RESEARCH ARTICLE

ISSN: 0331 - 670X
https://doi.org/10.51412/psnnjp.2021.4

The Nigerian Journal of PHARMACY
AS MEN OF HONOUR WE JOIN HANDS
THE OFFICIAL ORGAN OF THE PHARMACEUTICAL SOCIETY OF NIGERIA FOUNDED 1927

EFFECTS OF *CARISSA EDULIS* LEAF EXTRACT ON HEMATOLOGICAL PARAMETERS OF CCL_4 INDUCED ALBINO RATS.

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ARTICLE INFO

Article history:

Received	25 Dec 2020
Revised	11 Jan 2021
Accepted	8 Feb 2021
Online	31 Mar 2021
Published	-

Keywords:

Carissa edulis, Erythropoietin, Thrombopoietin, Hematological Parameters and CCl_4

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ABSTRACT

Background: The aim of this study was to investigate the effect of *Carissa edulis* leaves extract on some hematological parameters of rats treated with carbon tetrachloride.
Method: Thirty male albino rats were randomly allotted to six groups of five rats per group. The animals were treated with (250, 500 and 1000mg/kg) of Carissa edulis leaf extract. The extract was given daily by gavage to the animals for 28 consecutive days. The 50% V/V CCl4 Carbon tetrachloride and olive oil was gavaged through the gastric tube twice a week for 4 weeks to cover the duration of treatment (28days). The animals of the normal control group were given 5ml/kg of distilled water while the animal of the CCl4 treated group were administered with CCl4 only twice a week for 4 weeks. Hematological parameters investigated included; Red Blood Cell, White Blood Cell, Platelets, Packed Cells Volume, Neutrophils, Lymphocytes, Monocytes, Eosinophils, Basophils and Hemoglobin.
Results: Results of the study revealed that CCl_4 group caused a significant decrease in most of the hematological parameters while the extract treated groups showed an increase in the same parameters.
Conclusion: This suggests that the plant extract may possess some erythropoietin, thrombopoietin, and a positive effect on the immune system.

1. Introduction

Medicinal plants are central part of health care. Huge varieties of plants are accessible with identified therapeutic effects [1]. Approximately 70 – 80% of people globally rely on medicinal plant to cure various human ailments [2]. In addition, herbal drugs have gained much significance due to their easy adaptability, low cost and lesser side reactions on patients [3]. Medicinal plants have been employed to treat different disorders throughout the history of human life, but the use of synthetic drugs was extremely rampant since the middle of last century[4,5].
Hematological studies are of ecological and physiological concern in helping to comprehend the relationship of blood characteristics to the environment [6,7]. Erythrocytes have three main functions which include:- distribution of oxygen, removal of carbon dioxide and ensuring that the acidic and basic values of the body are normal [8]. For the metabolic requirements to be met, the suitable amount of nutrients and oxygen must be available and supplied to the tissue [9]. White blood cells are the cells of the immune system. All leucocytes are formed and originate from the hematopoietic stem cell [10]. Platelets are a component of blood whose main role is to stop bleeding by clumping and clogging blood vessel injuries [11].
Hematological complications comprise mainly of abnormalities in the function, morphology and metabolism of erythrocytes, leucocytes and platelets [12-14].
Liver diseases are one of the most important causes of mortality and morbidity globally, drug induced liver toxicity is a main cause of hepatic dysfunction [15]. Oxidative stress is considered as a mechanism in contributing to the commencement and development of hepatic damage in a variety of liver disorder [16-18]. Carbon tetrachloride (CCl_4) is one of the most studied environmental toxins. [19]. It is also one of the most commonly used experimental models for the study of Hepatotoxicity in acute and chronic liver failure [20-21].
Most standard ways of managing anemia, neutropenia and thrombocytopenia may be costly, have unwanted side effects, painful to patients or are not easily accessible. Blood transfusion confines its usefulness due to the risk of infection, formation of antibodies which could obscure a late transplant or causes hemoglobin fluctuation [22]. Iron supplement commonly used in anemia disorders often lead to diarrhea, epigastric abdominal discomfort and in some instances increase in infectious diseases morbidity in areas where bacterial infections are common [23]. Epogen used to treat anemia resulting from chronic Kidney disease can result to high blood

pressure, crippling cluster migraine, joint pain and clotting at the infection sites, skin rash, flu-like symptoms, allergic reactions, seizures, thrombotic events are among other likely side effects [24].

