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Haematological and Hepatotoxic Potential of Onion *(Allium cepa)* and Garlic *(Allium sativum)* Extracts in Rats

Enitan Seyi Samson[1*], Ajeigbe Kazeem Olasunkanmi[1], Josiah Sunday Joel[2] and Ehiaghe Friday Alfred[3]

[1]Department of Physiology, School of Basic Medical Sciences, College of Health Sciences, Igbinedion University Okada, P.M.B. 0006, Edo state, Nigeria.
[2]Department of Biochemistry, School of Basic Medical Sciences, College of Health Sciences, Igbinedion University Okada, P.M.B. 0006, Edo state, Nigeria.
[3]Department of Haematology, School of Clinical Medicine, College of Health Sciences, Igbinedion University Okada, P.M.B. 0006, Edo state, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. ESS designed the study, managed the literature searches, wrote the protocol and the first draft of the manuscript, AKO and JSJ managed the analyses of the study. EFA performed the statistical analysis. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: To evaluate and compare the effects of single and combined oral administration of fresh aqueous onion (*Allium cepa*) and garlic (*Allium sativum*) extracts at different concentrations (200mg/kg/d, 400mg/kg/d and 600mg/kg/d) on some haematological and hepatotoxicity indicator parameters in treated rats.
Study Design: Cross-sectional nonclinical study in animal model.
Place and Duration of Study: The study was carried out at the Department of Physiology, Department of Biochemistry and Department of Haematology, College of Health Sciences, Igbinedion University Okada, Edo state, Nigeria between the month of July and August, 2011.
Methodology: Following 30 days post-oral administration of extracts in 36 treated male albino rats as well as 4 controls, haematological parameters were determined using the

**Corresponding author: Email: realityoflifepublication@yahoo.com;*

Sysmex® Automated Haematology Analyzer, while serum levels of liver enzymes were evaluated using the Reflotron® Plus Auto-Analyzer and liver weight was determined using electronic sensitive weighing balance.

Results: Red blood cell count (RBC), Packed cell volume (PCV), Haemoglobin concentration (HGB), Total White blood cell count (TWBC), Absolute Count of Neutrophil (NEUT#), Absolute Count of Lymphocyte (LYM#), Absolute Count of the summation of Monocyte, Eosinophil and Basophil (MXD#) and Platelet count (PLT) were significantly increased ($P<0.05$) to varied extent, except Mean cell volume (MCV), Mean cell haemoglobin (MCH) and Mean cell haemoglobin concentration (MCHC) while Alanine aminotransferase (ALAT) and Aspartate aminotransferase (ASAT) serum levels were significantly decreased ($P<0.001$) and liver weight (LW) was non-significantly ($P>0.05$) reduced in a dose-dependent manner when compared to the control. Synergistic effect was not observed in the haematological parameters when the two extracts were combined.

Conclusion: The results of this study reveal the haematological potential of onion and garlic extracts with no potential risk of hepatotoxicity (at the concentrations tested) as earlier anticipated. It also further confirms the higher efficacy of garlic over that of onion, but the molecular mechanism behind their combined effect would require further investigation.

Keywords: Onion; garlic; haematological parameters; hepatotoxicity indicator parameters; rats.

1. INTRODUCTION

The potency of onion (*Allium cepa*) and garlic (*Allium sativum*) as medicinal plants, due to their high content of vitamins, trace elements, amino acids and several organosulphur compounds has been acknowledged over 5000 years (Balick and Paul, 1996). These "*magic*" drugs are well known for their: fibrinolytic effects (Chutani and Bordia, 1981), hemodynamic and hemostatic effects (Brosche et al., 1990), platelet effects (Apitz-Castro et al., 1992), immunologic effects (Chisty et al., 1996), lipid-lowering effects (Ide and Lau, 1997), anti-artherosclerotic effects (Koscielny et al., 1999), anti-oxidative effects (Borek, 2001), anti-cancer effects (Milner, 2001), vascular effects (Ashraf et al., 2004), anti-microbial effects (Benkeblia, 2004), haematological effects (Fazlolahzadeh et al., 2011) and hepatoprotective effects (Ugwu and Omale, 2011) among other health benefits.

It is quite alarming that the use of some medicinal plants seems to pose some health hazards despite their overwhelming health benefits either due to their inherent toxic effect or abuse resulting from over-dosage or prolong usage. Concerns over their safety were raised after incidents of toxicity were reported following consumption of some Herbal preparations but the underlying pathogenesis remained cryptic (Favreau et al., 2002; Stickel et al., 2005). While the health benefits of most herbs have been strongly demonstrated, their safety is poorly documented, and the awareness of consumers and health professionals towards herbal preparations as a potential source of health damage is low. More worrisome are recent reports about adverse effects, following the intake of some herbal preparations containing ephedrine and green tea extracts resulting in acute and chronic liver injury. Severe liver injury associated with long-term consumption of Herbalife-products possibly by a yet unknown profibrotic component was described ranging from reversible cholestatic

hepatitis to acute liver failure requiring liver transplantation, and death from post-transplant complications (Duque et al., 2007; Elinav et al., 2007).

