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Paracetamol and hydrogen peroxide-induced tissue alterations are reduced by *Costus afer* extract

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ABSTRACT

Aim/Objective: To evaluate the effect of the methanolic extract of *Costus afer* stem on paracetamol-induced tissue injury in rats (*in-vivo*) and its ability to reduce hydrogen peroxide-induced erythrocyte damage (*in-vitro*).
Methods: Twenty-five rats were acclimatized for two weeks and randomized into five groups. Group I was untreated controls, while each rat in groups III–V was given a single intraperitoneal administration of paracetamol (800 mg/kg b.w.). Prior to the administration of paracetamol, rats in groups II and IV were orally given *Costus afer* extract at 200 mg/kg b.w, while rats in group V were given 400 mg/kg b.w. extract once daily for four days. In the other experiment, hydrogen peroxide was used to induce erythrocyte damage (after isolating erythrocytes from whole blood) and subsequently incubated with the extract at different concentrations. Markers for tissue and erythrocyte damage were assessed in serum, tissue homogenates, and erythrocytes.
Results: Paracetamol caused marked elevations ($p < 0.05$) in the levels of alanine aminotransaminase (ALT), aspartate aminotransaminase (AST), alkaline phosphatase (ALP), urea, and creatinine in serum. The levels of thiobarbituric acid reactive species (TBARS) in both the liver and the kidney were also raised when compared to untreated control ($p < 0.05$) while the activities of superoxide dismutase (SOD) and catalase (CAT) were reduced when compared to untreated controls ($p < 0.05$). Pretreatment of rats with *Costus afer* extract (p.o.) caused a significant reduction in the serum levels of ALT, AST, ALP, urea, creatinine, and tissue TBARS closer to the control values ($p < 0.05$). Pretreatment of rats with the plant extract also increased the activities of SOD and CAT in both the liver and the kidney. Apart from the levels of TBARS in the liver and CAT in the kidney, rats pretreated with 400 mg/kg b.w. extract showed greater response than in pretreatment with 200 mg/kg b.w. The extract also reduced the hydrogen peroxide-induced erythrocyte damage assessed as hemolysis and lipid peroxidation, which was concentration-dependent ($p < 0.05$).
Conclusion: This indicated that *Costus afer* possesses the ability to protect the various tissues against chemical-induced injury.

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KEYWORDS

Costus afer; paracetamol; urea; creatinine; antioxidant

Introduction

Efforts have been made to show empirical proof on the use of plants, especially tropical plants as alternative medicine. This is also backed up by the less frequent side effects associated with their use, when compared with conventional medicine. However, there still exists a near-negative correlation between the reported medicinal potentials and the utilization of these plants, coupled with the fact that the bioactive potentials of so many others are yet to be explored.

Costus afer Ker Gawl (Family Costaceae) is a relatively tall monocot, perennial, herbaceous, unbranched tropical plant with a creeping rhizome commonly found in the moist or shady forests of West Africa [1]. The plant is commonly known as

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ginger lily or bush cane [2]. This and other species of the genus, such as *C. lucanusianus* and *C. spectabilis,* are used as a remedy for cough, inflammation, and arthritis, as a laxative, purgative, and a diuretic, and for rheumatism and the treatment of other diseases [3,4]. Various parts of these plants have also been used as a remedy for adjuvant-induced polyarthritis, rheumatic pains, and as a fodder to treat goats with retained placenta [5,6]. The plant is also used to treat hypertension, worms, and hemorrhoids [7]. It has been revealed that the plant is rich in flavonoids, anthraquinones, terpenoids, steroidal saponins, oxalates, furan derivatives, and diogenin [4,5,8].

Paracetamol (or acetaminophen) is widely used as an analgesic and antipyretic but produces liver and kidney damage if an overdose is administered [9,10]. The toxic property of paracetamol has been ascribed to a minor but alkylating metabolite *N*-acetyl–p–benzo–quinone imine (NAPQI) [11,12]. This work investigates the effect of *Costus afer* extract on paracetamol-induced alterations on some indices of tissue and erythrocyte damage.

