1974, Degruchy 1976) and an assessment of hematological parameters can be employed to determine the extent of harmful effect of foreign compound including plant extract on blood. Also cells naturally contains enzymes for their functions and damages to cellular membrane could lead to the run off of the enzymes into the blood where their occurrence or activities can simply be considered as an index of cellular integrity (Coles 1989, Coppa *et al.,* 2002). Further, certain plasma biochemistry might be used to identify tissue injury (Patti & Kulkarni 1993).

1.3. Hepatotoxicity

Drug induced liver toxicity is a major rationale for hepatic dysfunction (Abound 2007). Oxidative stress is measured as a mechanism contributing to the commencement and development of hepatic damage in a variety of liver disorders. Cell damage takes place when there is a surplus of reactive species resulting from oxygen and nitrogen on deficiency of antioxidants (Girish & Pradhan 2008).
Oxidative stress involving enhanced production of reactive oxygen species (ROS) has been implicated in the etiology of many human diseases. Antioxidants capable of neutralizing ROS and their actions are known to be beneficial. In this context, natural dietary components with antioxidants actions can be significant (Bandyopadhyay *et al.,* 1999, Yamamoto 2000).

1.4. Background and justification of the study

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Hematology refers to the study of the number and morphology of the cellular components of blood basically the red blood cells (erythrocytes), white blood cells (leucocytes) and the platelets (thrombocytes) in addition to the application of the outcome in the diagnosis and monitoring of disease (Merck 2012).
Hematological studies are of ecological and physiological importance in helping to understand the connection of blood characteristics to the environment (Ovuru & Ekweozor 2004).
Hematological complications consist primarily of irregularities in the functions morphology and metabolism of erythrocytes, leucocytes and platelets (Comazzi *et al.,* 2004). Blood disorders that have an effect on the RBC consist of anemia which is characterized by low number of RBC and symptoms include; fatigue, pale skin and shortness of breathe with exertion. Cases of anemia include; Iron deficiency anemia, anemia of chronic diseases, pernicious anemia, B_{12} deficiency aplastic anemia, autoimmune hemolytic anemia, thalassemia, sickle cell anemia and polycythemia (Richard *et al.,* 2008).
A range of disorders can cause diseases in white blood cells. This type of white blood cells decrease is normally the neutrophils, in this instance the decrease may be called Neutropenia or granulocytopenia (Lee *et al.,* 2006). Lymphocytopenia is defined as total lymphocyte count below 10 x 10^4 g/l, the cells most frequently affected are the $CD4^+$T cells (Heinz 2008).
Most platelets disorders are as a result of an inadequate number of platelets, a condition identified as thrombocytopenia (Heinz 2008).The categorization of these disorders can be divided into two; congenital and acquired disorder (Heinz 2008).
Most conventional ways of managing anemia, neutropenia and thrombocytopenia may be expensive, have undesirable side effect, hurting to the patient or are not readily accessible. Blood transfusion restricts its usefulness because of the risk of infection, formation of antibodies which could make a late transplant difficult or cause hemoglobin fluctuation (Saunders *et al.,* 2007).
Iron supplements regularly used in anemic disorders often lead to diarrhea, epigastric abdominal discomfort and in some instances increase in infectious diseases and morbidity in areas where bacterial infection are widespread(Tobilli & Brignoli 2007).
Epogen used to treat anemia resulting from Chronic kidney disease can lead to high blood pressure, crippling cluster migraine, joint pains and clotting at the infection sites, skin rash, flu-like symptoms, allergic reactions, seizures, thrombotic events along with other possible side effects (Drueke *et al.,* 2006).
In view of all these setbacks, there is therefore need to develop agents that are effective, cheaply available with minimal side effect as the alternative medical intervention. Unlike conventional drugs which are based on a single active ingredient aiming just one hematological component, the plant derived agents encompasses a cocktail of chemical components that act together to bring back a normal physiological state. Necessitating an urgent need to develop agents that are effective, cheaply available with minimal side effect as alternative means of treatment.
Like other terrestrial plants, *Carissa edulis* has ethno pharmacological significance and has also been exploited by the local people in search of remedies for different ailments to increase strength and manage hematological disorders (Tolo *et al.,* 2006). Its effect in these activities has not been scientifically studied or validated. It was against this background that this study was undertaken to scientifically test the hematological claimed effect of the plant extract in rats.
Carissa edulis (CE) belong to the family Apocynaceae; it was formerly identified as C. pubescence (Irvine 1961, Hutchinson & Dalziel 1963). The plant is regularly known among Hausa people in Northern Nigeria as "Cizaki" and in Somalia as Adishawel. The English name of the plant is Arabic numnum. Other common names include: endelkoring-noeminaem (Africana) 'agam' (Tigrigna and Amharic), 'emir' (Arabic), Muyonzo (Luganda), and 'mianoo-mboo, (Swahili) and Impambalamyoloko, (Sofowara 1986).
Carissa edulis is a spiny, much branched small tree shrub, up to 105m in height, with a milky sap, bark grey, smooth young branchlets with or devoid of hairs, pine simple, straight, 2-5cm long. Leaves ovate to ovate-elliptic lateral in veins, obscure, apex tapering, often with a bristle like tip, base rounded to shallowly. Flowers white tinged with purple, red or pink. Fruits ovoid to just about spherical. The fruits are red black ripening to purplish black.
Carissa edulis is broadly used for the treatment of a variety of ailment but little or no interest has been shown on its hematological benefits. Hence the need to investigate the effect of *Carissa edulis* stem bark on some hematological parameters on carbon tetrachloride induced Wistar albino rats.