Controversies exist regarding the health benefit and toxic effect of onion and garlic extracts. Very little is known about their potential toxic effects on body organs, however garlic has been shown to cause bad breath and body odor, Irritation of the stomach lining, nausea, intestinal gas, diarrhea, heart burn (Desai et al., 1990), decrease of serum protein and calcium (Shashikanth et al., 1986), anemia (Nakagawa et al., 1980) and inhibition of spermatogenesis (Qian et al., 1986) particularly in people who lack the liver processing system for detoxifying allicin (Mader, 1990), also significant damage to the epithelial mucosal membrane, resulting in bleeding, shrinkage and ulcers, after 24 hours of exposure has been reported in rats, while Onion on the other hand, has been reported to exacerbate hyperacidity in patients with active gastric and duodenal ulcers and similarly worsen existing heart burn (Allen et al., 1990). Consuming large quantities of onions can also lead to stomach distress, gastrointestinal irritation and allergy manifesting as skin rash, redness and itchy eyes (Valdivieso et al., 1994) particularly in people who lack the liver processing system for detoxifying N propyl disulphide (toxin, sometimes found in onion and other alliums).

Though, some studies have shown that regular intake of garlic and onion can help treat haematotoxicity (resulting from chemicals, microbal or plant toxins in the blood), as well as potentiate the haematopoietic activities of the bone marrow in the treatment of anemia and leucopenia, nevertheless, adequate data on the potential risk of hepatotoxicity seems to be lacking. Causes of hepatotoxicity include viral and parasitic infections, autoimmune diseases and intoxication with various xenobiotics such as chlorinated solvents, alcohol, fungal toxins, industrial pollutants, radioactive isotopes, drugs and herbal medicines (Evans, 2002). Liver injury do occur, not necessarily as a result of over-dosage as earlier reported, but sometimes it does occur, even when drugs/herbs are used within therapeutic limits. The predominant pathologies in a given country depend on nature (genetics) and nurture (environment).

A primary purpose of nonclinical studies using animal model is to monitor for the onset of adverse effects, discover target organ toxicity, determine the margin of safety, determine whether the risk is acceptable for the therapeutic effect of the drug and for the patient population and from this information stop the development of the compound or to utilize this information for monitoring possible toxicities in human studies. Presently, there is a lack of sufficiently organized data to make an informed conclusion on the predictivity of nonclinical studies for identifying the risk of significant hepatotoxicity in clinical trials and in the post-marketing population. Besides, lack of standardization, quality and safety shortcomings of some herbal preparations and supplements are major concerns of advocates of a tighter regulation of these products. If the use of herbal remedies must be fully embraced, they must be subjected to toxicological scrutiny from time to time in order to ensure their safe use both on short and long term bases.

To address this issue it is necessary to determine whether there are data that would allow accurate predictions to be made, whether there are early signals in animal studies, that indicates a drug (in this case, plant extract) will later cause serious hepatotoxicity in humans, and whether there are signals that indicate that the hepatotoxicity of the drug poses an acceptable risk. In an attempt to explore the haematological potentials of onion and garlic extracts, we also see the need to determine if these extracts cause hepatotoxicity at the varied concentrations (200mg/kg/d, 400mg/kg/d and 600mg/kg/d) tested at the end of 30 days of oral administration. It is hope that these animal data can be extrapolated to predict

and prevent unanticipated hepatotoxicity in man. Besides, even though garlic and onion extracts have been shown to possess haematological potential in some previous studies, the controversy regarding which of them exhibit a higher efficacy needs to be investigated and clarified. And owing to the fact that plausible evidence exist that garlic may interfere with the action of some herbal remedies in modulating haematopoietic and hepatic activities (Zlotogorski and Littner, 2004), it becomes paramount to investigate the combined effects of garlic and onion extracts on some haematological and hepatotoxicity indicator parameters in experimental models, with a view to establish if the anticipated effects are elicited either singly or combined and if also dose-dependent. It is hoped that the outcome of this investigation will provide adequate scientific justification on the continuous use of garlic and onion extracts as haematopoietic modulating agents with little or no hepatotoxic potential.

2. MATERIALS AND METHODS

2.1 Study Area

This study was carried out at the College of Health Sciences, Igbinedion University Okada located in Okada wonderland (a commercial, but costly recreational center equipped with housing venture, comprehensive education institute and 300-bed space medical center) in Okada, a town beside Benin City (capital city of Edo state), located in the Ova-North East local government area of Edo state, coordinates: $6^{O}30, N\ 6^{O}00E$ in central southern Nigeria. Predominant occupation among the people is farming and they are noted for the growing of the following agricultural products: rubber, cocoa, cashew nuts, plantain etc. Despite the availability of reliable medical service, the local populaces still rely on the use of herbs as medicines for both curative and prophylaxis purpose.

2.2 Study Design

A total of 40 male albino rats weighing 150±40g (mean±SD) were purchased from the Animal Production and Health Department, Federal University of Technology, Akure (Ondo State, Nigeria) and housed in the Experimental Animal House, College of Health Sciences, Igbinedion University Okada (Edo state, Nigeria) separately in well ventilated wire-bottom steel cages under hygienic conditions, with proper aeration at 25±2ºC, and a relative humidity of 45–50%. The rats were randomly assigned into 10 groups of 4 rats each and fed on standard rat diet (10g/100g body weight) twice daily and tap water ad libitum. Prior to commencement of administration, the rats were allowed to stabilize in the Animal House with standard 12-hour light-dark cycle, for a period of 14 days, and were treated for 30 days with different doses of the plant extracts. All studies on animal experimentation were conducted in accordance with the Current Animal Care Regulations and Standards approved by the Institute for Laboratory Animal Research (ILAR, 1996).