In view of all these setbacks, there is hence a need to develop agents that are effective, cheaply available with insignificant side effect as the alternative medical intervention. Unlike conventional drugs which are based on a single active ingredient aiming at just one hematological component, the plant derived agents comprise of a combination of chemical components that act collectively to restore a normal physiological state. There is as a result an urgent need to develop agents that are effective, cheaply available and with minimal side effect as alternative means of treatment.

Carissa edulis has ethnobotanical importance and has also been exploited by the local people in the search for remedies for various ailments to increase vitality and manage hematological disorders [25]. However, 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 extracts in rats.

2. Method

2.1 Identification and authentication of Carissa edulis

Fresh leaves of Carissa edulis were collected from Fursum east, Jos east local government of plateau state on 25th January 2017. 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 deposited in the Faculty of Pharmaceutical Science University of Jos Herbarium for reference. The leaves were detached from the stems, and air dried at room temperature for fourteen (14) days. The dried part was powdered using mortar and pestle and sieved with 29 mesh size. The powder was soxhlet extracted with 700ml 70% V/V ethanol for 72 hours. The resultant filtrate was evaporated to dryness on steam bath at 40°C to give greenish extract that was stored in air tight container and preserved at 4°C until required for use.

2.2 Preliminary Phytochemical Studies of Carissa edulis Extract

Phytochemical evaluation of the ethanolic leaf extract of Carissa edulis was carried out to identify the presence or absence of secondary metabolites (Alkaloids, Anthraquinones, and Cardiac glycosides, Carbohydrate, Flavonoids, Saponins, Tannins and Steroids) using standard method [26,27].

2.4 Determination of LD50 of Carissa edulis

The acute toxicity study of the extract was determined using the method of Lorke (1983) [28] this was done in two phases, in the first phase, nine rats were randomly divided into three groups of three rats per-group and were given 10,100 and 1000 mg/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 divided into 3 (three) groups of one rat each, based on Lorke,s method (1983) given 1600, 3900 and 5000 mg/kg body weight of the extracts respectively for 14 days. The rats were observed for signs of toxicity which include 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 Animals Studies

Wistar albino rats of both sexes weighing between (120 – 220) grams obtained from experimental 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.

Thirty-six (36) albino rats weighing between (150 – 220 g) were randomly divided into six groups comprising of six rats each. Normal control (consisting of animals who do not receive the experimental treatment, but were administered distilled water 5mL/kg body weight daily for 28 days) standard drug, carbon tetrachloride treated group and the three test groups (250, 500 and 1000 mg/kg) for the leaves extract. The standard drug used was (silymarin) a standardized extract from the dried seeds of Silybum marianum clinically used as an antihepatotoxic agent. To study the hematological effect of the leaves extract of Carissa edulis against carbon-tetrachloride induced liver toxicity in rats, Carissa edulis leaves extract (250, 500 1000 mg/kg body weight) was given daily by gavage to the animals for 28 consecutive days. Animals in the Silymarin group were treated with Standard drug silymarin in water at a dose of 50mg/kg daily for 28 consecutive days. The 50%V/V CCl4 in olive oil was introduced via oral gavage using a gastric tube to the CCl4 group, the silymarin group and to the three treatment groups twice a week on the third and seventh day of each week for 28 days.

On day 29, each rat was anaesthetized with ethyl ether. The animals were then sacrificed, and the blood sample collected via the jugular vein into heparinized capillary tubes for hematological evaluation. Histological evaluations were carried out using blood samples collected via the jugular veins into heparinized capillary tubes. Red blood cell count, white blood cell count, platelet count, packed cell volume, neutrophils, lymphocyte, monocytes, eosinophils, basophils and hemoglobin concentration were evaluated.

