Pharmacogn. Commn. 2018; 8(1): 29-36
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcogcommn.org

Original Article

Hepatic Oxidative Stress and Haematological Parameters of Wistar Rats Following Infusion of Carbon Tetrachloride and Treated with Raw and Hydrothermal Processed Herbs

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ABSTRACT

Background and Aim: The present study evaluated hepatic oxidative stress and haematological parameters of Wistar rats following infusion of carbon tetrachloride (CCl_4) and treatment with extracts of raw and hydrothermal processed *Monodora myristica* (Gaertn.), *Chromolaena odorata* (Linn), *Buccholzia coriacea* (Engl.) and *Sphenostylis stenocarpa* (Hochst ex A. Rich). **Materials and Methods:** Hydrothermal processing of the herbs was according to indigenous traditional methods. Quantitative compositions of alkaloids, flavonoids, tannins and saponins of the herbal extracts were measured using standard methods. Fibrosis was induced in the rats by single dose intra-peritoneal (i.p) injection of CCl_4 in paraffin oil as vehicle {1:1 *v/v*; dose = 1.0 mL/kg body weight (b.wt.)} for 2 consecutive days. The rats received treatments (dose = 250 mg/kg b.wt.; i.p. of the herbal extracts and silymarin or otherwise 1.0 mL/kg b.wt.; i.p. of phosphate buffered saline solution, paraffin oil and CCl_4/paraffin oil mixture) for 28 consecutive days. Liver homogenates were measured for malondialdehyde (MDA) content, catalase (CAT) and superoxide dismutase (SOD) activities as well as haematological parameters using spectrophotometric methods. **Results:** Hydrothermal processing of the raw herbs resulted in losses in their contents for all measured phytochemical classes except for saponins content. The administration of raw and hydrothermal processed herbal extracts reversed the changes in hepatic MDA contents, CAT and SOD activities as well as haematological parameters in the fibrotic rats. **Conclusion:** Hydrothermal processing of the raw herbs did not adversely affect their capacities to ameliorate hepatic oxidative stress and haematological disorders in the fibrotic rats.

Key words: Carbon tetrachloride, Catalase, Haematological parameters, Malondialdehyde, Oxidative stress, Superoxide dismutase.

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DOI: 10.5530/pc.2018.1.6

INTRODUCTION

Oxidative stress is elicited following persistent generation of reactive oxygen and nitrogen species (RONS) such that the antioxidant capacity of the cell is overwhelmed.[1,2,3,4,5] Major generators of RONS are the mitochondrial electron transfer chain, cytochrome P450 family and peroxisomes as well as activities of lipoxygenase, xanthine oxidase and membrane-bound reduced nicotinamide adenine dinucleotide phosphate (NADPH)-dependent oxidase (NADPH oxidase).[6,7,8] For instance, the superoxide ($O_2^{\bullet-}$) radicals are largely generated from complexes I and III of the mitochondrial electron transfer chain and, for the most part, responsible for the cellular oxidative damage that underlie degenerative diseases, inflammation, mutation, carcinogenesis and senescence.[9,10] The pathophysiology of degenerative diseases revealed that oxidative tissue damage is mediated by activating a number of cellular stress-sensitive pathways, which include nuclear factor-κB (NF-κB), p38 mitogen-activated protein kinase (MAPK), NH_2-terminal Jun kinases/stress-activated protein kinases (JNK/SAPK) and hexosamines.[1,11,12]

Superoxide dismutase (SOD) activity in concert with spontaneous dismutation of $O_2^{\bullet-}$ are the major sources of cellular hydrogen peroxide (H_2O_2).[9,13] The oxidizing potential of H_2O_2, by its capability to act as substrate to produce other oxidizing species like hydroxyl ($OH^{\bullet-}$) radicals and hypochlorous acid (HClO),[14,15] is largely responsible for the deleterious actions of H_2O_2. Furthermore, H_2O_2 in the presence of metallic ions like Fe^{2+} and Cu^{2+}, in the so-called Fenton reaction, is converted to reactive $OH^{\bullet-}$, whereas in the Haber-Weiss reaction, $O_2^{\bullet-}$ and H_2O_2 generates more $OH^{\bullet-}$.[9,10] The H_2O_2 is capable of degrading haem proteins, inactivate enzymes, oxidize DNA, lipids and sulphydryl groups (-SH).[13,15] Specifically, $OH^{\bullet-}$ reacts with membrane lipid components yielding alkoxyl ($RO_2^{\bullet-}$) radicals, which undergo auto-oxidation in a self-propagating chain reaction to form lipid peroxides ($LOO^{\bullet-}$) and hydroperoxides (LOOH).[9] In the presence of $O_2^{\bullet-}$, nitric oxide (NO) is converted to highly potent oxidant molecule- peroxynitrite (^-ONOO).[1,9,16] The pro-radicals like peroxynitrite (^-ONOO) are relatively stable compared to free radicals. However, protonated ^-ONOO forms the highly reactive peroxynitrous acid (ONOOH).[17] Generation of excess ^-ONOO leads to oxidative damage and tissue lesion.[9]