2.3 Preparation of Aqueous Onion and Garlic Extracts

A bulk of fresh onion bulbs and garlic cloves (single batch) sufficient for the study was purchased from a retail store in Okada market (Edo state, Nigeria). Aqueous onion extract was prepared from fresh onion by modified method of Martha et al., (1998). The light scaly leaves were peeled with knife and thoroughly washed. 60g of the onion was then weighed, sliced into tiny pieces and homogenized in 100 ml of cold sterile distilled water. This gave a concentration of 600mg/ml, on the basis of the weight of the starting material (60g/100 ml). The homogenates were filtered three times through sterile cheese cloth and centrifuged at

200 xg for 10 minutes and the supernatant was collected and then filtered into separate sterile containers using a funnel containing sterile Whatman No. 1 filter paper for clarification. The liquid filtrates were transferred into sterile Universal bottles. After preparation of the crude extracts as described; additional concentrations (400mg/ml and 200mg/ml) were made from the stock (600mg/ml) with sterile distilled water. All these were done aseptically. Protocol was repeated for garlic. It was ensured that fresh plant extracts were prepared each day from the single batch of onion and garlic samples purchased before administration as the active ingredients are well known to lose most of their potency with time after extraction when exposed to higher temperature; otherwise, extracts were stored in the refrigerator at 4ºC to maintain their potency.

2.4 Animal Treatment

A total of 40 rats were randomly assigned into 10 groups (n=4/group) and treated as shown in Table 1.

Table 1. Experimental pharmacological protocol for onion and garlic treated rats

Groups (n=4)	Treatments
G1	Control Group received no extracts at all
G2	200mg/kg/d aqueous extract of onion
G3	400mg/kg/d aqueous extract of onion
G4	600mg/kg/d aqueous extract of onion
G5	200mg/kg/d aqueous extract of garlic
G6	400mg/kg/d aqueous extract of garlic
G7	600mg/kg/d aqueous extract of garlic
G8	200mg/kg/d combined aqueous extract of onion and garlic
G9	400mg/kg/d combined aqueous extract of onion and garlic
G10	600mg/kg/d combined aqueous extract of onion and garlic

The volume of extract (2ml/100g body weight) to be administered to individual rat in each group using intragastric tube was calculated, recorded and adjusted daily with changes in body weight throughout the treatment period which lasted for 30 days. They were observed daily for any observable change. Overnight prior to euthanasia, the animals were starved of food. The animals were sacrificed by cervical dislocation as described by Ochei and Kolhatkar (2006). Cardiac blood specimen was taken from each rat by terminal bleeding from the heart. The first half of the blood collected was transferred into a clean edta-container (thoroughly mixed) ready for evaluation of haematological parameters while the second portion was transferred into an anticoagulant-free test tube and allowed to clot and subsequently centrifuged at 750 g for 15 min to obtain serum component ready for evaluation of serum level of liver enzymes.

2.5 Evaluation of Haematological and Hepatotoxicity Indicator Parameters

Haematological parameters were determined using the Sysmex® Automated Haematology Analyzer kx-21n, Sysmex Corporation, (Kobe-Japan) as described by Samuel et al. (2010). while, the serum level of alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) were evaluated photometrically with Reflotron® Plus auto-analyzer (Boehringer, Mannheim, FRG, Roche, Germany) according to the method as described by Deneke and Rittersdorf (1984, 1985). The auto-analyzers were operated as instructed in the user's

operational manual. Following dissection of the peritoneal cavity, the liver was isolated as described by Fricker et al. (1994) and weighed with the aid of electronic sensitive analytical balance.

2.6 Statistical Analyses

All numerical results were obtained from the ten (10) groups (control and treated). Data were presented as mean±SEM and analysed using one way analysis of variance (ANOVA) and Tukey-Kramer Multiple Comparisons Test using SPSS-18.0 (Statistical packages for social Scientists – version 18.0) statistical program. P values<0.05 were considered significant.

3. RESULTS AND DISCUSSION

RBC, PCV, HGB, TWBC, NEUT#, LYM#, MXD#, and PLT were significantly increased, while MCV, MCH and MCHC were significantly decreased to different extent ($P<0.05$) in a dose-dependent manner in both onion and garlic treated groups when compared to the control. Extract of garlic at 600mg/kg/d proved to be the most potent while the combined extract of onion and garlic at 200mg/kg/d was the least effective. A partial antagonistic effect on haematological indices was observed when the two extracts were combined (Table 2). The serum level of Liver enzymes (ALAT and ASAT) were significantly decreased ($P<0.001$) in a dose-dependent manner in both onion and garlic treated groups when compared to the control. The extracts appear to be non-hepatotoxic when used singly and combined. Meanwhile, a non-significant decrease ($P>0.05$) in liver weight (LW) was observed in the onion, garlic and combined and treated groups when compared to the control (Table 3).

The outcome of this present study shows that extracts of fresh garlic and onion (especially at higher concentration) have significant ($P<0.05$) impacts on some haematological parameters as evident by the active proliferations of blood components to varied extent as measured in the treated groups, compared to the control. While, the significant increase ($P<0.001$) in RBC, TWBC and PLT counts observed in this present study did not agree with that of Michael et al. (2009) who reported a significant decrease ($P<0.05$) in total WBC and Ugwu and Omale (2011), who reported a non-significant decrease ($P>0.05$) in the WBC and RBC counts; it does agrees with the works of: Iranloye (2002), who reported increases in total leucocyte count, neutrophils, lymphocytes, monocytes, RBC and Hb concentration in rats following 30-days of garlic consumption; Sahu (2004) observed an increase in erythrocytic count and Salah et al. (2008) reported a significant increase in leucocytic count after administering garlic.

It also strongly confirms the findings of Tatfeng and Enitan (2012) who reported significant increases in total leucocyte count, absolute count of lymphocytes, neutrophils and summation of monocytes, eosinophils and basophils in their work on effects of onion and garlic extracts on some immunologic cells.