Materials and Methods

Chemicals and reagents

Paracetamol (inj) was obtained from Dana Pharmaceuticals (Nigeria). Methanol, hydrogen peroxide, diethyl ether, and sodium chloride were obtained from BDH Chemicals (Poole, England). Sodium hydroxide, thiobarbituric acid, and trichloroacetic acid were purchased from Lab. Tech Chemicals (UK). All other chemicals and reagents were of analytical grade and were thus used without further purification. All the buffers were prepared in double-glass distilled water.

Animals

Twenty-five albino rats of the Wistar strain weighing 80–100 g were purchased from the animal house of the Department of Physiology, College of Health Sciences, University of Port Harcourt. The animals were kept in a separate animal room, at a temperature of 22 ± 2 °C on a 12 hours light/dark cycle, and had free access to food and water *ad libitum*. They were acclimatized to laboratory conditions for two weeks, housed in steel cages. All the experiments were performed according to the Helsinki declaration in 1964 as amended in 2000, and approved by the Institutional Research Committee.

Preparation of extract

Fresh stems of *Costus afer* were collected from a farm in Agudama-Epie in Yenagoa, Bayelsa State, Nigeria. They were cut to pieces, sun-dried, and pulverized using a blender. A total of 250 g of the pulverized sample was soaked in 2 l methanol for six days and filtered. The filtrate was further concentrated using a rotary evaporator set at 40 °C. The residue was further dried in-vacuo to a constant weight. A recovery rate of 9.4% was finally obtained. Solutions of the extract were prepared in distilled water and used immediately.

Treatment of animals

The rats were grouped into five groups (groups I, II, III, IV, V), with five rats each. After acclimatization, *Costus afer* extract was orally given to rats in groups II, IV, and V. Rats in groups II and IV were given 200 mg/kg b.w. of the extract, while rats in group V were given 400 mg/kg b.w. of the extract once daily for four days. Twenty four hours after the last administration of the extract, rats in groups III, IV, and V were given a single intraperitoneal administration of paracetamol (800 mg/kg b.w.).

Throughout the experiment, the rats were allowed unlimited access to growers' mash and tap water.

Blood and tissue collection

Twenty four hours after the administration of paracetamol, each rat was subjected to light anesthesia in a diethyl ether saturated chamber, and dissected. The thoracic region was opened and blood was drawn through direct cardiac punctures and delivered into sterile sample bottles having no anticoagulant. Blood was allowed to stand for 10 min and centrifuged at 4 °C for 10 minutes at 2880 × *g* in order to collect serum. The liver and the kidney were also excised immediately and washed in ice-cold phosphate buffer (0.2 M, pH 7.0). A 10% tissue homogenate was prepared using the same buffer and perinuclear fraction was obtained by centrifugation at 2880 × *g* for 30 minutes at 4 °C.

Determination of biochemical parameters in serum

The activities of alanine aminotransaminase (ALT), aspartate aminotransaminase (AST), alkaline phosphatase (ALP), urea, and creatinine were measured in serum according to the instructions on the diagnostic kits purchased from Randox laboratories (UK).