Toxicological evaluation showed that the metabolism of certain xenobiotics elicits the production of overwhelming levels of RONS.[9,18] Carbon tetrachloride (CCl_4) is a toxic lipophilic molecule that easily absorbs into lipid structural compartments of the cell and is often used to provoke organ lesion in animal models by virtue of its capability to elicit microsomal *CYP2E1*–induced formation of trichloromethyl ($CCl_3^{\bullet-}$) and trichloromethylperoxyl ($Cl_3COO^{\bullet-}$) radicals.[19,20,21,22] In a concerted manner, $CCl_3^{\bullet-}$ binds to -SH group of glutathione and thiol entities of proteins, whereas $Cl_3COO^{\bullet-}$ initiates a chain reaction of lipid peroxidation, and thereby alters the permeability of mitochondria, endoplasmic reticulum and the plasma membrane.[23] Additionally, *CYP2E1*-derived RONS, like H_2O_2, LOOH, malondialdehyde (MDA) and 4-hydroxynonenal (HNE), may diffuse from the hepatocytes to other cell types where fibrotic collagen Type I and III molecules are activated, eliciting fibrotic changes in the affected tissues.[24] These alterations in homeostasis lead to tissue lesion, necrosis and ultimately apoptosis.[25]

Blood is a tissue comprised of the corpuscles (erythrocytes, leucocytes and platelets) suspended in fluid medium called plasma and circulating within a close system in vertebrates referred to as vascular system. The plasma mostly consists of water with dissolved metabolites, proteins, lipoproteins, hormones, electrolytes and nutrients. By virtue of the

fact that the polyunsaturated fatty acids (PUFAs), which are potent targets of RONS cytotoxicity, account for the majority of the erythrocyte membrane structural lipid components, the erythrocyte is highly vulnerable to the vagaries of oxidative stress.[26,27] Erythrocytes in particular, are further predisposed to oxidative damage as a result of their continual exposure to oxygen in the presence of transition metal catalysts, especially in the form of free iron and haemin.[26,28,29,30] Oxidative damage to erythrocyte structural components is exacerbated in the event of exposure of the vascular system to xenobiotics that promote or elicit the generation of RONS,[31,32] and thereby cause profound perturbation of blood homeostasis.

Protective mechanisms have evolved to limit overwhelming levels of RONS and their detrimental outcome.[1,9,33,34,35,36,37] Nevertheless, endogenous antioxidant systems may not confer absolute protection in the event of overwhelming levels of RONS. Therefore, additional protective mechanisms in the form of dietary antioxidants supplementation and intake of herbal products are required to stave off oxidative stress-induced pathologic conditions.[37,38] The use of herbs, which are rich in antioxidant phytochemicals and vitamins, has been suggested to be an effective therapeutic strategy for the management and amelioration of pathologic conditions link with oxidative stress.[37,38,39] Many Nigerian indigenous herbs such as *Monodora myristica*, *Chromolaena odorata* (Linn), *Buccholzia coriacea* and *Sphenostylis stenocarpa* are commonly used among traditional herbal medicine practitioners as remedies for pathologic conditions and disorders. The phytochemical contents and medicinal usefulness of these Nigerian indigenous herbs have been reported elsewhere.[40,41,42,43,44,45,46,47] In traditional herbal medicine practice, these herbs are either administered raw or subjected to hydrothermal processing prior their application.

Previous studies have shown that raised levels of products of lipid peroxidation and alterations in antioxidant enzymes activities in plasma are diagnostic of oxidative stress and serve as biomarkers for evaluating therapeutic interventions.[33,36] The present study evaluated the differentials in phytochemical contents of raw and hydrothermal processed Nigerian indigenous herbs, namely, *M. myristica*, *C. odorata*, *B. coriacea* and *S. stenocarpa* as well as their capacity to ameliorate CCl_4-induced oxidative stress in hepatic tissues of Wistar rats, in which the levels of products of lipid peroxidation and antioxidant enzymes activities served as measure of therapeutic interventions. Furthermore, the levels of adjustments of some haematological parameters of the rats were also investigated following the administration of the herbs.

MATERIALS AND METHODS

Collection and preparation of samples

High-grade raw seeds of *M. myristica*, *B. coriacea* and *S. stenocarpa* were purchased from Relief and Obazu-Mbieri Markets located in Owerri Capital Territory, Imo State, Nigeria. Fresh leaves of *C. odorata* were harvested from a private garden in Amakihia, Owerri-North Local Government Area, Imo State, Nigeria. The samples were transported to the laboratory, identified and authenticated by Dr. E.S. Willie at the Herbarium of the Department of Agronomy, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. All samples were collected between the months of February and March, 2015. Voucher specimens were deposited at the Herbarium for reference purposes.