Table 2. Haematological parameters of rats after onion, garlic and combined extracts were administered

Group Parameters	1 CONTROL	2 200mg/ Kg ONION	3 400mg/ Kg ONION	4 600mg/ Kg ONION	5 200mg/ Kg GARLIC	6 400mg/ Kg GARLIC	7 600mg/ Kg GARLIC	8 200mg/ Kg O+G	9 400mg/ Kg O+G	10 600mg/ Kg O+G
RBC COUNT	2.58	4.51	6.07	7.76	4.83	6.30	8.02	4.33	6.02	6.70
($X10^6$/UL)	±0.01	±0.02^c	±0.13^c	±0.05^c	±0.11^c	±0.23^c	±0.19^c	±0.03^c	±0.09^c	±0.17^c
PCV	18.67	24.07	40.43	45.13	26.60	43.47	47.40	24.17	35.23	40.60
(%)	±1.45	±0.58^a	±0.52^c	±0.68^c	±0.47^b	±0.59^c	±0.84^c	±0.97^a	±1.71^c	±1.75^c
HGB	8.03	10.07	12.40	13.23	11.00	14.10	14.90	9.70	10.93	12.07
(g/dl)	±0.49	±0.29^a	±0.27^c	±0.67^c	±0.32^c	±0.27^c	±0.15^c	±0.40^d	±0.29^a	±0.35^c
MCV	72.36	53.37	66.61	58.16	55.07	69.00	59.10	55.82	58.52	60.60
(fl)	±1.16	±0.99^c	±0.56^a	±0.65^c	±1.19^c	±1.39^d	±0.71^c	±0.69^c	±0.61^c	±1.68^c
MCH	31.12	22.33	20.43	17.05	22.77	22.38	18.58	22.40	18.16	18.01
(Pg)	±0.23	±0.47^c	±0.57^c	±1.09^c	±1.27^c	±1.03^c	±2.11^c	±0.38^c	±0.26^c	±0.57^c
MCHC	43.01	41.84	30.67	29.32	41.35	32.43	31.44	40.13	31.03	29.73
(g/dl)	±0.86	±0.77^d	±0.62^b	±2.11^c	±0.74^d	±0.68^b	±3.10^b	±2.49^d	±0.95^b	±2.13^c
TWBC COUNT	4.74	5.51	6.01	7.27	7.29	9.65	10.30	5.22	5.28	6.33
($X10^3$/UL)	±0.05	±0.27^b	±0.07^c	0.03^c	±0.04^c	±0.11^c	±0.15^c	±0.09^d	±0.04^d	±0.04^c
NEUT#	2.22	2.73	2.77	2.83	2.95	3.02	3.38	2.53	2.64	2.75
($X10^3$/UL)	±0.02	±0.04^c	±0.04^c	±0.06^c	±0.01^c	±0.04^c	±0.09^c	±0.04^b	±0.02^c	±0.04^c
LYM#	1.22	1.61	1.75	1.90	1.62	1.83	1.96	0.32	0.48	0.77
($X10^3$/UL)	±0.04	±0.01^c	±0.03^c	±0.06^c	±0.01^c	±0.01^c	±0.02^c	±0.02^c	±0.04^c	±0.02^c
MXD#	1.03	1.18	1.23	1.30	1.19	1.25	1.32	1.15	1.19	1.26
($X10^3$/UL)	±0.02	±0.01^b	±0.01^c	±0.02^c	±0.01^c	±0.01^c	±0.02^c	±0.02^a	±0.02^c	±0.05^c
PLT	278.33	376.33	391.00	759.67	369.33	823.67	922.33	90.33	172.33	709.00
($X10^3$/UL)	±0.88	±1.86^c	±2.08^c	±2.03^c	±2.33^c	±2.40^c	±9.06^c	±10.17^c	±6.12^c	±18.08^c

Automated Method (Samuel et al., 2010)

Keys: *CONC. = Concentration, RBC= Red blood cell count, PCV= Packed cell volume, HGB= Haemoglobin concentration, MCV= Mean cell volume, MCH= Mean cell Haemoglobin, MCHC= Mean cell haemoglobin concentration, TWBC= Total White blood cell count, NEUT#= Absolute neutrophil count, LYM#= Absolute lymphocyte count, MXD#= Absolute count of the summation of monocyte, eosinophil and basophil, PLT= Platelet count, O+G = combined extracts of onion and garlic. All values are expressed in Mean ±SEM of 4 animals. Group values differ significantly from control at 5 percent level (^{a} = P<0.05), 1 percent level (^{b} = P<0.01), 0.1 percent level (^{c} = P<0.001) and ^{d}= P>0.05 (Not Significant).*

Table 3. Hepatotoxicity indicator parameters of rats after onion, garlic and combined extracts were administered

Group Indices	1 CONTROL	2 200mg/ Kg ONION	3 400mg/ Kg ONION	4 600mg/ Kg ONION	5 200mg/ Kg GARLIC	6 400mg/ Kg GARLIC	7 600mg/ Kg GARLIC	8 200mg/ Kg O+G	9 400mg/ Kg O+G	10 600mg/ Kg O+G
ALAT (U/L)	12.66 ±0.05	11.47 ±0.01[c]	9.75 ±0.03[c]	9.32 ±0.03[c]	12.34 ±0.03[c]	11.48 ±0.04[c]	10.34 ±0.01[c]	9.83 ±0.02[c]	9.06 ±0.02[c]	7.76 ±0.06[c]
ASAT (U/L)	13.30 ±0.30	9.11 ±0.02[c]	8.55 ±0.70[c]	8.15 ±0.01[c]	12.20 ±0.06[b]	11.72 ±0.14[c]	10.64 ±0.25[c]	8.54 ±0.20[c]	8.18 ±0.03[c]	8.11 ±0.01[c]
LW (g)	5.62 ±0.01	4.80 ±0.16[d]	4.64 ±0.22[d]	4.23 ±0.48[d]	3.99 ±0.60[d]	4.40 ±0.43[d]	4.15 ±0.53[d]	4.67 ±0.12[d]	3.90 ±1.23[d]	4.20 ±0.36[d]