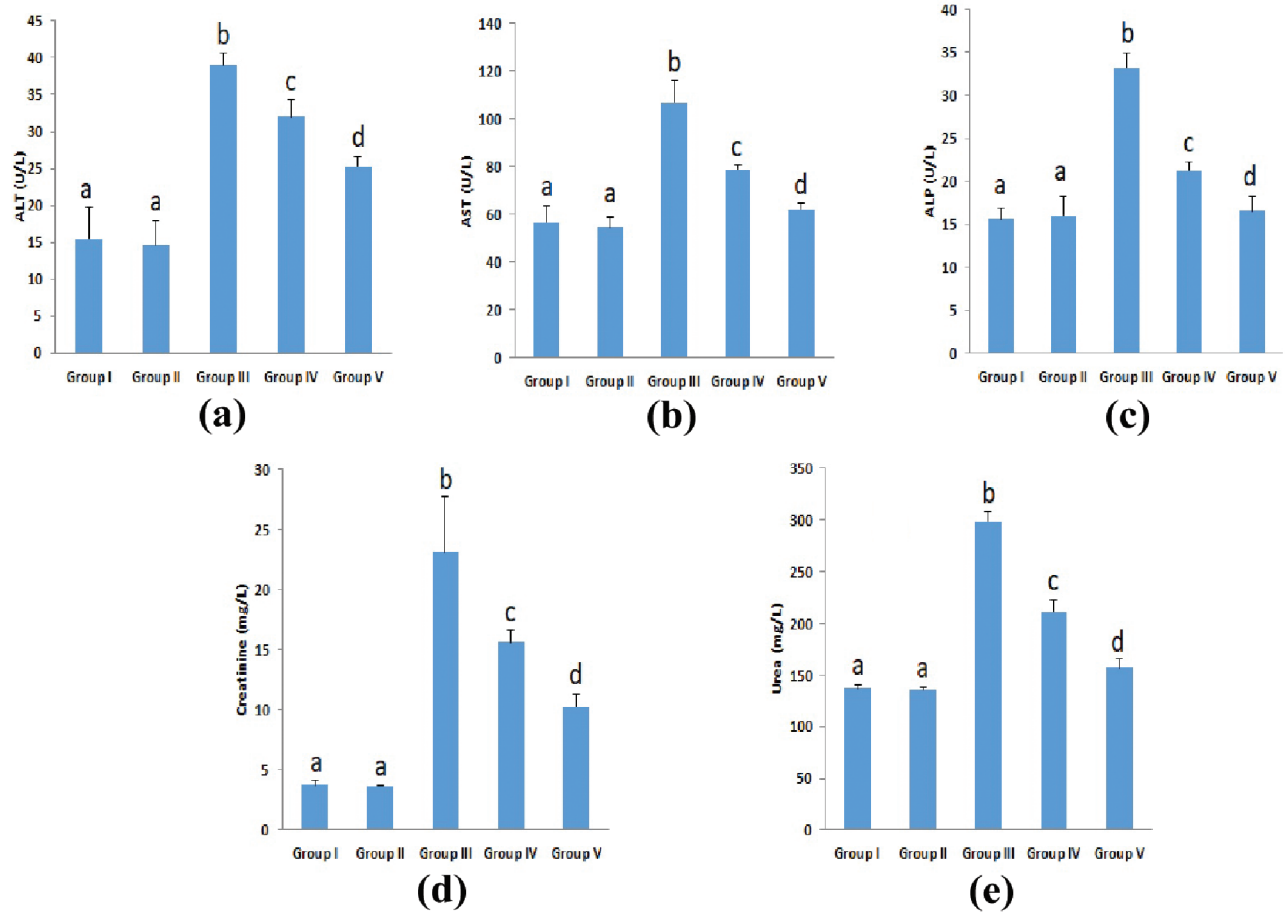

Figure 1. The effect of *Costus afer* extract on serum alanine aminotransaminase (a), serum aspartate aminotransaminase (b), serum alkaline phosphatase (c), serum urea (d), and serum creatinine (e) in rats treated with paracetamol. Values are mean ± SEM of five rats. Values having different superscript letters differ significantly ($p < 0.05$).

Thiobarbituric reactive substances (TBARS) assay

A total of 1 ml of the perinuclear fraction of each tissue was incubated with 2 mL of the thiobarbituric acid reagent (0.375% w/v thiobarbituric acid, 50% v/v conc. hydrochloric acid, and 5% trichloroacetic acid). The solution was boiled for 15 minutes, cooled, and later centrifuged at 2880 × *g* for 5 minutes. The absorbance of the supernatant was later read at 532 nm. TBARS was expressed as nanomoles of malondialdehyde per milligram of protein using a molar extinction coefficient of 1.56×10^{-5} $m^{-1}cm^{-1}$.