The various samples were washed separately in a continuous flow of distilled water for 15 min and allowed to dry at laboratory ambient temperature ($T = 24 \pm 5$°C). The samples were divided into two portions on an equal weight basis and designated as follows:

Group R: Raw samples

Group H: Hydrothermal processed samples

Appropriate separate quantities of Group R samples were pulverized using Thomas-Willey milling machine (ASTM D-3182, India). The ground samples were transferred into corresponding vacuum desiccators and allowed to dry at laboratory ambient temperature until a constant weight was achieved. Appropriate separate quantities of Group H samples were boiled in distilled water in corresponding conical flasks (sample/water ratio = 1:4 *w/v*). According to local traditional medicine practice, hydrothermal processing of the seed samples was in the following durations: *M. myristica* = 10 min, *B. coriacea* = 1 h and *S. stenocarpa* = 1.5 h, whereas leaves of *C. odorata* were subjected to hydrothermal processing for 5 min. Next, the Group H samples were dried separately in an oven (Gallenkamp Oven 300 plus series, England) at 50°C until a constant weight was achieved. Finally, Group H samples were ground using the Thomas-Willey milling machine (ASTM D-3182; India), after which the samples were stored in air-tight plastic bottles with screw caps pending extraction.

Extraction of samples

Extraction of Group R and Group H samples was according to the methods previously described.[48] Portion of 10 g each of the ground and dried Group R and Group H samples were subjected to repeated soxhlet extraction cycles for 2 h using 96% CH_3OH (BDH, U.K) as solvent to obtain final volume of 250 mL of corresponding extracts. The volumes of the extracts were concentrated and recovered in a rotary evaporator (Büch Rotavapor R-200) for 12 h at 50°C under reduced pressure. The extracts were dried in vacuum desiccators for 24 h, wrapped in aluminum foil and stored in air-tight plastic bottles with screw caps at ≤ 4 °C. The yields were calculated to be as follows:

- Extract R1; Raw seeds of *M. myristica* = 8.94% (*w/w*).
- Extract R2; Raw leaves of *C. odorata* = 6.22% (*w/w*).
- Extract R3; Raw seeds of *B. coriacea* = 8.07% (*w/w*).
- Extract R4; Raw seeds of *S. stenocarpa* = 14.02% (*w/w*).
- Extract H1; Hydrothermal processed seeds of *M. myristica* = 6.41% (*w/w*).
- Extract H2; Hydrothermal processed leaves of *C. odorata* = 4.39% (*w/w*).
- Extract H3; Hydrothermal processed seeds of *B. coriacea* = 7.12% (*w/w*).
- Extract H4; Hydrothermal processed seeds of *S. stenocarpa* = 13.51% (*w/w*).

A portion of the each extract was measured for phytochemical contents. Also, each extract was reconstituted in phosphate buffered saline (PBS) solution that was osmotically equivalent to 100 g/L PBS (90.0 g NaCl, 17.0 $Na_2HPO_4.2H_2O$ and 2.43 g $NaH_2PO_4.2H_2O$) and an appropriate dose was administered to corresponding experimental animals.

Phytochemicals

Quantitative compositions of alkaloids, flavonoids, tannins and saponins were measured using standard methods. The concentration of alkaloids and saponins were measured using the methods of Harborne.[49] The flavonoids content was according the methods of Boham and Kocipal.[50] The concentration of tannins was measured using the methods of Van-Burden and Robinson,[51] as reported by Belonwu *et al.*[52]

Experimental animals

Healthy male Wistar rats (90 days old) weighing between 150-260 g were maintained at laboratory ambient temperature of 30–55% relative humidity on a 12-h light/12-h dark cycle, with access to water and standard commercial feeds (SCF) (Ewu Feed Mill, Edo State, Nigeria) *ad libitum*, for 2 weeks acclimatization period. The Institutional Review Board of the Department of Biochemistry, Federal University of Technology, Owerri, Nigeria, granted approval for this study. The care and handling of the

animals conformed to the standard principles of laboratory animal care of the United States National Institutes of Health (NIH, 1978).

Carbon tetrachloride infusion/experimental design

A total of 72 male Wistar rats were allotted into 12 groups of 6 rats each. Fibrosis was induced in the Wistar rats by single dose intra-peritoneal (i.p) injection of CCl_4 in paraffin oil as vehicle {1:1 *v/v*; dose = 1.0 mL/kg body weight (b.wt.)} for 2 consecutive days.[53] The animals were deprived of feed only for an additional 16 h before commencement of treatment as described.[54,55] The animal groups were randomly designated on the bases of diets and treatments (dose = 250 mg/kg b.wt.; i.p. of the extracts and silymarin or otherwise 1.0 mL/kg b.wt.; i.p. of PBS, paraffin oil and CCl_4/paraffin oil mixture) received for 28 consecutive days. Silymarin (Medical Union Pharmaceuticals Company) was used as the standard drug for reference treatment of fibrotic rats.[54]

- Group 1: Normal rats received SCF + water *ad libitum* + PBS.
- Group 2: Normal rats received SCF + water *ad libitum* + paraffin oil.
- Group 3: Fibrotic rats received SCF + water *ad libitum* + CCl_4/paraffin oil mixture.
- Group 4: Fibrotic rats received SCF + water *ad libitum* + silymarin.
- Group 5: Fibrotic rats received SCF + water *ad libitum* + Extract R1.
- Group 6: Fibrotic rats received SCF + water *ad libitum* + Extract R2.
- Group 7: Fibrotic rats received SCF + water *ad libitum* + Extract R3.
- Group 8: Fibrotic rats received SCF + water *ad libitum* + Extract R4.
- Group 9: Fibrotic rats received SCF + water *ad libitum* + Extract H1.
- Group 10: Fibrotic rats received SCF + water *ad libitum* + Extract H2.
- Group 11: Fibrotic rats received SCF + water *ad libitum* + Extract H3.
- Group 12: Fibrotic rats received SCF + water *ad libitum* + Extract H4.