2. Materials and methods

2.1. Collection and identification of plant extract

The stem bark of *Carissa edulis* were collected from Fursum East, Jos East Local government of Plateau state on the 30th of January 2016. The plant was identified and authenticated by Mr. J.J Azila of the Federal College of Forestry Jos. A voucher specimen number UJ/PCG/HSP/95A09 was issued and the specimen put in the faculty herbarium for reference. The stem bark were removed from the stems and air dried at room temperature for fourteen (14) days, the dried parts were powered using mortar and pestle and sieved with 29 mesh size. The powdered part was extracted with 700ml 70%v/v aqueous ethanol by means of the soxhlet extraction method for 72 hours. The resultant filtrate was evaporated to dryness on steam bath at 40^0C to provide a brownish extract that was stored in air tight-containers and preserved in refrigerator.

2.2. Animals

Wistar albino rats weighing between (150-220) grams obtained from experiment animal house of the department of Pharmacology University of Jos were used for the study. The rats were fed with standard animal pellets (Pfizer Feeds Nigeria), given water *ad libitum* and maintained in a well-ventilated room.

2.3. Preliminary phytochemical studies of Carissa edulis extract

Phytochemical assessment of the ethanolic stem bark extract of *Carissa edulis* was carried out to identify the presence or absence of secondary metabolites. (Alkaloids, Anthraquinone, cardiac glycosides, carbohydrate, Flavonoids, saponins, Tannins and steroids) by means of standard methods.

2.3.1. Test for alkaloids

The extract (0.5g) was tainted with 5ml of 1% aqueous hydrochloric acid on a steam bath, the resultant mixture was filtered and treated with a small amount of Mayer's reagent and the final 1ml of the filtrate was treated with picric acid solution, precipitation with either of the reagents was taken as a preliminary indication for the presence of alkaloids in the extract (Trease & Evans 1989).

2.3.2. Test for anthraquinones

Borntrager's test was used to find out the presence of anthraquinones. Half gram of the extract was measured into the test tube 5ml chloroform was added into the test tube and shaken for 5 minutes and the final filtrate was shaken with the same volume of 100%ammonia solution. A pink violet or red color in the ammonia (lower layer) signifies the presence of free hydroxyl anthraquinones (Trease& Evans 1989).

2.3.3. Test for cardiac glycosides

Salkowski test was employed in which 0.5g of the extract was weighed and dissolved in 2ml of chloroform. Sulphuric acid was carefully added to form a lower layer. A reddish brown color at the interface indicates the presence of a steroidal ring of a glycone portion of cardiac glycosides (Sofowara 1993)

2.3.4. Test for carbohydrates

Few drops of Molisch's reagents were added to 2ml of the dissolved *Carissa edulis* extract, concentrated sulphuric acid was allowed to run down the tending tube slowly. The interface was observed for a purple color, which is a typical of carbohydrates (Trease& Evans 1989).

2.3.5. Test for flavonoids

Carissa edulis extracts (0.5g) was dissolved in 2ml of dilute sodium hydroxide solution. A few drops of concentrated sulphuric acid were added to it and the presence of flavonoids was pointed out if the solution becomes colorless (Trease& Evans 1989).