Activities of superoxide dismutase (SOD) and catalase (CAT)

Superoxide dismutase (SOD) was measured in perinuclear fractions according to the method of Kapoor and Kakkar [13], while the activity of catalase (CAT) was determined according to Aebi [14] with slight modifications. Briefly, assay mixture was made up of perinuclear fraction (0.1 ml), 1.5 ml phosphate buffer (0.02 M, pH 7.4), and 1.4 ml of distilled water. The reaction was initiated by the addition of 0.1 ml of H_2O_2. After 30 seconds, the amount of H_2O_2 consumed was determined by measuring the absorbance at 240 nm.

Both the enzyme activities were expressed as k/g protein.

Studies on erythrocyte peroxidation

Blood was collected from a healthy individual and erythrocytes were isolated as described [15]. Later, hydrogen peroxide (100 µM) was used to induce erythrocyte hemolysis and lipid peroxidation (measured as malondialdehyde) and the effect of *Costus afer* stem extract (0.5mg/ml, 1 mg/ml, 2 mg/ml, 4 mg/ml, 8 mg/ml) on hydrogen peroxide-induced alterations was determined according to the method of Okoko and Ere [15]. The results were expressed as percent inhibition. For these experiments, vitamin C was used as the control.

Table 1. The effect of *Costus afer* extract on thiobarbituric acid reactive species (TBARS), superoxide dismutase (SOD), and catalase (CAT) in the liver of rats treated with paracetamol.

Group	TBARS	SOD (k/g)	CAT (k/g)
I	1.63 ± 0.38[a]	96.54 ± 2.19[a]	33.65 ± 2.06[a]
II	1.61 ± 0.29[a]	97.23 ± 4.67[a]	34.72 ± 2.17[a]
III	3.86 ± 0.31[b]	25.24 ± 3.45[b]	20.17 ± 2.78[b]
IV	2.45 ± 0.15[c]	45.65 ± 5.78[c]	25.05 ± 1.93[c]
V	2.35 ± 0.06[c]	77.97 ± 6.11[d]	30.55 ± 2.79[a]

TBARS is measured as nanomoles of malondialdehyde per milligram of protein. The values are presented as mean ± S.E.M. from five rats in a group. The mean having different alphabets in superscripts differ significantly ($p < 0.05$).

Table 2. The effect of *Costus afer* extract on thiobarbituric acid reactive species (TBARS), superoxide dismutase (SOD), and catalase (CAT) in the kidney of rats treated with paracetamol.

Group	TBARS	SOD (k/g)	CAT (k/g)
I	1.25 ± 0.12[a]	69.22 ± 4.77[a]	26.22 ± 2.18[a]
II	1.22 ± 0.64[a]	71.37 ± 5.36[a]	25.92 ± 2.05[a]
III	3.33 ± 0.37	22.32 ± 4.27[b]	11.26 ± 2.00[b]
IV	2.36 ± 0.45[c]	43.84 ± 3.14[c]	15.22 ± 2.18[c]
V	1.89 ± 0.39[a]	56.33 ± 5.43[d]	16.36 ± 3.55[c]

TBARS is measured as nanomoles of malondialdehyde per milligram of protein. The values are presented as mean ± S.E.M. from the five rats in a group. The mean having different alphabets in superscripts differ significantly ($p < 0.05$).

Statistical analysis

All data are expressed as mean ± S.E.M. Statistical significance was analyzed using analysis of variance followed by Tukey's range test. A confidence level exhibited at $p < 0.05$ was considered statistically significant.

Results

Alterations on serum enzymes, creatinine, and urea

The effect of *Costus afer* on ALT, AST, ALP, urea, and creatinine in serum is presented in Figure 1. The results revealed that when the rats were intraperitoneally treated with paracetamol alone, the levels of the markers were significantly ($p < 0.05$) higher than in the untreated controls. The levels that were elevated were reduced close to the untreated control values when the rats were pretreated with the *Costus afer* extract. The levels of the markers in the rats given 400 mg/kg b.w. were significantly ($p < 0.05$) lower than the rats given the 200 mg/kg b.w. (i.e., for the rats treated with paracetamol). There was no significant ($p > 0.05$) difference in the markers between the rats in groups I and II.