At the end of the feeding and treatment period, the rats were subjected to fasting for 12 h, after which time the animals were sacrificed and autopsy samples of the liver were excised for assessment of oxidative stress parameters. Blood samples were drawn from the orbital sinus.[56] for measurement of haematological parameters.

Preparation of liver homogenates

Organ homogenate was prepared according the procedures of Adekunle *et al*.[57] as previously described.[58] Briefly, the liver was excised and placed between blotting papers to remove accompanying blood. Next, the organ was rinsed in 1.15% KCl solution to obliterate residual hemoglobin molecules. The sample was homogenized using a Teflon homogenizer in aqueous K_2PO_4/$KHPO_4$ buffer (0.1 M; pH = 7.4); in 4:1 volume of buffer to organ weight. Subsequently, the homogenate was centrifuged at 10,000 ×g for 20 min at 4°C to obtain the post mitochondrial supernatant (PMS) fraction and collected into sample bottles. The PMS fraction was stored at 4°C until further analysis.[59] The homogenate was used to assay the following oxidative stress parameters: MDA content, CAT activity and SOD activity. Protein concentration was measured at maximum wavelength (λmax) = 595 nm by the methods of Bradford,[60] using bovine serum albumin as standard.

Malondialdehyde

Measurement MDA content of PMS fraction was according to the methods of Tjahjani *et al.*,[61] as described.[62] A mixture of 20% trichloroacetic acid (TCA) and 0.67% thiobarbituric acid (TBA) in a ratio of 2:1 was added into a test tube. A volume of 0.2 mL of PMS fraction was introduced in the mixture and boiled for 10 min in a water bath. After cooling to 24°C, the mixture was centrifuged at 3,000 ×*g* for 10 min. The absorbance of supernatant was read with a spectrophotometer (SPECTRONIC 20, Labtech–Digital Blood Analyzer®) at λmax = 532 nm. The absorbance of the samples was converted to MDA concentrations using the MDA standard curve.[63]

Catalase

Measurement CAT activity of PMS fraction was according to the methods of Luck.[64] The final reaction volume of 3.0 mL contained 0.05 M Tris-buffer, 5 mM EDTA (pH = 7), and 10 mM H_2O_2 (in 0.1 M K_2PO_4/$KHPO_4$ buffer; pH = 7). A hundred micro milliliter (100 µL) aliquot of the PMS fraction was added to the above mixture. The rate of change of absorbance per min at λmax = 240 nm was recorded for 5 min. CAT activity was calculated using $\Sigma = 43.6\ M^{-1}cm^{-1}$ and expressed in terms of mole H_2O_2 consumed/min/mg protein (U/mg protein).

Superoxide dismutase

SOD activity of PMS fraction was estimated according to the methods of Kono,[65] as previously reported.[58] Briefly, the reaction mixture contained solution A (50 mM Na_2CO_3, 0.1 mM EDTA, pH = 10), solution B (96 µM nitroblue tetrazolium [NBT] in solution A), and solution C (0.6% Triton X-100 in solution A) were incubated at 37°C for 10 min. The reaction was started by introducing 100 µL of solution D (20 mM hydroxylamine hydrochloride, pH = 6) to the reaction. The rate of NBT dye reduction by $O_2^{\bullet-}$ generated due to photo-activation of hydroxylamine hydrochloride was measured at λmax = 560 nm in the absence of PMS fraction. Next, a 10 µL aliquot of PMS were added to the reaction mixture and 50% inhibition in the rate of NBT reduction by SOD present in the enzyme source was measured. A unit (U) of SOD activity was defined by the 50% inhibition of NBT. SOD activity was expressed in U/mg protein.

Haematology

Measurement of plasma haemoglobin concentration was the cyanomethaemoglobin method.[66] Red blood cells count (RBC), white blood cells count (WBC) and platelets count (PC) were measured by direct enumeration using a haemocytometer with improved neubauer slide.[67]

Statistical analysis

The data collected were analyzed by the analysis of variance procedure while treatment means were separated by the least significance difference (LSD) incorporated in the statistical analysis system (SAS) package of 9.1 version, (2006).

RESULTS

Table 1 shows the levels of disparities in some phytochemical contents between the raw and hydrothermal processed herbal extracts. Extract R1 gave the highest concentration of alkaloids, but showed 35.84% loss in alkaloids in corresponding hydrothermal processed herbal extract (Extract H1). Likewise, hydrothermal processed herbal extracts (Extract H2, Extract H3 and Extract H4) represented 55.58%, 78.51% and 55.07% loss in alkaloids compared with their corresponding raw extracts (Extract R2, Extract R3 and Extract R4); $p < 0.05$. Similarly, the hydrothermal processed herbal extracts exhibited lower concentrations of flavonoids compared with their corresponding raw herbal extracts. However, the loss in flavonoid contents in Extract H3 and Extract H4 was not significantly different ($p > 0.05$) from their corresponding raw herbal extracts (Extract R3 and Extract R4). The concentrations of tannins in the four hydrothermal processed herbal extracts (Extracts H1-H4) were significantly lower ($p < 0.05$) than their corresponding raw herbal extracts (Extracts R1-R4). For instance, concentration of tannins in Extract H1 was 21.1 fold lower than that of Extract R1. The concentrations of saponins in all the hydrothermal processed herbal extracts were not significantly different ($p > 0.05$) from their corresponding raw herbal extracts.

