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Short communication

Hematopoietic Alteration in Carbon tetrachloride-Induced Liver Injury and Amelioration by *Vitex agnus*-castus Extract

***Ajani R.S. and Akpovwovwo N.A.**

Division of Gastrointestinal and Morphological Anatomy, Department of Anatomy, College of Medicine, University of Ibadan, Nigeria.

Summary: Although the liver is not a primary hematopoietic site after the period of embryogenesis, however liver diseases in human can have adverse effects on hematopoiesis. This study evaluated the ability of *Vitex angus*-castus extract to ameliorate hematopoietic alteration occasioned by carbon tetrachloride (CCl_4) induced acute liver injury. Varying doses of *V. angus*-castus plant extract were administered to groups of rats with CCl_4 Induced acute liver injury. The low dose group (LEL) had 200mg/Kg body weight; the medium dose (MEL) had 400 mg/kg while the high dose group (HEL) had 600 mg/Kg of the extract once daily for 21 days. Each group had composite control without liver injury ie LE, ME and HE. The packed cell volume (PCV), hemoglobin concentration (Hb%), red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular hemoglobin (MCH) were similar across the groups. Groups LEL and ME had significantly elevated white blood cell count (WBC). Amongst the composite groups ie LE: LEL; ME:MEL; & HE:HEL, there was significant difference within the composite group. The WBC differential was lymphocyte predominant without significant difference. The two low extract groups (LE & LEL) had significant thrombocytopenia ie low platelet count. The ethanolic extract of *V. angus*-castus only affects the white cell and platelet components of the blood and not the erythrocyte parameters.

Keywords*: Carbon tetrachloride- induced liver injury, Vitex angus castus extract, red blood cell, white blood cell.*

**Authors for correspondence: rs.ajani@mail1.ui.edu.ng rsaajani@yahoo.co.uk , Tel: +234-8023007362*

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INTRODUCTION

The human circulatory system consists of the blood, lymph, body fluids and electrolytes, blood vessels, lymphatics and the heart. The two main components of the blood are cells and plasma which is fluidly. The cellular component of the blood consists of leukocytes (white blood cells), erythrocyte (red blood cell) and platelets. All these cell series have specific and diverse functions with structural or physiologic alterations resulting in a myriad of pathologies with variable dimensions. Hematopoiesis is the process by which these cells are formed.

During embryogenesis, hematopoiesis starts in the yolk sac mesoderm and subsequently involves the liver and spleen. Postnatally, hematopoiesis involves the stem cells precursors located in the bone marrow of the long bones, however, lymphocyte production also involves the lymphoid organs such as spleen, lymph nodes and spleen. Hematopoiesis can be altered by nutritional derangement, bone marrow diseases, chemical/drug toxicity, exposure to ionizing radiation, abnormal renal function and liver diseases. Deranged hematopoiesis may result in either low or elevated values of all or specific blood cells depending on the underlying pathology. Low cell counts may require transfusion of whole blood or its component, iron (ferrous) supplement or even bone marrow transplant. While exchange blood transfusion or chemotherapy may be needed to correct elevated levels of blood cells counts.

Many chemical substances such as paracetamol, thioacetamide and carbon tetrachloride (CCl_4) have been used to induce acute liver injury in experimental animals (Butterworth *et al*., 2009), the most widely used being CCl_4 (Cong *et al*.,2017; Zhang *et al*., 2017). The hepatic cytochrome P450 enzyme system metabolizes CCl_4 to produce highly active trichloromethyl radicals and reactive oxygen species (Wei *et al*., 2023). All these molecules will lead to lipid peroxidation, oxidative stress and along with up regulation of proinflammatory cytokines, acute liver injury becomes established.

Vitex agnus-castus, a deciduous shrub that grows naturally in several countries including Nigeria has several phytochemicals such as flavonoids and diterpenoids that had been documented to have antioxidant activity (Hajdú Z *et al*.,2007) and oestrogenic activity (Jarry H *et al*., 2003) while its essential oils said to be bactericidal (Senatore F *et al*.,2003). Other bioactivities attributable to the plant in literature include anti-inflammatory, antihistamine, analgesia and cytotoxicity against certain cell lines (Kamal N, *et al*., 2022).

There is however no available information documenting its role in hematopoietic alteration occasioned

by carbon tetrachloride liver toxicity. This study was thus premised on this observation.