2.3.6. Test for tannins

0.5 gram of *Carissa edulis* extract was dissolved in 1ml of distilled water, stirred and filtered. 5% of ferric chloride was added to the filtrate a blue-black or green precipitation was indicative of the presence of tannins (Trease& Evans 1989).

2.3.7. Test for saponins

0.5 gram of *Carissa edulis* extract was dissolved in 5ml of water in a test tube. The resultant mixture was shaken well and warmed for one minute, frothing which persist on warming was taken as a preliminary indication for the presence of saponins (Trease& Evans 1989).

2.3.8. Test for steroids and terpenes

Carissa edulis (2.0g) was dissolved in 15ml of 95% alcohol and boiled in steam bath the resulting filtrate was evaporated to dryness. The residue was then dissolved in 10% of anhydrous chloroform and filtered. The resulting filtrate was mixed with 1ml acetic anhydride followed by the adding of 1ml of concentrated sulphuric acid to run-down the wall of the test tube once the test tube was inclined and a layer beneath was formed. The test tube was observed for green and reddish coloration which is an indication of the presence of steroids and terpenes (Trease& Evans 1989).

2.4. Determination of ld50 of Carissa edulis

Acute toxicity study was carried out for the stem bark extract using the method of Lorke 1983.This was done in two phases. In the first phase, nine (9) rats were randomly alienated into 3 groups of three (3) rats per group and were given 10, 100 and 1000mg/kg of the extract of *Carissa edulis* orally (via a cannula) respectively. The rats were observed for signs of adverse effects and death for 24 hours. Based on the result of the phase one study, the procedure was repeated using another set of three rats randomly alienated into 3 (three) groups of one rat each, given 1600, 2900 and 5000mg/kg bodyweight of the extracts respectively for 24 hours. The rats were observed for signs of toxicity which comprise of but not limited to paw ticking, salivation, stretching, rubbing of nose on the floor and walls of the cage, change in body weight and death.

2.5. Hematological studies

Thirty-six (36) albino rats weighing between (150 – 220g) were randomly divided into six groups comprising of six rats each. Normal control, standard drug, carbon tetrachloride treated group and the three test groups for the stem bark extracts. The standard drug used was silymarin. To study the hematological effect against carbon tetrachloride induced albino rats. *Carissa edulis* stem bark (250, 500 and 1000mg/kg body weight) were given daily by gavage to the animals for 28 consecutive days. The 50%v/v CCl_4 in olive oil was gavaged through gastric tube twice a week on the third and seventh day of each week and with vehicle on the rest of the days while the animals of the silymarin standard group were treated with standard drug silymarin in water at a dose of 50mg/kg daily for 28 consecutive days alongside CCl_4 on the third and seventh day of each week.

On the 29thday each rat was anaesthetized with ethyl ether, the animals were then sacrificed and blood sample collected via the jugular vein into heparinized capillary tubes for hematological evaluations.

Hematological Evaluations

Blood samples collected via the jugular veins into heparinized capillary tubes were used to evaluate the hematological parameters, these include, the red blood cells count, white blood cell count, platelet count, packed cell volume, neutrophils, lymphocyte, monocyte, eosinophil, basophil and hemoglobin concentration.

2.6. Statistical analysis

The results were expressed as mean SEM where applicable. The data used were subjected to ANOVA (Analysis of Variance) using SPSS software (version-20) and each stage ANOVA was significant a Post-Hoc test (Fishers Least Significant Difference) was carried out. The difference was taken to be statistically significant at $p < 0.05$

3. Results

Phytochemical Screening of the Ethanolic Stem-Bark Extract of Carissa edulis

The preliminary phytochemical test for the stem bark extracts was positive for saponins, tannins, flavonoids, carbohydrate cardiac glycosides and steroids. Tannins, flavonoids, carbohydrates and steroids were highly present while alkaloids and anthraquinones were absent.

Determination of LD_{50} of the Stem Bark Extract of Carissa edulis in Rats

Even at a dose of 5000mg/kg there was no record of death, the animal was calm, non-aggressive, decreased exploratory activities and non-stereotype behaviors.