Alterations on TBARS, superoxide dismutase (SOD) and catalase in liver

The level of the TBARS (measured as malondialdehyde), SOD, and CAT in the liver of rats treated with paracetamol are shown in Table 1. Paracetamol caused marked elevations in malondialdehyde when compared to the untreated controls. Treating the rats with paracetamol alone also resulted in a significant ($p < 0.05$) decrease in the activities of SOD and CAT. However, pretreatment of the rats with the *Costus afer* extract reduced the levels of TBARS and increased the activities of the enzymes closer to the group I levels.

There was no significant difference in the liver malondialdehyde level when the rats were given either 400 mg/kg b.w. or 200 mg/kg b.w. extract. However, pretreatment with a 400 mg/kg extract gave a greater response ($p < 0.05$) in the restoration of SOD and CAT than pretreatment with 200 mg/kg b.w.

Alterations on TBARS, superoxide dismutase (SOD), and catalase in kidney

Table 2 shows the effect of *Costus afer* extract on the levels of TBARS, SOD, and CAT in the kidneys of rats given paracetamol. The results reveal that the drug caused marked elevations in malondialdehyde and also caused a significant ($p < 0.05$) reduction in the activities of SOD and CAT. However, pretreatment of the rats with the *Costus afer* extract reduced the levels of TBARS and increased the activities of the enzymes closer to the group I levels. While the levels of TBARS and SOD were significantly ($p < 0.05$) different for rats pretreated with 200 mg/kg b.w. and 400 mg/kg b.w. of the extract, the activities of CAT were not significantly different.

Effect on erythrocyte damage

Figure 2 shows that *Costus afer* stem extract reduced hydrogen peroxide-induced erythrocyte hemolysis and lipid peroxidation and was concentration-dependent ($p < 0.05$).

Discussion

Cells basically have the capacity to tolerate the production of free radicals/reactive oxygen species (due to the inherent antioxidant systems), since it is a basic feature of metabolism. However, serious pathological consequences occur when there is an imbalance between the antioxidants/antioxidant