Figure 1 shows that hepatic MDA concentration of Group 1 was not

Table 1: Some phytochemical contents of raw and hydrothermal processed herbal extracts.

	Concentration (mg/100 g dry sample)			
Extract	**Alkaloids**	**Flavonoids**	**Tannins**	**Saponins***
R1	31.33 ± 0.29^{a}	14.17 ± 0.10^{b}	13.28 ± 0.11^{a}	1.67 ± 0.04
R2	$16.50 \pm 0.10^{b,c}$	21.00 ± 0.18^{a}	$12.63 \pm 0.09^{a,b}$	2.23 ± 0.02
R3	$7.00 \pm 0.09^{d,e}$	$6.33 \pm 0.08^{d,e}$	$7.39 \pm 0.07^{c,d}$	2.33 ± 0.03
R4	$4.83 \pm 0.03^{d,e,f}$	$6.23 \pm 0.07^{d,e,f}$	8.10 ± 0.12^{c}	2.83 ± 0.03
H1	20.01 ± 0.21^{b}	6.50 ± 0.07^{d}	0.62 ± 0.01^{e}	1.33 ± 0.09
H2	7.33 ± 0.07^{d}	$12.33 \pm 0.09^{b,c}$	$0.42 \pm 0.01^{e,f,g,h}$	1.00 ± 0.01
H3	$1.50 \pm 0.01^{e,f,g,h}$	$4.33 \pm 0.03^{d,e,f,g}$	$0.46 \pm 0.01^{e,f,g}$	1.67 ± 0.02
H4	$2.17 \pm 0.03^{e,f,g}$	$3.17 \pm 0.03^{d,e,f,g,h}$	$0.52 \pm 0.01^{e,f}$	2.33 ± 0.02

The mean (X) ± S.D of six ($n = 6$) determinations. Means in the column with the same letter are not significantly different at $p > 0.05$ according to LSD. *Concentrations of saponins showed no significant difference $p > 0.05$ according to LSD.

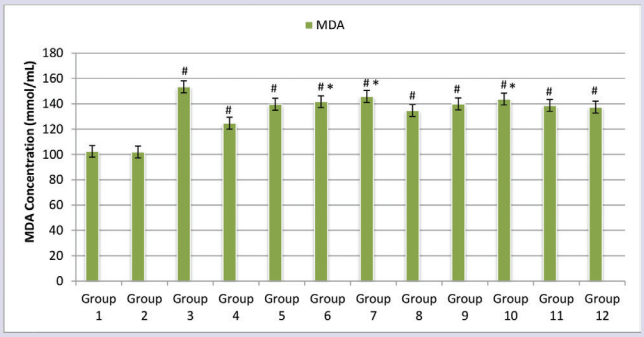

Figure 1: Hepatic malondialdehyde concentrations of experimental rat groups

#: MDA concentrations are significantly different ($p < 0.05$) from control rat groups (Group 1 and Group 2)

***:** MDA concentrations are not significantly different ($p > 0.05$) from untreated fibrotic rats (Group 3).

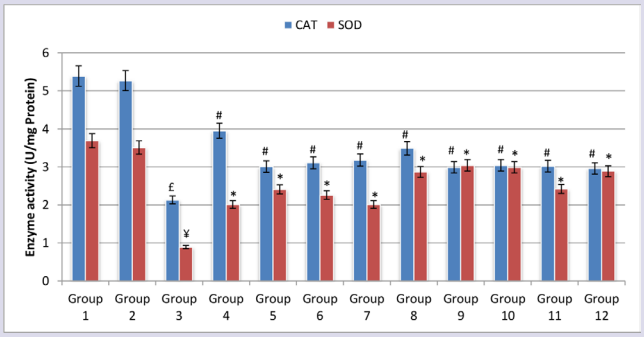

Figure 2: Hepatic catalase and superoxide dismutase activities of experimental rat groups

#: SOD activities are significantly different ($p < 0.05$) from untreated fibrotic rats (Group 3)

***:** CAT activities are significantly different ($p < 0.05$) from untreated fibrotic rats (Group 3)

£: SOD activities are significantly different ($p < 0.05$) from control rat groups (Group 1 and Group 2)

¥: CAT activities are significantly different ($p < 0.05$) from control rat groups (Group 1 and Group 2).

significantly different ($p > 0.05$) from that of Group 2. Hepatic tissue of Group 3 gave the highest MDA concentration, which was significantly different ($p < 0.05$) from those of Group 1 and Group 2. However, hepatic MDA concentration of Group 3 was not significantly ($p > 0.05$) from those of Group 6, Group 7, Group 10 and Group 11. Hepatic tissue of Group 4 accounted for the lowest MDA concentration amongst the CCl_4 treated groups (Groups 3-12). In terms of the herbal treated experimental rat groups (Groups 5-12), hepatic MDA concentrations of Group 5 and Group 6 were not significantly different ($p > 0.05$) from those of corresponding Group 9 and Group 10. Conversely, hepatic MDA concentrations of Group 7 and Group 8 were not significantly different ($p > 0.05$) from those of corresponding Group 11 and Group 12.