Table 1: Effect of the Ethanolic Stem Bark Extract of Carissa Edulis on Hematological Parameters of Ccl_4 Treated Rats

Treatment	WBC	RBC	PLATELETS	PCV	NEU	LYM	MONO	EOSIN	BASO	HB
A	5.43±0.15	4.83±0.45	148.00±36	32.00±3.46	17.66±3.06	75.66±2.13	6.00±2.54	0.33±0.21	0.50±0.22	18.78±9.26
B	5.65±0.68	6.10±0.45^{a}	118.50±6.03	39.00±2.95^{a}	18.50±1.87	76.50±4.66	3.00±1.34	1.00±0.44	0.83±0.16	11.60±0.84
C	9.70±55^{ab}	3.35±0.20^{ab}	144.66±8.5	23.00±93a^{b}	50.00±4.08^{ab}	46.50±4.67	2.00±0.44^{a}	1.50±0.22	0.16±0.1	6.67±0.73
E	8.26±1.72^{ab}	7.30±0.38^{abc}	261.33±34.95^{abc}	48.33±2.95^{abc}	24.33±2.01^{ac}	71.00±2.84^{c}	3.33±1.42^{c}	1.33±021	-abc	13.33±0.53
G	7.88±1.61	6.60±0.07^{ac}	162.33±10.99	43.66±0.76^{abc}	28.00±2.06^{abc}	69.33±3.10^{c}	0.66±0.21a	1.33±0.55	0.66±0.21	12.13±0.34
I	8.55±0.14^{ab}	5.45±0.13^{c}	158.50±3.13	37.50±2.04^{bc}	23.00±0.93^{c}	72.50±0.76^{c}	0.50±0.22^{a}	3.00±0.89^{abc}	1.00±0.00ac	10.20±0.06
LSD	2.61	0.81	63.71	6.87	6.46	8.20	3.07	1.15	0.44	NS

KEY:
Groups (A) =Normal Control (distilled water)
Groups (B) =Standard Drug (silymarin)
Groups (C) =CCl_4,
Groups (E) =250mg/Kg (Bark) + CCl_4,
Groups (G) =500mg/Kg (Bark) + CCl_4,
Groups (I) =1000mg/Kg (Bark) + CCl_4
LSD = Least Significant Difference
Means tagged with superscript 'a' are significantly different compared to Normal Control at $p<0.05$
Means tagged with superscript 'b' are significantly different compared to Standard Drug at $p<0.05$
Means tagged with superscript 'c' are significantly different compared to CCl_4 at $p<0.05$
Means tagged with superscript a, b and 'c' are significantly different compared to Normal control, Standard Drug and CCl_4 at $p<0.05$; value are Mean ±SEM

Table 1: Effect of the Ethanolic Stem Bark Extract of Carissa edulis on Hematological Parameters of CCl_4 treated Rats.

The values of the blood indices were significantly difference between the treatment groups at $p<0.05$ except for HB which had the same level between the treatment groups.
In WBC, there was a significant increase in all the groups compared to the normal control group.
For RBC, the CCl_4 group had the most significantly lower RBC count than the remaining groups. Other groups were significantly higher than the CCl_4 group.
For Platelet, 250mg of the stem bark extract had the highest concentration of platelets compared to the CCl_4 group the remaining groups (500 and 1000mg/kg) had the same level of platelets with the CCl_4 and normal control group.
For PCV, the least value was in the CCl_4 group which was significantly lower than normal control at (32.00±3.46) i.e. the extract treated groups were significantly higher than the CCl_4 and normal control groups.
For Neutrophils, the highest level was observed in the CCl_4 group at (50.00±4.08) which was significantly higher than the other groups.
In the case of Lymphocytes, the CCl_4 group was also significantly lower compared to the other groups at (46.50±4.67). The normal control groups had the same level with the other groups except CCl_4 group. The groups treated with the three concentrations of the stem bark extract showed significant increase in Lymphocyte level.
For monocytes, 500 and 100mg of the stem bark extract groups and also the Silymarin group had the same level with the CCl_4 group. The values for all the other groups were significantly lower than the normal control group.
In eosinophils, the highest level was observed in 1000mg bark extract group which was significantly higher compared to the other groups at (3.00±0.89).The remaining groups except 1000mg of the stem bark had the same level with the normal control group.
For Basophils, all the treatment groups had the same level with normal control except 1000mg group of the bark extract which is significantly higher than the CCl_4 and the normal control groups.

4. Discussion

The blood is a very important fluid found in humans and other animals. It conveys gases and nutrients to all organs and tissues of the body and carries away waste materials as well as regulation of body temperature. The blood transports foreign body which might be

harmful to all parts of the body leading to initiation and development of diseases. The echelon of blood components is characteristics of one's health status (Ohaeri & Eluwa 2011, Sexena *et al.,* 2011).