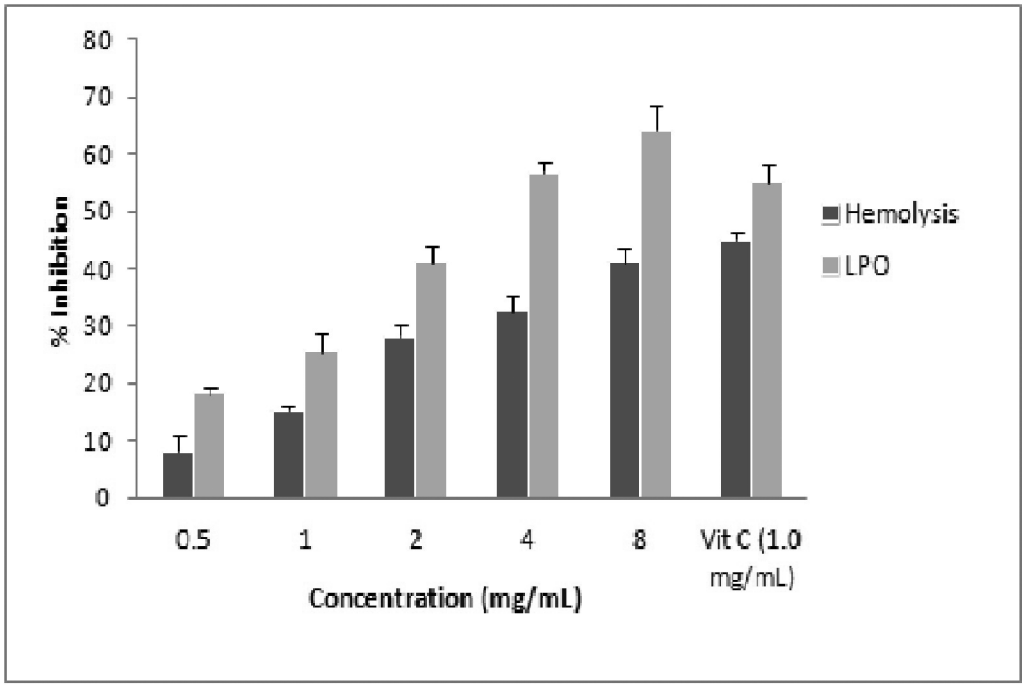

Figure 2. Inhibitory effect of *Costus afer* on erythrocyte damage.

systems and the free radicals/reactive oxygen species—a condition known as oxidative stress.

The results reveal that paracetamol caused both liver and kidney damage at high concentrations, which can be linked to lipid peroxidation (Fig. 1, Tables 1 and 2).

One of the ways in which paracetamol causes tissue injury is by depletion of cytosolic and mitochondrial glutathione, which leads to the loss of cellular homeostasis [16]. This may further result in a reduced ATP content that will hamper a lot of biochemical events. The results reveal that paracetamol reduced the activities of SOD and CAT, and this could be attributed to the consumption of these enzymes in detoxication and antioxidation, in order to deal with lipid peroxidation as suggested [17].

Treating the rats with the extract (200 mg/kg b.w.) alone did not affect the blood and tissue levels of all the markers when compared with the untreated controls. This signifies that the plant extract was not toxic and did not significantly affect tissue pathology.

From the results obtained in this study, it was obvious that *Costus afer* ameliorated the toxicity of paracetamol, which could be dose-dependent. *Costus afer* contains flavonoids and flavonoid glycosides which can act synergistically to reduce the toxicity induced by paracetamol [18,19]. Flavonoids have generally been shown to protect against various forms of disorders such as coronary heart diseases, liver and kidney disorders [20,21]. This is thought to be a result of the induction of detoxifying enzymes such as epoxide hydroxylase, glutathione S-transferase, and UDP-glucuronosyltransferases [22,23]. Although the molecular mechanism through which detoxification enzymes are induced is not well known, it has been suggested that these phytochemicals interact with various intracellular signalling cascades [24].

Erythrocytes possess a high content of polyunsaturated lipids coupled with a rich oxygen supply. In the presence of transition metals (which are redox active), the erythrocytes are vulnerable to oxidative attack and, thus, have been used to study the effects of reactive oxygen species/antioxidants [25,26]. Hydrogen peroxide on the other hand is a potent reactive oxygen species that degrades heme, which leads to the release of redox active Fe ions that can initiate free radical production [27]. Hydrogen peroxide is highly diffusible along various cellular compartments in which it oxidizes various cellular targets [28]. Although produced by routine biochemical processes, it can cause cellular damage by generating the very potent hydroxyl radical via the Fenton reaction [28,29].

Oxidative damage on erythrocytes could manifest as hemolysis, which is a common mechanism in some hemoglobinopathies and suppressed antioxidant systems [30].

From the results, the plant extract possessed substances that reduced the hydrogen peroxide-mediated effects, and thus could ameliorate the morphological changes that accompany erythrocyte hemolysis and lipid peroxidation. Direct scavenging of the ROS may also be a possible mechanism.

In addition to flavonoids, other phytochemicals such as terpenoids, alkaloids, and terpenes also exhibit significant antioxidant potential and also chemoprotective [31–35], thus the observed bioactivity in the current experiment could be attributed to synergy between the various phytochemicals that have been detected in *Costus afer* [4,5,8]; however, further experiments are required to evaluate this possibility.

In conclusion, the bioactive properties of the stem extract could justify the reported traditional uses of the plant, thus its controlled consumption should be encouraged. The presence of *Costus afer* in the biosphere is nature's generosity in providing mankind with effective and natural nutraceuticals that could be exploited pharmacologically.