Figure 2 shows that the hepatic SOD and CAT activities of Group 1 and Group 2 were not significantly different ($p > 0.05$). The lowest hepatic SOD and CAT activities were observed in Group 3 and were significantly different ($p < 0.05$) from those of Group 1 and Group 2. Furthermore, hepatic SOD and CAT activities of Group 4 were significantly increased ($p < 0.05$) compared with that of Group 3. Hepatic CAT activities of Group 5, Group 6 and Group 7 were not significantly different ($p > 0.05$) from those of corresponding Group 9, Group 10 and Group 11, whereas hepatic CAT activity of Group 8 was significantly higher ($p < 0.05$) than that of corresponding Group 9. Conversely, hepatic SOD activities of Group 9, Group 10 and Group 11 were not significantly higher ($p > 0.05$) than those of corresponding Group 5, Group 6 and Group 7, whereas hepatic SOD activities of Group 8 and correspond Group 12 showed no significant difference ($p > 0.05$).

Plasma haemoglobin concentration of Group 1 was not significantly different ($p > 0.05$) from that of Group 2. Figure 3 showed that Group 3 gave the lowest plasma haemoglobin concentration. Furthermore, plasma haemoglobin concentrations of Group 1 and Group 2 were significantly higher ($p < 0.05$) than that of group 4. Similarly, plasma haemoglobin concentrations of groups (5-8) were not significantly different ($p > 0.05$) from those of corresponding Groups (9-12) (Figure 3).

Figure 4 shows that RBC of Group 1 was not significantly different ($p > 0.05$) from that of Group 2. Group 3 exhibited the lowest RBC amongst the experimental rat groups and was significantly different ($p < 0.05$) from those of Groups 4-12. Additionally, RBC of Group 4 was not significantly different ($p > 0.05$) from those of Groups 5-12. However, RBCs of Group 9 and Group 10 were significantly higher ($p < 0.05$) to those of corresponding Group 5 and Group 6.

The WBC of Group 1 was significantly different ($p < 0.05$) from that of Group 2 (Figure 5). Group 3 gave the highest WBC compared with other experimental rat groups ($p < 0.05$). Additionally, WBCs of Group 5, Group 8, Group 9, Group 11 and Group 12 were not significantly

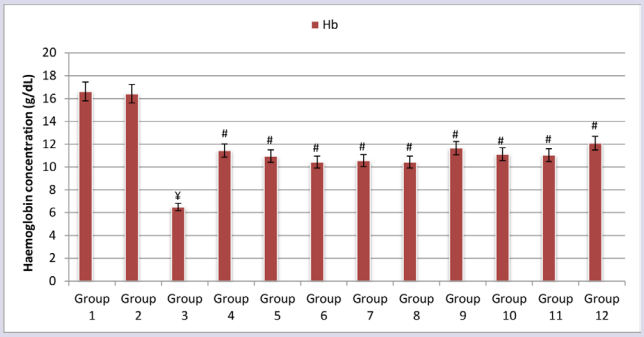

Figure 3: Plasma haemoglobin concentrations of experimental rat groups

#: Haemoglobin concentrations are significantly different ($p < 0.05$) from untreated fibrotic rats (Group 3)

¥: Haemoglobin concentrations are significantly different ($p < 0.05$) from control rat groups (Group 1 and Group 2).

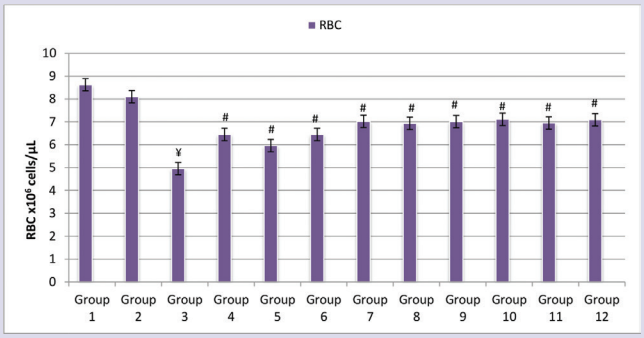

Figure 4: Plasma red blood cell counts of experimental rat groups.