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 state ANOVA was significant a POST-HOC test (Fishers Least Significant Difference) was carried out. The difference was taken to be statistically significant at $P < 0.05$.

3.0 Results

3.1 Phytochemical Screening of Ethanolic Leaf Extracts of Carissa edulis

The preliminary phytochemical test for the leaf extracts was positive for saponins, flavonoids, carbohydrates and steroids. Tannins is more present while flavonoids is highly present in the extracts. Alkaloids, cardiac glycosides and anthraquinones were absent.

3.2 Determination of LD50 of the Leaf Extract of Carissa edulis

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

3.3 Effect of the Leaf Extract of Carissa edulis on Hematological Parameters of CCl4 treated Rats.

The values of the blood indices were significantly different between the treatment groups at $P<0.05$ except for HB which had same level between the treatment. The WBC showed significant increase in all the treatment groups compared to the normal control group. Red blood cell count in the CCl_4 group was significantly lower compared to the other groups. The extracts treated groups had significantly higher platelet count than CCl_4 and the normal control group.

For PCV, the extract treated groups were significantly higher than the CCl_4 and the normal control group. For Neutrophils, the highest value was observed in the CCl_4 group at (50 ± 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 group had the same level with the other group except the CCl_4 group. Groups treated with the three concentration of the leaf extract showed significant increase in Lymphocyte level. For monocytes, 250 mg of the leaf extract and the Silymarin group had the same level of monocytes with CCl_4 group the levels for 500 and 1000 mg of extract were significantly lower than the normal control group. For Eosinophils, the 250 mg of the leaf extract had zero eosin level while the remaining groups had the same level with the normal control. In the case of Basophils, 250 and 500 mg of the leaf extract had the same level with normal control group except 1000 mg extract group which was significantly higher than the CCl_4 and the normal control groups. See Table 1.

Table 1: *Effect of the Ethanolic Leaf extract of Carissa edulis on Haematological Parameters of CCl4 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±0.16	6.67±0.73
D	11.65±0.33[ab]	6.15±0.23[ac]	273.50±12.67[abc]	42.00±1.89[abc]	24.00±1.09[c]	73.00±0.93[c]	0.50±0.22[a]	-	0.83±0.16	10.85±0.21
F	7.80±0.95	6.66±0.18[ac]	192.66±39.73	45.66±1.87[abc]	18.66±1.87[c]	80.33±2.23[c]	-[a]	0.33±0.21	0.66±0.21	11.90±0.26
H	11.35±0.56[ab]	6.10±0.21[ac]	191.00±3.29	39.50±83[abc]	21.50±1.87[c]	75.50±1.83[c]	-[a]	2.00±0.00[ab]	1.00±0.00ac	11.20±0.40
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) = CCl4,
Groups (D) = 250mg/Kg (Leaves) +CCL_4,
Groups (F) = 500mg/Kg (Leaves) +CCL_4,
Groups (H) = 1000mg/Kg (Leaves) +CCL_4.
LSD = Least Standard Deviation

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 CCl4 at $p<0.05$

Means tagged with superscript a, b and 'c' are significantly different compared to Normal control, Standard Drug and CCl4 at $p<0.05$; value are Mean ±SEM

WBC = White blood cell,

RBC = Red Blood cell,

PVC = Packed cell volume, Neut = Neutrophils, Lymph = Lymphocyte, Mono = Monocytes, Eosin Eosinophil, BASO = Basophils ,HB = haemoglobin concentration.

4. Discussion

Hematological indices have been reported to be reliable parameters for the assessment of health status of animals [29], [30]. The primary reason for assessing the RBC is to check the level of anemia and to evaluate normal erythropoiesis [31]. The result of this study shows that the (CCl_4) group had the least (RBC) and (PCV) while the extract treated groups showed significant increase in the level of these parameters. This is consistent with the study of [32] which shows that the administration of the leaf extract of Carissa edulis significantly increases the levels of PCV, RBC and other parameters in normal rats [32].