Automated method (Deneke and Rittersdorf, 1984 and 1985)

Keys: *CONC. = Concentration, ALAT = Alanine aminotransferase, ASAT = Aspartate aminotransferase, LW= Liver weight, O+G = combined extracts of onion and garlic. All values are expressed in Mean ±SEM of 4 animals. Group values differ significantly from control at 1 percent level ([b] = P<0.01), 0.1 percent level ([c] = P<0.001) and [d]= P>0.05 (Not Significant).*

Still, the significant increase observed in the PCV of rats in this present study, concur with that of Salah et al., (2008) who reported a non-significant increase in PCV values of treated fish (*Oreochromis Niloticus*), but, however, contradicts the results of Banerjee and Maulik (2002), Adebolu et al. (2011) and Ugwu and Omale (2011), who all observed significant decreased PCV in the treated rats when compared with that of the control and thereby conclude that garlic is toxic to red blood cells and at very high concentration may induce anaemia in animals on prolong feeding.

Meanwhile, red cell indices (MCV, MCH and MCHC) on the other hand, are particularly important for the diagnosis of anemia in humans and most animals. Significant decreases ($P<0.01$) in these red cell indices as observed in the treated rats in this current study is in agreement with the findings made by Corzo-Martinez et al., 2007), who similarly observed a significant decrease of MCH and MCV in fish fed on the highest dose of Allium sativum. It is assumed that the decrease or increase of blood indices may be attributed to a defence reaction against garlic or onion, which occurs by stimulation of erythropoiesis.

According to Lampe (1999), the mechanisms of action under-pining these diverse haematological effects of plant extracts remain disjointed and relatively poorly understood. Nevertheless, postulated mechanisms of action supposedly revolve around their stimulatory effects on some cytokines, their role in iron bio-availability, and presence of some vitamins, essential amino acids and phytochemicals.

First, garlic and onion compounds seem to have a stimulatory effect along particular pathways on some haematopoetic growth factors (cytokines) which interact with specific receptors on the surface of haematopoietic cells, regulating the proliferation and differentiation of progenitor cells and the maturation and functioning of mature cells. Normal erythropoiesis for instant; is dependent on there being adequate amounts of erythropoietin among other factors. Chemical components of garlic and onion seem to act as active oxygen scarvenger in vivo, It is thus possible that they compete with haemoglobin in the RBC for oxygen resulting in tissue hypoxia which in turn stimulates the kidney directly to cause formation and secretion of erythropoietin or that the end product of metabolism of garlic and onion in the body may step up Hb synthesis and RBC production by their indirect effect on erythropoietin. The hormone erythropoietin is crucial to erythropoesis by increasing the number of erythrocytes precursors. This is made possible by stimulating stem cells to transform into pro-erythroblast (the first morphologically recognisable cell of the erythrocyte series). Production of erythrocytes is restricted to the bone marrow in adult life, however Akgul et al. (2010), reported that dietary garlic can enhance erythropoiesis in the spleen (i.e garlic-induced extramedullary haemopoiesis) but not in the bone marrow. Stimulation of splenic erythropoiesis gene expression in mice by garlic components is most likely mediated by hypoxia which steps up the production of erythropoietin. Similarly, effects of garlic and onion extracts on leucopoiesis may be attributed to their ability to stimulate production of some colony stimulating factors (CSF) like the Granulocyte-CSF, Monocyte-CSF and Granulocyte-Macrophage-CSF, or it may be the effect of their end product of metabolism on the regulation of the cytokine production system, maintenance of the normal systemic defence and bio-regulation. This may explain why these cytokines are used in boosting immune function and in the treatment of some infectious diseases. These soluble membrane-bound glycoproteins of the hematopoietic microenvironment are produced in the cells lining of the blood vessels and they serve to stimulate the bone marrow to synthesize white blood cells. They activate intracellular signaling pathways that can cause the myeloid and lymphoid cells to proliferate and differentiate into a specific kind of white blood cells (Albert et al., 2002). Garlic has been found to stimulate proliferation of several white blood

cells lines and induced the infiltration of tumours by white blood cells such as natural killer cells and macrophages (Kandil et al., 1987). Mean while, the effects of garlic and onion extracts on thrombopoiesis may be attributed to their ability to stimulate production of thrombopoietin. This cytokine (also known as megakaryocytes growth and development factor) is a glycoprotein hormone produced mainly by the liver (parenchymal cells and sinusoidal cells), the kidney (proximal convoluted tubule cells) and bone marrow (stromal cells). It is similarly believed that the chemical components of garlic and onion or the end products of their metabolism seem to act on thrombopoietin which is responsible for the production, proliferation and maturation of megakaryocytes (megakaryocytopoiesis) and differentiation of megakaryocytes into large numbers of platelets (thrombopoiesis). Reduced platelet number (thrombocytopenia) in the blood circulation is often therapy resistant in individuals with AIDS; yet, Platelets numbers have been reported to increase, sometimes greater than 100 000 during 4 months of garlic supplementation.