MATERIALS AND METHODS

Plant collection and authentication: *Vitex agnus* castus plant was sourced from a herbal garden located in Aladja, Delta State, South-South Region of Nigeria. Botanical identification and specie confirmation were done at the Herbarium Unit of the Department of Botany, University of Ibadan, Nigeria. For reference purpose, a sample of the plant was banked with the Herbarium with voucher number UIH-22953.

Extract preparation: The flowering stems of *V. agnus* castus plant were initially washed under running water and then allowed to dry under ambient temperature till moisture content was zero. The dried sample was subsequently milled into fine powder 1.2 kg of which was used for the ethanolic extraction with a 9.6 % yield. A portion of the powdery sample was used for phytochemical analyses.

Animals: Forty adult male Wistar rats weighing 190 to 290 g were sourced from the Central Animal house of the College of Medicine, University of Ibadan. They were acclimatized for three weeks in a well-ventilated and illuminated environment with optimal ambient temperature (26±2°C, 12 hours light / dark cycle) that was conducive for the study. The animals were fed liberally with locally sourced but standard pelletized rat feed and had unrestricted water intake.

Design of the Experiment: The confounding factors of the study were induction of liver injury and dosage of extract administered consequent upon which eight groups with five animals each were created as follows: (1) Normal control (NC)- liver injury not induced; (2) Liver injury (LI)- extract not administered; (3a) Low extract without liver injury (LE); (3b) Low extract with liver injury (LEL) **;** (4a) Medium extract without liver injury (ME)**;** (4b) Medium extract with liver injury (MEL); (5a) High extract without liver injury (HE)
(5b) High extract with liver injury (HEL)

Induction of Liver Injury: Based on empirical evidence from available literature, liver injury was induced by single intraperitoneal administration of carbon tetrachloride at a dose of 1.6mg /kg (Maiti K *et al*., 2005; Ohta Y *et al*.,2000). The induction of acute liver injury was done for animals in groups LI, LEL, MEL and HEL.

Conduct of the Experiments: The ethanolic extract of the plant was administered once daily via oral steel canula to the following groups- low dose extract (LE &LEL), medium dose extract (ME & MEL) and High dose extract (HE & HEL) at respective dosage of 200 mg/kg, 400 mg/kg and 600 mg/kg for 21 days. The normal control (NC) and the liver injury (LI) groups had only rat chow and water for the same period. On day 22 of the study, venous blood was collected through intraocular puncture from the animals and stored in the appropriate specimen bottle for subsequent hematological analysis.

Ethical Conduct: In the conduct of this study, the animals were handled in accordance to the guidelines as prescribed by the ethical conduct of animal research of the University of Ibadan. Also, the principles of laboratory animal care as contained in the 8th edition (2011) of the Guide for the Care and Use of Laboratory Animals by the National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals were observed (16).

Data Analysis and Processing: The numerical aspects of the results were analyzed with Statistical Package for the Social Sciences (SPSS) version 24 and expressed as percentages, means plus standard deviation of means (SD). The student t- test was used for inter group comparison and level of significance was set at $p<0.05$.

RESULTS

The results of the haematological changes in control and extract treated rats are shown in Table 1. The packed cell volume (PCV) was least in the high extract dose (HE) group (49.2±3.19 %) and highest in the liver injury (LI) group (53.2±2.23 %). The differences in the PCV between the groups were insignificant.

Table 1:
Haematological Parameters

	GROUPS							
TESTS	**CN**	**LI**	**LE**	**LEL**	**ME**	**MEL**	**HE**	**HEL**
PCV(%)	52.6±1.85	53.2±2.23	50.0±0.1	51.25±2.59	51.75±2.05	51.4±4.27	49.2±3.19	50.25±2.28
Hb (g/dl)	17.1±0.31	17.3±0.42	16.6±0.8	17.10±0.84	16.94±0.61	17.21±0.9	16.3±1.4	16.81±0.6
RBC (10^6/µL)	8.61±0.11	8.72±0.12	8.23±0.2	8.51±0.31	8.71±0.14	8.52±0.31	8.32±0.62	8.51±0.31
MCV(μ^3)	60.82±1.72	60.78±2.17	59.44±1.25	60.44±2.15	59.35±1.95	60.69±3.09	59.58±1.11	59.44±0.91
MCHC (g/dl)	32.51±0.67	33.04±0.33	31.53±0.32	32.57± 0.34	31.83± 1.76	33.10± 0.55	33.51± 0.6	32.53± 0.73
MCH (µg)	19.97±0.14	19.87± 0.3	18.76±0.48	19.96± 0.52	19.33± 0.46	19.28± 0.58	19.72±0.55	19.92± 0.15
WBC (10^3/µL)	3.57±0.35	3.68±0.44	3.9±0.45	$4.66±0.37^{\alpha\,\delta}$	$4.75±0.99^{\alpha}$	$3.83±0.31^{\delta}$	3.52±0.20	$4.39±0.54^{\beta}$
Lymphocyte (%	76±0.89	74.4±2.66	75.4±2.2	74.75±1.92	75.25±0.83	74.4±1.85	73.6±2.58	$74.0±0.82^{\alpha}$
Neutrophil (%)	20.2±2.04	22.2±3.37	21.2±3.3	22.75±3.83	20.5±1.5	21.2±1.47	22.8±2.71	$23.5±1.12^{\alpha}$
Platelet (10^3/µL	100.8±7.08	107± 27.13	74.5±18.57	$76.5±19.87^{\varphi}$	$119.25±0.99^{\varphi}$	$117.6±12.39^{\varphi}$	106.2±8.38	$111.75±8.84^{\varphi}$