The decrease in the CCl_4 group may be ascribed to the cytotoxic effect and the repression of erythropoiesis while the increases in the level of RBC and PCV upon administration of the extract imply that the extract could have stimulated erythropoietin release in the kidney which is the hormonal regulator or RBC production [33].

The presence of phytochemicals like flavonoids, tannins and Terpenes in the leaf extract may be accountable for the Hematopoietic stimulating effects [34]. This is in line with previous studies which revealed that therapeutic and prophylactic oral administration of antioxidant supplement of plant extracts significantly increase cell hematopoietic origin in animals exposed to a possible toxic dose of radiation [35]. Flavonoids, tannins and terpenes have been found to guard the erythrocytes from oxidative damage [36-39], flavonoids have a variety of benefits for human health owing to their antioxidant and free radical scavenging activities alongside anti-inflammatory, antiviral and anticancer properties [39].

White blood cell count results obtained from the study revealed that there was a significant increase in the level of WBC in all extract treated groups compared to the normal control group. The elevation in WBC may be due to the stimulation of the immune defense system [40] resulting to improvement in the rate of entry of leucocytes into the blood from the bone-marrow and a reduced rate of elimination from circulation. Granulocyte-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 liable for the production of WBC [41].

A low level of monocytes in the blood can be caused by something that decrease the overall WBC such as blood stream infection, chemotherapy, or blood marrow disorders. The result of this study shows that there was no significant difference between the CCl_4 and the extract treated groups probably due to the presence of the CCl_4 that must have triggered the elevation of the monocytes.

Platelets are blood cells implicated in coagulation [43]. Coagulation of blood entails that the platelets should be in sufficient size, number, and function. The lowest concentration of the extract produced significant increase in platelet level compared to the $CCl_4$and the normal control group. The increase in platelet levels, observed in the group treated with 250mg of the leaves extract may be due to the stimulatory effect on thrombopoietin [44]. The significant increase in platelets suggests that the extract may contain compounds and photochemical that may have stimulated the thrombopoietic process in rats. The significant increase in platelets may be accredited to the presence of tannins which has been shown to confer anti-hemorrhagic properties in animals [45]. The effect of the extract on platelets suggest that it may be beneficial in Thrombocytopenia which is a disorder typified by relative decrease in thrombocytes commonly known as platelets present in the blood [46].

Lymphocytes are the major effectors cells of the immune system [47]. They are markers of infection autoimmune disorders and some kind of cancers. Some infectious diseases like viral infection and hepatitis can lower the lymphocyte level. The significant decrease in lymphocyte level in the CCl4 group may be due to the inhibition of the effector cells of the immune system while the increase in the other groups may be due to the ability of the extract to activate the effector cells of the immune system resulting to an increase in lymphocytes level.

In neutrophils, the highest level was observed in the CCl_4 group compared to other groups, this may be due to the capability of CCl_4 to enhance blood component of phagocytosis [40]. The result of this study may suggest that Carissa edulis extract possess hematopoietic activity and is not haemato-toxic.

For Basophils, there was a significant increase between the CCl_4 group, and the extract treated group at a dose of 1000mg/kg of the leaves extract which propose positive effect on the immune system [32].

For eosinophils, there was a significant increase between the CCl_4 group and the extract treated group at a dose of 1000mg/kg, which propose positive effect on the immune system [32].

5. Conclusion

The ethanolic leaves extract of Carissa edulis showed a significant increase in the levels of RBC, PCV and platelets which suggest that the extract stimulates erythropoietic and thrombopoeitic process in rats. The study also revealed a significant increase in the level of WBC, Lymphocyte, basophils and eosinophils among the extract treated group which suggest a positive effect on the immune system. The hematological effect of Carissa edulis leaves extract was probably due to the presence of some endogenous substances with Hematopoietic, erythropoietin and positive effect on the immune system.

6. ACKNOWLEDGEMETS

Prof. SO, Otimeyi, Prof. NN, Wannang for their academic mentoring, Mr. S, Azi and Mr. DA, Bulus for their technical assistance.

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