Second, it is also believe that both garlic and onion compounds help to improve iron metabolism. The bone marrow produces over 2 million RBC per seconds. This tremendous effort is only possible when there is enough iron available, because it is needed to produce the haem component of haemoglobin and it is therefore crucial to synthesis of haemoglobin. Besides, it also assists in cellular immune response by stimulating the production of T-cells in our immune system. There is considerable variation in the availability of iron for absorption in different foodstuffs. Haem iron from animal origin is said to be easily absorbed than non-haem iron of plant origin. However, recently, some Researchers in India found that compounds in both onion and garlic seem to have a promoting influence on the bioavailability of iron from food grains. These alliums, they believe can help the body absorb more dietary iron from cereals and pulses than it otherwise would. This is possible because the diallyl sufides in garlic and onion may induce ferroportin mRNA, this can result in increased production of ferroportin located on the basolateral surface of gut enterocytes and the plasma membrane of reticuloendothelial cells. Ferroportin is an iron channel, a protein that runs across the cell membrane, forming a passageway that allows stored iron to leave the cells and become available for the synthesis of haemoglobin. This is important because the bio-availablity of iron from plant foods like cereal is low. Research has shown that if onion and garlic are consumed alongside cereal, the body absorbs up to 70 percent more iron from the cereal. This discovery has the potential for global health, as non-availability of iron for Hb production leads to iron deficiency anaemia, a serious problem worldwide. It is therefore assumed that the extracts of garlic and onion administered in this current study might have facilitated the absorption of more dietary iron (30-35mg/kg) present in the pelletized feed upon which the rats were fed, thereby stepping up Hb synthesis and resulting in an elevated haemoglobin concentration in the treated group compared to the control. It is also speculated that garlic and onion compounds may inhibit hepcidin mRNA responsible for the transcription of hepcidin (an iron shuttling protein, critical to iron homeostasis, which prevents absorption of iron by binding to ferroportin), resulting in little or no production of hepcidin. The lower the concentration of hepcidin, the more iron channels that are opened for more iron to be absorbed by the hepatic portal system and can be made available for Hb synthesis. Anemia of inflammation has been linked to high activity of hepcidin (Papanikolaou et al., 2005).

Third, Fenwick and Hanley (1985) reported that several vitamins like vitamin B_1, B_2, C and E are present in both garlic and onion while vitamin B_6 and B_9 are exclusively found in garlic. Of particular interests are those that help with red blood cell formation and maintenance. For instance, deficiency of Vitamin B_2 (Riboflavin) has been reported to results in a decrease in red cell glutathione reductase activity, since this enzyme requires flavin adenine dinucleotide

for activation (Powers et al., 1983). Glutathione reductase is an enzyme that reduces glutathione disulfide (GSSG) to the sulphydryl form (GSH), which is an important cellular antioxidant scavenging reactive oxygen species in cells like red blood cells. It plays an important role in protecting Hb, red cell enzymes and biological cell membrane against oxidative damage by increasing the level of reduced glutathione (GSSGR) in the process of aerobic glycolysis. The enzyme deficiency may result in mild to moderately severe haemolytic anemia upon exposure to certain drugs or chemicals. Still, Researchers at the University of Maryland Medical Center, believe that cloves of garlic contain vitamin B_6 (Pyridoxine) and deliver around 6% of the pyridoxine require per day by the average adult. A hypochromic microcytic anemia has been associated with Vitamin B_6 deficiency induced in children. While others believe that garlic in addition, contain trace amount of folic acid (vitamin B_9) which is essential for DNA synthesis and are therefore needed by all dividing cells in the body, particularly haematopoietic cells in the bone marrow. Deficiency of folate leads to the death of many precursor cells in the bone marrow resulting in megaloblastic anaemia. Vitamin C (ascorbic acid) is believed to help in the absorption of non-haem iron from plant origin for the synthesis of Hb. Deficiency of dietary Vitamin C is associated with deficiency of dietary iron, because Vitamin C serves to facilitate intestinal iron absorption. Combination of iron and Vitamin C is needed to correct a hypochromic microcytic anemia. Besides, Vitamin C is required for the maintenance of folic acid reductase in its reduced or active form. Impaired folic acid reductase activity results in an inability to form tetrahydrofolic acid, the metabolically active form of folic acid. It also serves to prevent the irreversible oxidation of methyltetrahydrofolic acid to formylfolic acid. Failure to synthesis tetrahydrofolic acid or protect it from oxidation ultimately results in the appearance of a megalobastic anemia. While Vitamin E (tocopherol) strictly serves as an antioxidant; its role had been demonstrated in its deficiency to compensate for genetic defects that limit the eryhtrocytes's defense against oxidant injury. Vitamin E has been found to lengthen the red cell life span and increase level of haemoglobin. Deficiency of Vitamin E may result in haemolytic anaemia, associated with morphologic alterations of the eryrocytes and more irreversibly sickled cells in children with sickle cell anemia (Fishman et al., 2000).

Fourth, garlic and onion have been screened to possess some important phytochemicals such as flavonoids, steroidal glycosides, alkaloids, saponins, tannins, phenolics, pectin, amino acids etc, with their biological and physiological roles well documented by Fenwick and Hanley (1985). They are believed to play a crucial a role in gene expression, enzyme activity, stimulation of the immune system and organs related to blood cell formation particularly the bone marrow as reported by Jeorg and Lee (1998). Flavonoids, glycosides and alkaloids in particular act as antioxidants. They have been demonstrated to possess good free radical scavenging properties, protecting the red blood cells against oxidative damage. Addition of extract of aged garlic to red blood cell suspension prior to the addition of an oxidant has been found to minimize oxidation and cell ruptures. About 0.07% of Steroid saponins found in garlic have been shown to have antinutritive effects, particularly mid chronic hemolysis without anaemia resulting in accelerated RBC turnover. However, as the dosage of saponins (garlic) exceeds the rodent' erythropoeitic capacity, anemia may ensue. However, there appears to be no evidence of harmful effects of Alllium saponins in humans (Sparg et al., 2004). According to Matsuura, (2001) and Oboh, (2004), accelerated RBC turnover has been proposed as the crux of the mechanisms by which garlic sustains and amplies its multiple biological effects. As garlic enhanced rates of turnovers, each degraded RBC may release up to approximately $6X10^8$ heme molecules, of which a significant proportion are then converted to CO, to alleviate any heme toxicity. Normally, CO is produced through metabolic processes and CO by itself can stimulate increase in RBC counts, Hb content and PCV level. It is believe that metabolism of garlic likely result in