Conflict of Interest Statement

The author declares that there are no conflicts of interest.

References

[1] Omokhua GE. Medicinal and socio-cultural importance of *Costus afer* (Ker Grawl) in Nigeria. Afr Res Rev 2011; 5:282–7.

[2] Anyasor GN, Onajobi FD, Osilesi O, Adebawo O. Proximate composition, mineral content and in vitro antioxidant activity of leaf and stem of *Costus afer* (Ginger lily). J Intercul Ethnopharmacol 2014; 3:128–34.

[3] Nyananyo BL. Plants from the Niger Delta. Onyoma Research Publications, Port Harcourt, Nigeria, 2006.

[4] Anyasor GN, Onajobi F, Osilesi O, Adebawo O, Oboutor EM. Anti-inflammatory and antioxidant activities of *Costus afer* Ker Gawl. hexane leaf fraction in arthritic rat models. J Ethnopharmacol 2014; 155:543–51.

[5] Ezejiofor AN, Orish CN, Orisakwe OE. Effect of aqueous leaves extract of *Costus afer* Ker Gawl (Zingiberaceae) on the liver and kidney of male albino Wistar rat. Anc Sci Life 2013; 33:4–9.

[6] Cousins D, Huffman MA. Medicinal properties in the diet of gorillas: an ethno-pharmacological evaluation. Afr Stud Monogr 2002; 23:65–89.

[7] Akpantah AO, Akpan U, Eluwa MA, Isamoh TE, Udonkang M, Ekanem TB. Blood parameters and spleen histology following chronic consumption of ethanolic extract of *Costus Afer* stem and juice on albino Wistar rats. Br J Med Med Res 2016; 13:1–10.

[8] Marles RJ, Farnsworth NR. Antidiabetic plants and their active constituents. Phytomed 1995; 2:137–89.

[9] Boyd EM, Bereczky GM. Liver necrosis from paracetamol. Br J Pharmacol Chemother 1996; 26:606–14.

[10] Graham GG, Davies MJ, Day RO, Mohamudally A, Scott KF. The modern pharmacology of paracetamol: therapeutic actions, mechanism of action, metabolism, toxicity and recent pharmacological findings. Inflammopharmacol 2013; 21:201–32.

[11] Salomone F, Barbagallo I, Puzzo L, Piazza C, Volti GL. Efficacy of adipose tissue-mesenchymal stem cell transplantation in rats with acetaminophen liver injury. Stem Cell Res 2013; 11:1037–44.

[12] Kalinec GM, Thein P, Parsa A, Yorgason J, Luxford W, Urrutia R, et al. Acetaminophen and NAPQI are toxic to auditory cells via oxidative and endoplasmic reticulum stress-dependent pathways. Hearing Res 2014; 313:26–37.

[13] Kapoor R, Kakkar P. Naringenin accords hepatoprotection from streptozotocin induced diabetes in vivo by modulating mitochondrial dysfunction and apoptotic signaling cascade. Toxicol Reports 2014; 1:569–81.

[14] Aebi H. Catalase in vitro. Methods Enzymol 1984; 105:121–6.

[15] Okoko T, Ere D. Reduction of hydrogen peroxide–induced erythrocyte damage by *Carica papaya* leaf extract. Asian Pac J Trop Biomed 2012; 2:449–53.

[16] Shankar K, Vaidya VS, Apte UM, Manautou JE, Ronis MJ, Bucci TJ, et al. Type 1 diabetic mice are protected from acetaminophen hepatotoxicity. Toxicol Sci 2003; 73:220–34.

[17] Hamza RZ, Al-Harbi MS. Amelioration of paracetamol hepatotoxicity and oxidative stress on mice liver with silymarin and *Nigella sativa* extract supplements. Asian Pacific J Trop Biomed 2015; 5:521–31.

[18] Lin RC, Lacaille-Dubois MA, Hanquet B, Correia M, Chauffert B. New diosgenin glycosides from *Costus afer*. J Nat Prod 1997; 60:1165–9.