$^{\alpha}$ significantly different from the Control(NC); n=5
$^{\beta}$ significantly different from that of its non-injured counterpart.
$^{\varphi}$ of significant difference when compared with LEL
Values with $^{\delta}$ symbol are of significant difference
PCV- Packed cell volume, WBC-White blood cell count, MCV-Mean corpuscular volume, MCH- Mean corpuscular hemoglobin; MCHC-Mean corpuscular hemoglobin concentration; RBC- Red blood cell count, Hb- Hemoglobin concentration.

The mean hemoglobin concentration values parallel those of the PCV. Although no significant difference was observed in the red blood cell (RBC) counts, the LI group had the highest mean RBC count while group HE had the least count (8.72±0.12 vs 8.32±0.62 x 106). The leucocyte (white blood cell) counts of the medium extract (ME) and low dose extract with liver injury(LEL) groups were significantly elevated when compared with that of the control (4.75±0.99, 4.66±0.37 vs 3.57±0.35 x 103). The high dose extract with liver injury (HEL) group had a significantly elevated WBC count than its non-injury counterpart (HE) (4.39±0.54 vs 3.52±0.20 x 103). The WBC count of LEL was also observed to be markedly elevated in comparison with that of the MEL (4.66±0.37 vs 3.83±0.31 x 103). In terms of differentials, the white blood cells were predominantly lymphocytes. Both the lymphocyte and neutrophil counts of the HEL group were significantly different from those of the control group (74.0±0.82 vs 76±0.89; 23.5±1.12 vs 20.2±2.04 %). The platelet count of group CN was similar to those groups ME, MEL, HE and HEL but higher than those of groups LE and HEL. Also, the platelet count of group LEL was significantly lower to those of the other extract groups. As for the mean corpuscular volume (MVC), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular hemoglobin (MCH), the results were not significantly different from those of the control or even between the groups.

DISCUSSION

The hemoglobin moiety of the red blood cell is responsible for oxygen carriage and delivery to the organs and tissue for proper metabolic function. Administration of carbon tetrachloride to induce hepatic injury triggers off an acute inflammatory response this creates a stressful milieu. As a coping strategy, there will be kan increased tissue metabolism with consequent elevated oxygen demand. This will stimulate increased red blood cell production (erythropoiesis) by the stem cells. This may explain why the liver injured group of this study had the highest packed cell volume (PCV). Also, the PCVs were not significantly different amongst the groups this might be due to the durations of the acute liver insult and administration of the extracts. If these had been chronic (ie long durations) the picture might have been different.

The total circulating red cell mass may be elevated or reduced absolutely or relatively. Relative alternation known as hemoconcentration (elevation) or hemo-dilution (reduction) usually results from altered volume of circulatory body fluid. Absolute reduction in circulating red cell mass known as anemia presents clinically as low PCV and is much more common than absolute elevation which is usually due to polycythemia. Classification of anemia is premised on alterations of the red cell morphology. Such alterations include size (normocytic, microcytic and macrocytic), degree of hemoglobinazation (normochromic or hypochromic) and shape. Each of these types of anemias has different aetiology, abnormal hemoglobin synthesis usually causes microcytic hypochromic anemias while macrocytic varieties result from abnormalities that impair maturation of erythroid precursors in the bone marrow. Treatment of anemia depends on the type, thus proper classification is crucial and a prerequisite for effective therapy that will ultimately transform to an excellent prognosis. Evaluation of the red cell mass is very important for correct classification of anemia. Laboratory parameters used to evaluate red cell mass include-: the (i) Mean cell volume which is the average volume of a red cell, (ii) Mean cell hemoglobin which is the average mass of the hemoglobin content of a red cell, (iii) Mean cell hemoglobin concentration- this is the average concentration of hemoglobin in a given volume of packed red cells and (iv) Red cell width-this is the coefficient of variation of red cell volume (Notta F *et al*., 2016). In this study, all these red cell parameters of the experimental groups were similar to those of the control and were neither affected by the chemical toxin nor by the plant extract. This might be due to the fact that erythropoiesis exutero occurs in the bone marrow and not in the liver.

