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Original Research

Hepatoprotective and in vivo Anti-oxidant Activity of Costus Afer Leaf Extracts against Acetaminophen-Induced Hepatotoxicity in Rats

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Abstract

This study investigated the hepatoprotective and in vivo antioxidant activity of orally administered chloroform and ethanol Costus afer leaf extracts (20 - 60 mg/kg b.w.) on 1 g/kg b.w. acetaminophen-induced acute hepatic injury in albino rats for 7 days. Result showed no significant difference in plasma and hepatic superoxide activities. Plasma catalase (CAT) activity was significantly reduced in 20 and 40 mg/kg chloroform extracts and 20 mg/kg ethanol extract treated groups. Hepatic CAT activity was reduced significantly at co-administered 20 mg/kg ethanol extract and 1 g/kg ACT treated group. Plasma and hepatic glutathione levels were elevated significantly in chloroform and ethanol leaf extracts treated groups. The plasma and liver glutathione S-transferase activity in the chloroform extract groups were significantly reduced while ethanol leaf extract showed no significant difference. The plasma and hepatic MDA concentrations in the chloroform and ethanol extracts treated groups were found reduced. Further studies showed that the reduction in AST and ALT elevated activities induced by ACT was more profound in ethanol treated groups than chloroform treated groups. Therefore, findings from this present study indicate that the chloroform leaf extract possess high antioxidant effect than ethanol leaf extracts while ethanol leaf extract possess high hepatoprotective potentials than chloroform leaf extract.

INTRODUCTION

Acetaminophen (ACT) (Tylenol or N-acetyl para-aminophenol or APAP or Paracetamol) is a widely used analgesic and antipyretic drug [1]. It is often found in prescription formulas and as over-the-counter-drug including cold-and-cough remedies and narcotic pain relievers [2]. As such it is often reported as product responsible for both acute and chronic overdose associated with significant hepatotoxicity [3, 4]. Under normal conditions, orally administered ACT is rapidly absorbed from gastrointestinal tract to the liver where it is metabolized through glucuronide and sulphate conjugation. About 4-5% of the drug is metabolized via the cytochrome P_{450} oxidase pathway resulting into the production of reactive intermediate N-acetyl-p-benzoquinone imine (NAPQI) metabolite. Subsequently, NAPQI is detoxified by glutathione (GSH) both non-enzymatically and enzymatically into mercapturic acid and cysteine conjugates. During chronic use or overdose, the glucuronide and sulphate conjugation metabolic routes are saturated and GSH store is depleted by NAPQI [4]. This leaves the cytochrome P_{450} pathway (involving CYP1A1, 1A2, 2E1, 3A1 and 3A2) to accumulate toxic NAPQI metabolites. NAPQI induces oxidative stress and binds covalently to hepatocyte membranes and sulfhydryl proteins [5, 6]. Although the exact mechanism of ACT induced cell toxicity is still unresolved, it is suggested that mitochondria may play an important role in the ACT induced liver apoptosis [7, 8].

Hepatic disease remains a global health problem [9]. However, an appreciable amount of hepatic protective drugs from ethno medicine used in treating serious liver disorders remains scientifically unresolved and hence, the search for new drugs is still on-going [10]. The development of liver protective agents is of paramount importance in the protection from liver damage [10]. The literature has constantly shown that hepatoprotective effects are associated with plant extracts rich in antioxidants [3; 13]. The uses of traditional medicine from plants still represent a large source of natural anti-oxidants that might serve as leads for development of novel drugs [14; 15].

The plant, *Costus afer* Ker Gawl (Family: Zingiberaceae) is a tall perennial herbaceous, unbranched medicinal plant with creeping rhizome, commonly found in moist or shady forest and river banks of tropical West Africa [16; 17]. *C. afer* is commonly called gingerlily or bush cane [18]. It is known as "Okpete" or "Okpoto" in Igboland, "Kakizawa" in Hausa "ireke omode" in Yoruba and "Mbritem" in Efik all in Nigeria, Anglophone Cameroon calls it "Monkey sugar cane" [18]. It is commonly used as a medicinal herb, especially the leaf, stem, seeds and rhizomes which are harvested from the wild [19]. It has been used in folkloric medicine to treat ailments such as inflammation, rheumatism, arthritis, cough, hepatic disorders, helminthic, miscarriages, epileptic attack, haemorrhoids, as laxative, diuretics, and also had served as an antidote for poison [20; 21]. The plant stem extracts had been studied in our laboratory and shown to possess potent antioxidants *in vitro* [22]. Other studies include: *in vitro* and *in vivo* pharmacological activities of methanol leaf extract [21], leaf essential oil [23], and topical anti-inflammatory activity [24]. However, no work has been reported on the hepatoprotective properties of this plant leaf. Therefore, the present study was undertaken to investigate hepatoprotective and *in vivo* antioxidant activity of the chloroform (CFECA) and ethanol leaf extracts (ETECA) of *C. afer* on acetaminophen-induced liver injury in rats.