production of extra CO resulting in accelerated RBC turnover. Besides, the free amino acids (mainly arginine) present in garlic and onion is also believed to be contributory to the amino-acid pool needed for the synthesis of Hb. The globin part of the Hb molecule consists of chains of amino-acids. The larger the amino-acid pool is, the more chances there are that more Hb will be synthesized and more RBC produced (Block, 1985).

On the other hand, the complex chemistry of alliums necessitates assessments of their potential toxic effects on body organs, particularly the liver because of the crucial role it plays in drug-detoxification. The liver is the central site for the biotransformation of xenobiotics and therefore is involved in the detoxifying mechanism of the body. It is responsible for detoxifying the chemical substances in the blood and in this process it is exposed to high concentrations of toxicants and toxic metabolites making it susceptible to injury. Recently, liver injury after consumption of herbal remedies was reported but the underlying pathogenesis remained cryptic (Evans, 2002).

In Clinical chemistry, indicator parameters routinely implemented during nonclinical assessments for hepatotoxicity include: ALAT, ASAT (Hepatocellular leakage enzymes), bilirubin, ALP, GGT (Cholestasis indicators), albumin, urea, nitrogen (Function indicators), electrolytes, total CO_2, glucose, triglyceride, and cholesterol (Metabolism indicators) among others. Increases in these indicator parameters, even in the absence of histologic changes, are considered adverse, unless the pathogenesis indicates to the contrary. It is difficult to identify an absolute cut-off between ‘adverse’ and ‘not adverse’ for an individual parameter. However, certain findings in individual parameters are considered adverse, unless they can be associated with a mechanism that is non-adverse. It is known that serum level of liver enzymes reflect liver cell function and integrity and increase in their levels indicate hepatic cellular damage. The biochemical analysis of the liver can provide some valuable information on the status or condition of the liver. >3-5X increase in the serum level of ALAT and ASAT in particular, has been found to be associated with hepatocellular degeneration and hepatocellular necrosis (including apoptosis) respectively (Ballet, 1997; Zbinden, 1991).

In this present study, the serum levels of ALAT and ASAT were significantly reduced ($P<0.001$) in the treated group following 30 days administration of onion and garlic extracts when compared to the control. Here, a rise in the serum level of the liver enzyme (i.e transminitis) as was initially anticipated was absent, it can therefore be inferred that the extracts did not cause hepatotoxicity at the concentration tested. The outcome of this present study, appear to agree with the results of other similar studies: Hamlaoui-Gasmi et al. (2011) reported a decrease in the plasma level of transaminases following oral treatment of garlic (5g/Kg bw) against intra-peritoneal route treatment which resulted in hepatotoxicity as indicated by the elevation in the enzymes level; Sankaran et al. (2010) showed that administration of raw garlic significantly decreased the activity of markers enzymes in alcoholic patients with severe liver damage; El-Demerdash et al. (2005) also found out that the activities of ASAT, ALAT, LDH, AlP and AcP were significantly ($P<0.05$) decreased in the liver tissue of alloxan-diabetic rats fed garlic when compared to the control values; Augusti et al. (2001) reported that lipid parameters and enzyme activities (ASAT, ALAT and ALP) in rat serum decreased significantly when they were fed a diet containing 5% garlic; while Ohaeri (2001) showed that the levels of various enzyme in the serum and tissue of streptozocin diabetic rats were significantly reduced by garlic oil.

Plasma concentration of liver enzymes is normally low, but is elevated in the event of hepatotoxcity, which may occur in alcohol, drug or herb-induced liver damage leading to necrosis of hepatocytes, increased permeability and leakage of cellular enzymes from the

liver cytosol into the blood stream. The main value of the Aminotransferases is in detecting hepatocellular damage and monitoring the patient's progress as the levels rapidly return to normal following resolution of the factors causing hepatocellular damage.

The elucidating mechanism of action behind the non-toxic effects of onion and garlic extracts is also not fully understood and the wide discrepancy in the literatures may be due to many factors such as the species of garlic and onion used its concentration, route of administration or the nutritional status of rats. However, most researchers believe that the presence of essential nutrients like the vitamins, trace elements and phytochemical contents are responsible for their inherent-antioxidant activities as reported by Rahman (2003) and Yang et al. (2004). These nutrients seem to chelate metal ion, inhibit lipid peroxidation, increase the amount or increase the activity of antioxidant and detoxifying enzymes thereby accelerate the regenerative capacity of the hepatocytes (under the influence of hepatocyte growth factor), cause stabilization of the cell membrane of the hepatocytes and ultimately protect the liver cells against deleterious agents and free radical-mediated toxic damages. According to Gibson (1998), high concentration of fructans (35-40% dry weight), which constitute a major portion of the water-soluble carbohydrates in onion also contribute to the reduction of toxic metabolites and detrimental enzymes, protection of liver function and reduction of serum cholesterol. Another side to it; is that hepatotoxins (like N propyl disulphide) might be entirely absent or seem to be present in a very low concentration (i.e, not significant enough to induce hepatotoxicity) in the extracts or that the liver is well fortified to detoxify them. The ability of the extracts to induce a significant decrease in liver enzymes activities hereby suggests that: (1) they can be considered safe and non-hepatotoxic. (2) They pose no risk of hepatotoxicity at the concentration tested, however the use of higher dosage is recommended for further investigation in the future.