[19] Anyasor GN, Ogunwenmo O, Oyelana OA, Akpofunure BE. Phytochemical constituents and antioxidant activities of aqueous and methanol stem extracts of *Costus afer* Ker Gawl. (Costaceae). Afr J Biotechnol 2010; 9:4880–4.

[20] Manach C, Scalbert A, Morand C, Rémésy C, Jiménez L. Polyphenols: food sources and bioavailability. Am J Clin Nutr 2004; 79:727–47.

[21] Scalbert A, Manach C, Morand C, Rémésy C, Jiménez L. Dietary polyphenols and the prevention of diseases. Crit Rev Food Sc Nutr 2005; 45:287–306.

[22] Chen C, Kong AN. Dietary chemopreventive compounds and ARE/EpRE signaling. Free Rad Biol Med 2004; 36:1505–16.

[23] Lee YY, Westphal AH, de Haan LH, Aarts JM, Rietjens IM, van Berkel WJ. Human NAD(P)H: quinone oxidoreductase inhibition by flavonoids in living cells. Free Rad Biol Med 2005; 39:257–65.

[24] Schroeter H, Boyd C, Spencer JP, Williams RJ, Cadenas E, Rice-Evans C. MAPK signaling in neurodegeneration: influences of flavonoids and of nitric oxide. Neurobiol Aging 2002; 23:861–80.
[25] Zhu QY, Holt RR, Lazarus SA, Orozco TJ. Inhibitory effects of cocoa flavanols and procyanidin oligomers on free radical-induced erythrocyte hemolysis. Exp Biol Med 2002; 227:321–9.
[26] Kuhn V, Diederich L, Keller IV TS, Kramer CM, Lückstädt W, Panknin C, et al. Red blood cell function and dysfunction: redox regulation, nitric oxide metabolism, anemia. Antioxid Redox Signal 2017; 26:718–42.
[27] Morris JD, Wong KM, Penaherrera CD, Payne CK. Mechanism of the biomolecular synthesis of PEDOT:PSS: importance of heme degradation by hydrogen peroxide. Biomat Sci 2016; 4:331–7.
[28] Peralta IN, Cogoi L, Filip R, Anesini C. Prevention of hydrogen peroxide-induced red blood cells lysis by *Ilex paraguariensis* aqueous extract: participation of phenolic and xanthine compounds. Phytother Res 2013; 27:192–8.
[29] Ninh Pham A, Xing G, Miller CJ, David Waite T. Fenton-like copper redox chemistry revisited: Hydrogen peroxide and superoxide mediation of copper-catalyzed oxidant production. J Catalysis 2013; 301:54–64.
[30] Ko FN, Hsiao G, Kuo YH. Protection of oxidative hemolysis by demethyldiisoeugenol in normal and β-thalassemic red blood cells. Free Rad Biol Med 1997; 22:215–22.
[31] Gülçin İ, Elias R, Gepdiremen A, Chea A, Topal F. Antioxidant activity of bisbenzylisoquinoline alkaloids from *Stephania rotunda*: cepharanthine and fangchinoline. J Enzyme Inhib Med Chem 2010; 25:44–53.
[32] Chung HS, Woo WS. A quinolone alkaloid with antioxidant activity from the aleurone layer of anthocyanin-pigmented rice. J Nat Prod 2001; 64:1579–80.
[33] Gu HF, Li CM, Xu YJ, Hu WF, Chen MH, Wan QH. Structural features and antioxidant activity of tannin from persimmon pulp. Food Res Intern 2008; 41:208–17.
[34] Stahl W, Sies H. Antioxidant activity of carotenoids. Mol Aspect Med 2003; 24:345–51.
[35] Murthy KC, Vanitha A, Rajesha J, Swamy MM, Sowmya PR, Ravishankar GA. In vivo antioxidant activity of carotenoids from *Dunaliella salina*—a green microalgae. Life Sci 2005; 76:1381–90.