#: RBCs are significantly different (p < 0.05) from untreated fibrotic rats (Group 3)

¥: RBCs are significantly different (p < 0.05) from control rat groups (Group 1 and Group 2)

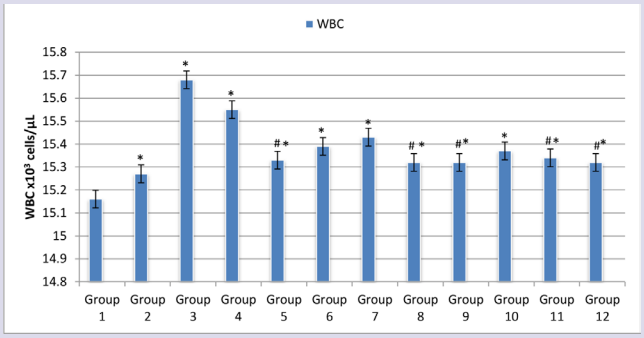

Figure 5: Plasma white blood cell counts of experimental rat groups

#: WBCs are not significantly different ($p > 0.05$) from Group 2

***:** WBCs are significantly different ($p < 0.05$) from control rat group (Group 1).

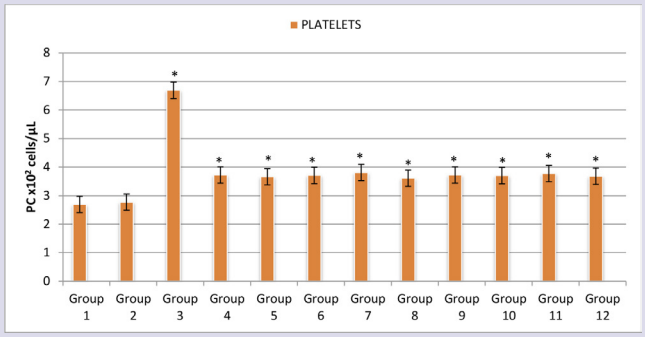

Figure 6: Plasma platelet counts of experimental rat groups

***:** RBCs are significantly different ($p < 0.05$) from control rat groups (Group 1 and Group 2).

different ($p > 0.05$) from that of Group 2. Likewise, Figure 6 shows that the PCs amongst the experimental rat groups (Groups 1-12) varied within the range of (6.69 ± 0.06 - 3.81 ± 0.06) x 10^2 cells/µL. The PCs of the experimental rat groups (Groups 4-12) showed no significant difference ($p > 0.05$). Conversely, PCs of Group 1 and Group 2 were significantly lower ($p < 0.05$) than those of Groups (3-12).

DISCUSSION

Bioactive principles from plants, namely the antioxidant phytochemicals, are of paramount importance in the diet because of their nutritional and medicinal benefits.[68,69] The impacts of hydrothermal processing of medicinal plants and vegetables on their therapeutic and nutritional benefits have been controversially discussed.[70,71,72,73,74] However, previous reports had noted that heat processing of animal feedstuffs and raw vegetables altered their phytochemical contents such that their nutraceutical properties were compromised.[75,76,77] For instance, Zhang and Hamauzu,[72] reported that ascorbic acid content in broccoli declined dramatically following cooking, whereas contrary reports revealed that heat treatment could actually improve the availability of polyphenols of frozen broccoli.[78] Furthermore, boiling of peas caused marginal losses in their water and lipid-soluble antioxidant activities.[79] The present study showed that hydrothermal processing of the experimental herbal extracts caused varying reductions in their phytochemical contents, of which the saponins contents were not affected.

The raised level of hepatic MDA of Group 3 was above baseline concentrations of those of Group 1 and Group 2, which was an obvious indication of exacerbated oxidative damage of the hepatocytes following CCl_4 intoxication as previously reported.[54,80,81,82,83] Furthermore, the raw and hydrothermal processed herbal extracts exhibited equivalent capacities to lower the severity of hepatic oxidative damage in the fibrotic rats but did not provide full therapeutic benefits. By implication, hydrothermal processing of the herbs did not alter their capacities to lower the severity of CCl_4-induced hepatic damage. Group 4 exhibited lower MDA concentrations than those of fibrotic rats treated with raw and hydrothermal processed herbal extracts, which were indications that silymarin offered better therapeutic benefits against hepatic oxidative injury than the experimental herbal extracts. Reports from a related study showed that bark extract of *Bathysa cuspidate* lowered hepatic MDA concentrations in male Wistar rats, which was a demonstration of the efficacy of the extract to scavenge RONS and conferred protection against biomembranes peroxidation.[84] Additionally, these findings corroborated the outcomes of previous studies using extracts of *Solanum* spp. (false "Jurubeba), *Artemisia capillaries,* and *Decalepis hamiltonii*.[85,86,87]

The present study revealed that fibrotic rats exhibited relatively lower hepatic CAT and SOD activities, which concurred with earlier reports.[58,59,82,88] According to Avti *et al*.,[59] overwhelming levels of RONS caused the inhibition of redox enzymes such as CAT and glutathione peroxidase activities as typified by relatively low CAT and SOD activities of Group 3. Therefore, reversion of hepatic CAT and SOD activities towards baseline levels of the control rat groups was diagnostic of the capacities of raw and hydrothermal herbal extracts as well as silymarin to neutralize overwhelming levels of RONS and ameliorate oxidative stress in the fibrotic rats. Furthermore, the present study showed that the hydrothermal processed herbal extracts exhibited greater capacities than the raw herbal extracts to improve SOD activity in the fibrotic rats. Conversely, the raw herbal extracts showed greater capacities than the hydrothermal processed herbal extracts to improve CAT activity in the fibrotic rats. However, the raw and hydrothermal processed herbal extracts did not exert full therapeutic benefits since hepatic CAT and SOD activities were comparatively lower than those of the control groups at the end of 28 consecutive days of treatment.