The fundamental reason for assessing the RBC is to determine the level of anemia and to evaluate usual erythropoiesis (Momoh & Damazio 2014). The result of this study showed that the CCl_4 group had the least RBC and PCV while the extract treated groups showed significant improvement in the levels of these parameters, this is consistent with the study of (Jorum 2016), which shows that the administration of the extract of *Carissa edulis* significantly boost the levels of PCV, RBC and other parameters in normal rats (Jorum 2016).The decrease in the CCl_4 group may be ascribed to the cytotoxic effect and the repression of erythropoiesis while the increase in the level of RBC and PCV upon administration of the extract suggests that the extract could have trigger erythropoietin release in the kidney, which is the humoral regular of RBC production (DeGruchy 1976).

The presence of phytochemical like Flavonoids, Tannins and Terpenes in the stem bark extract of *Carissa edulis* may be accountable for the Hematopoietic stimulating effects (Olsson & Aher 2006). This is in line with previous studies which showed the therapeutic and prophylactic oral administration of antioxidant supplement of plant extracts drastically increase cells hematopoietic source in animals exposed to potentially fatal dose of radiation (*Wambi et al.,* 2008). Flavonoids, tannins and terpenes have been established to protect erythrocytes from oxidative damage (Grassman 2005). Further (Ren *et al.,* 2003) reported that Flavonoids have a range of benefits for human health owing to their antioxidants and free radical scavenging activities as well as anti-inflammatory, antiviral and anticancer features (Naaz *et al.,* 2007).

In the case of WBC, the study showed that there was a significant increase in the level of WBC in all the extract treated groups and CCl_4 group compared to the normal control group. The elevation of WBC may be as a result of the stimulation of the immune defense system (Kashinath 1990) resulting to enhancement in the pace of deletion from circulation. Granulocytes-macrophage colony stimulating factor, macrophage colony stimulating factor interleukins (IL-2, IL-4 and IL-5) control the proliferation, differentiation and maturation of committed stem cells responsible for the production of WBC(Ganong 2001).In the same way, literatures have shown that increased concentration of antigen in the body results in high value of WBC(Schalm *et al.,* 1975).

A low level of monocytes in the blood can be due to whatever thing that decreases the total WBC such as chemotherapy, infection of the blood stream and bone marrow disorders (Dawkins 1963). The result from this studies suggest that there was no significant difference between the CCl_4 and the extract treated groups, probably due to the presence of CCl_4 that might have triggered the elevation of the monocytes.

The coagulation of blood requires that platelets be present in adequate size and functions. The lower concentration of the stem bark extract produced significant increase in platelet level compared to the CCl_4 and the normal control group. The increase in the platelet level observed may be due to the stimulation outcome on thrombopoietin (Li *et al.,* 1999). The significant elevations in platelet level suggest that the extract may contain compounds and phytochemicals that may have stimulated thrombopoietic process in rats. The presence of Tannins has been shown to bestow antihemorhagic properties in animals (Viyaya *et al.,* 1995, Weber *et al.,* 2003). The effect of the extract on platelets may imply that it may be beneficial in thrombocytopenia which is a disorder characterized by relative decrease in thrombocytes usually known as platelets present in the blood. (DeGruchy 1976).

The significant decrease in lymphocyte level in the CCl_4 group may be due to the inhibition of the effectors cell of the immune system while the increase in the other groups may be due to the ability of the extract to stimulate the effectors cell of the immune system resulting to an increase in lymphocyte level.

In the case of neutrophils, the highest level was observed in the CCl_4 group compared to the remaining groups at (46.50 + 4.67), this may imply that the toxic agent CCl_4 probably has the ability to enhance blood component of phagocytosis (Kashinath 1990).

For the Basophil, the result from this study showed a significant increase between the CCl_4 and the extract treated groups at a dose of 1000mg/kg of the stem bark extract which suggest positive effect on the immune system. (Jorum 2016). For the eosinophils, the result showed a significant elevation in the eosinophil level at a dose of 1000mg/kg of the extract which may also propose a positive effect on the immune system (Jorum 2016).

5. Conclusion

The study showed that the extract treated group showed significant increase in the levels of RBC and PCV compared to the CCl_4 group which had the least value. This suggests that the extract could stimulate erythropoietin release in the kidney.

The stem bark extract also produced significant increase in the level of platelets, WBC, lymphocyte, basophil and eosinophil which suggest a positive effect on the immune system.

However, the active compound accountable for the hematological effect has so far not been identified in this study. The mechanism of action also remained unproven. Further research is therefore required to discover the constituents of the tested plant responsible for the hematological effect.

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