Blood cells are formed from stem cells through a series of oligopotent progenitors that progressively become unipotent. These oligopotent progenitors largely reside in the fetal liver while the unipotent progenitors that give rise to the specific three blood cell lines domicile mostly in the bone marrow (Notta F *et al*., 2016). The induction of hepatic injury did not appear to affect leucocyte counts as the values for both control and liver injury groups were similar. However, the extract appeared to stimulate production of white blood cells (WBC) as evidenced by significant leukocytosis in groups LEL, ME and HEL. The non-injured medium extract group (ME) had higher but insignificant WBC count than the LEL and HEL. Also the leucocyte count of ME was significantly higher that of its liver injured group (MEL). Also, the injured low and high extract groups had significant higher WBC counts than their respective non-injured groups. Thus low and high doses of V.angus castus extract stimulated leukocytosis while medium dose of the extract depressed it ie leukopenia. This may be due to the well-known erratic behaviour of plant extracts hence the need to exercise caution when recommending plant extracts either as supplement, therapeutic or preventive. Differential analysis of the WBC counts revealed lymphocyte predominance. Lymphocytes are largely responsible for the integrity of the immune system through synthesis of antibodies. This may be the mechanism via which V. angus-castus exerts its immunological function. High neutrophil count in the blood known as neutrophilic leukocytosis is mostly due to acute bacterial infections (Kumar *et al*.,2015). In this study, the HEL had a significantly higher neutrophil differential when compared with the control, this was not absolute but rather relative as the overall picture was that of lymphocyte predominance.

The liver has an excellent regenerative capability, this occurs through division of matured hepatocytes and cholangiocytes within the liver. These cells are usually mitotically dormant (Boulter *et al*., 2013). When there is severe liver injury, the ability of the hepatocytes and cholangiocytes to regenerate may become overwhelmed. In this situation, the hepatic stem (progenitor) cells become activated (Espanol-Suner *et al*., 2012). During liver injury, fibrogenesis is enhanced by some epithelial cells that undergo epithelial to- mesenchymal transition. However, certain mesenchymal cells can transform into epithelial cells and ultimately differentiate into either hepatocytes or cholangiocytes. This suggests that multiple cell types modulate the outcome of liver injury (Xie *et al*. 2013). These

hepatic progenitor cells are located in the canals of Hering (Theise ND *et al*.,1999), portal area (Furuyama K *et al*., 2011) space of Disse (Kordes C *et al*.,2013) and central vein (Tarlow BD *et al*., 2014). The space of Disse also contain haematopoietic stem cells that rarely divide, this may explain why haematopoiesis does not occur in the liver ex utero. In acute liver insult, the most damaged cell type is the hepatocyte and in humans, for hepatic stem cell activation there has to be minimum of 50 % loss of hepatocytes (Falkowski *et al*., 2013; Katoonizadeh A *et al*.,2006). Depending on the extent of the injury, the hepatic stem cell activation response may be extended. This extension is usually through the remodelling of the extracellular matrix via the production of metalloproteinases by the inflammatory cells (Fallowfield *et al*.,2007). Thus, the diversity and plasticity of hepatic stem cells and their environment coupled with the molecular signalling determine liver homeostasis, regeneration, fibrosis and cancers under physiological and pathological conditions (Chen *et al*., 2017). Hematopoietic stem cells are said to be observed in mice feotal liver from embryonic day 11 till day 6 post-partum after which they relocate to the bone marrow (Guo *et al*., 2009). Thus, the liver is not a hematopoietic site in the adult rodent.

In conclusion, the ethanolic extract of Vitex angus castus plant causes increase in circulating white blood cell count in rats with chemical induced acute liver injury. This alteration is in a non-specific dose dependent manner.

Acute liver insult, if not adequately managed may be a precursor of chronic liver disease which more often than not carries a dismal prognosis. Though the liver is not a hematopoietic organ ex utero, the symptomatology of chronic liver disease is usually multiorgan and multisystemic. Thus the finding of this study will contribute to reduction in the incidence of chronic liver disease by ameliorating the negative effect of acute liver insult

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