There are issues beyond the scope of this study that require further investigation. We hereby recommend that the toxic doses of these extracts be properly determined, that experimental subjects be exposed to extracts for a longer duration (>30days), the number of animals used in toxicity studies be increased, test for sex and specie differences in order to ease the detection of hepatotoxicity occurring at a low incidence. Also, an assessment of the histological profiles of the liver is very crucial, as liver architecture will help to elucidate the result of the liver enzymes. Besides a better understanding of the mechanism(s) of liver toxicity as well as the underlying reasons why nonclinical studies fail to prevent compounds which produce serious human hepatotoxicity from proceeding in the clinic could result in developing a more predictive nonclinical testing strategy. Where available, technologies such as In Vitro Cellular Assays, Covalent Binding or Toxicogenomics/Proteomics should be considered for delineating the mechanisms of hepatotoxicities. Such methods could aid in identifying the molecular pathways of hepatotoxicity, covalent binding of compounds or metabolites to proteins and possibly neoantigens (produced from drug treatment) eliciting immune mediated toxicity. Their utility needs to be further assessed.

It should also be bore in mind that nonclinical studies like this do not provide adequate assessment of all hepatotoxic liabilities in man. It is particularly important to understand unique factors responsible for human differences from laboratory animal models and to modify models or testing strategies to account for such differences. Such factors include: genetic variability, lifestyle factors, formation of unique metabolites with hepatotoxic potential, immune mediated events, drug-herb or drug-food or drug-environment interactions, or endogenous or exogenous factors that modify or compromise organ or tissue function. Further studies will also require the isolation and characterization of potential hepatotoxins in garlic and onion extracts and preparations. Since, adequate data on toxic

doses seems not to be available; patients' serum level of liver enzymes must be evaluated and monitored continuously in order to avoid the potential risk of hepatotoxicity associated with long term use of garlic and onion components. Besides, herb-drug interaction has been well reported, for instance, both garlic and acetaminophen are processed by the same set of enzymes in the liver when they are taken together, large amounts of garlic may interfere with the break of acetaminophen and as a result too much acetaminophen may stay in the blood, for this reason, patients are therefore advised to check with their doctors first before taking onion and garlic preparation. Critical assessment of their liver function status must be carried out and those with history of liver problem should be properly informed on the impact of herbal-drug interaction on their health.

Regarding the degree of efficacy, this present study showed garlic to be more potent than onion. Extracts of fresh garlic, particularly at the highest dose tested (600mg/kg/d) were shown to be more effective than that of onion; this confirmed a higher efficacy of garlic as reported by Micheal et al. (2009) who observed a significantly higher effect in garlic than ginger; Tatfeng and Enitan (2012) who also observed a significantly higher effect in garlic than onion. The significantly higher effect ($P<0.01$) on RBC and Total WBC counts observed in garlic treated rats at various concentrations tested contradicts the works of Ugwu and Omale (2011), who show that there were no significant differences ($P>0.05$) between the garlic and onion extracts consumption on the RBC and Total WBC counts. While the non-significant difference ($P>0.05$) in PCV between the garlic and onion treated rats strongly support the findings of the same on equal treatments. Lesser effects as observed in onion treated rats may be due to the presence of the extracted phytochemicals in lower degrees or concentrations, since the same phytochemicals have been found to be also present in garlic extracts. It is interesting to know that the effects of these spices on haematological parameters were dose-dependent as higher values were obtained for haematological parameters measured (except, MCV, MCH and MCHC) with increasing concentration of the extracts administered compared to the control; suggesting that there may be a correlation between these responses and dosage. This work agrees with the findings of Banerjee et al. (2002) and Tatfeng and Enitan (2012).

The combination of both onion and garlic extracts does not appear to be really beneficial to haematopoietic activities; as some of the haematological parameters were significantly reduced compared to the single outcome of each extract. The reason for this is not clear, however, it is possible that the action of some organosulphur compounds found exclusively in garlic counteract those of onion resulting in a partial antagonistic relationship between them. For example, quercetin is the main flavonol in onions while myricetin is the main flavonol in garlic. These two compounds differ slightly in structure and it is possible that they both compete for the same available receptors on the haematopoietic factors. The possible mutual opposition in action between them may have resulted in a lesser or reduced haematological effect as observed in this study. The molecular mechanism behind such herbal–herbal interaction needs further investigation.

The non-significant decrease ($P>0.05$) in liver weight (LW) observed in the treated groups when compared to the control, seems not to agree with the result of Abdullah et al. (2001), who observed a significant decrease ($P<0.05$) in the weights of liver, heart and spleen of rats administered aqueous extract of garlic (100mg/kg/day) for 90 days; it is therefore recommended that, the dosage and the cumulative length of administration, which are known to cause both toxicity and atrophy of the hepatocytes and the need for withdrawal or dosage adjustment based on plasma level, must be considered in order to optimize the

medicinal benefits of these extracts. This is a challenge for the present and future generations of hepatologists and therefore warrants further investigation.

4. CONCLUSION

The outcome of this current study further confirms the inherent-haematological and non-hepatotoxic potentials of these extracts and therefore they can serve as natural haematinics and antioxidants to step up haematopoiesis without risk of potential hepatotoxicity at the concentrations tested. This study further strengthened the earlier works on the efficacy of these extracts in this regard.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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