The present study showed that CCl_4 intoxication perturbed haematological parameters of the rats and the pattern of alterations in serum haemoglobin concentrations paralleled that of RBCs. These findings concurred with previous reports.[89,90] and appeared to suggest that $CCl_3^{\bullet-}$ and $Cl_3COO^{\bullet-}$ from hepatic metabolism of CCl_4 diffused into systemic circulation and rendered the erythrocyte vulnerable to oxidative damage. Accordingly, infusion of the experimental rats with CCl_4 provoked massive haemolysis with attendant anaemia. Additionally, the results of the present study showed that raw and hydrothermal processed herbal extracts exhibited equivalent limited capacities to protect the erythrocytes against to oxidative damage. By implication, hydrothermal processing of the raw herbs did not affect their capacities to offer limited protection against CCl_4-induced erythrocytes oxidative damage. Previous studies had shown that potent antioxidants such as melatonin, ascorbic acid and α-tocopherol as well as plant extracts that exhibited high antioxidant activity protected rats against experimentally-induced tissue oxidative damage.[88,89,90,91]

The elevations of plasma WBCs of the experimental rat groups following CCl_4 intoxication were consistent with previous findings,[92,93] in which they noted that increased plasma WBC paralleled the levels of tumor necrosis factor-α (TNF-α) and interferon (INF-γ) in CCl_4-induced fibrotic rats. Furthermore, acute oxidative stress had been widely reported to provoke increased plasma WBC, neutrophil count as well as the ratio of neutrophil to lymphocyte in experimental animals.[94] Previous studies had shown that antioxidant-containing diets caused significant reversion of elevated WBC to normal levels by inhibiting downstream pro-inflammatory factors associated with acute and chronic hepatic damages in animal models.[92,95] Additionally, elevated plasma WBC could also be diagnostic of the presence of pathogenic antigens in systemic circulation. The elevation of plasma PC in untreated fibrotic rats may not be unconnected with intravascular coagulation associated with CCl_4-induced acute hepatic injury as previously reported elsewhere.[96]

CONCLUSION

Hydrothermal processing of the raw herbs did not adversely affect their capacities to ameliorate hepatic oxidative stress and haematological disorders in the fibrotic rats.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

ACKNOWLEDGEMENT

The authors are grateful for the technical assistance offered by Dr. E.S. Willie of the Department of Agronomy, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

ABBREVIATIONS USED

CCl_4: Carbon tetrachloride; **CH_3OH:** Methanol; **NaCl:** Sodium chloride; **$Na_2HPO_4.2H_2O$:** Diso-dium hydrogen phosphate dihydrate; **$NaH_2PO_4.2H_2O$:** Sodium dihydrogen phosphate dihydrate; **K_2PO_4:** Potassium phosphate; **$KHPO_4$:** Potassium hydrogen phosphate; **Na_2CO_3:** Sodium carbonate; **KCl:** Potassium chloride.

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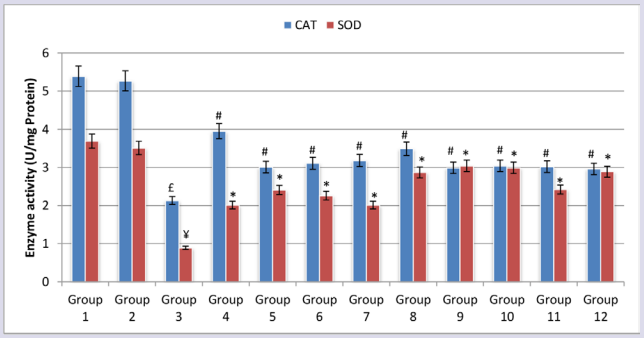
PICTORIAL ABSTRACT



SUMMARY

- Hydrothermal processing of the experimental herbal extracts caused varying reductions in their phytochemical contents, of which the saponins contents were not affected.
- Hydrothermal processing of the raw herbs did not adversely affect their capacities to ameliorate hepatic oxidative stress and haematological disorders in the fibrotic rats.
- The raised level of hepatic MDA was an obvious indication of exacerbated oxidative damage of the hepatocytes following CCl4 intoxication.
- Reversion of hepatic CAT and SOD activities towards baseline levels of the control rat groups was diagnostic of the capacities of raw and hydrothermal herbal extracts to neutralize overwhelming levels of RONS and ameliorate oxidative stress in the fibrotic rats.
- The raw herbal extracts showed greater capacities than the hydrothermal processed herbal extracts to improve CAT activity in the fibrotic rats.
- The raw and hydrothermal processed herbal extracts exhibited equivalent limited capacities to protect the erythrocytes against to oxidative damage. By implication, hydrothermal processing of the raw herbs did not affect their capacities to offer limited protection against CCl4-induced erythrocytes oxidative damage.
- The raw and hydrothermal processed herbal extracts exhibited equivalent capacities to lower the severity of hepatic oxidative damage in the fibrotic rats but did not provide full therapeutic benefits.

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