MATERIALS AND METHODS

Plant Collection

The whole plant *C. afer* was obtained from a farm land at Irolu, Ogun State, Nigeria and authenticated by Prof. E.B. Esan, Department of Biosciences, School of Basic Sciences, Babcock University, Ilisan-Remo, Ogun state, Nigeria.

Plant processing and extraction

The leaves were plucked off from the plant stem, air-dried under room temperature and pulverized mechanically. The pulverized leaf sample 100 g was weighed and soaked in 1500 ml of ethanol and chloroform respectively for 72 h. The extracts were filtered using Watman No. 1 filter paper and subsequently concentrated at 45°C using rotary evaporator (Buchi Rotavapor RE-3; Switzerland) under reduced pressure. The extracts derived were kept at 4°C until further use.

Drug and Assay kits

Emzor paracetamol tablets were purchased from Agram Pharmacy, Ilisan Remo Ogun State, Nigeria. Aspartate amino transferase (AST) and Alanine amino transferase (ALT) assay kits were purchased from QCA (UK). All other reagents used were of analytical grade.

Animals

Male albino rats (Wister strain) weighing between 100 – 120 g, total of 50 were obtained from the Preclinical Animal House, Physiology Department, University of Ibadan, Ibadan, Nigeria. The animals were acclimatized for two weeks at the Departmental Animal House, Babcock University. The animals were maintained and cared for following the National Institute of Health (NIH) animal care guideline.

At the end of the treatment period, the animals were starved overnight and then euthanized by cervical dislocation and subsequently sacrificed. The whole blood samples were collected through cardiac puncture using 5 ml hypodermic syringes into heparinized bottles and immediately centrifuged at 3000 rpm to obtain plasma for antioxidant enzyme assay, lipid peroxidation and liver function tests. The rat liver samples were also removed and perfused in sucrose solution and homogenized using Teflon homogenizer in 1.15% potassium chloride solution (KCl) and the homogenate centrifuged at 1500 revolution per minute to obtain supernatant used for the antioxidant enzyme assay, lipid peroxidation and liver function tests.

Table 1. Experimental Design

Experimental Groups	Treatment Type (Oral administration)
I	Normal control 1.0 ml 0.9% NaCl (normal saline)
II	1 g/kg b.w. Acetaminophen
III	40 mg/kg b.w Chloroform leaf extract
IV	40 mg/kg b.w Ethanol leaf extract
V	20 mg/kg b.w. Chloroform leaf extract + 1 g/kg b.w. Acetaminophen
VI	40 mg/kg b.w. Chloroform leaf extract + 1 g/kg b.w. Acetaminophen
VII	60 mg/kg b.w. Chloroform leaf extract + 1 g/kg b.w. Acetaminophen
VIII	20 mg/kg b.w. Ethanol leaf extract + 1 g/kg b.w. Acetaminophen
IX	40 mg/kg b.w. Ethanol leaf extract + 1 g/kg b.w. Acetaminophen
X	60 mg/kg b.w. Ethanol leaf extract + 1 g/kg b.w. Acetaminophen

Fifty male albino rats were randomly divided into 10 groups of five rats each and the various extracts and drug were co-administered for a period of 7 days. b.w.:- indicates body weight. 0.9% normal saline was used as vehicle in this study

Determination of antioxidant activity

The antioxidant activity in plasma and liver samples were assayed using spectrophotometric method for the determinations of superoxide dismutase (SOD) activity [25], Catalase (CAT) activity [26], Glutathione *S*-transferase (GST) activity [27], Glutathione (GSH) level was determined using Ellman's reagent [28; 29], Malondialdehyde level [30].

Protein determination: Plasma and tissue proteins were determined by the method of folin ciocateau method [31] using bovine serum albumin as a standard.

Biochemical parameters

Plasma and hepatic Aspartate amino transferase (AST) and Alanine amino transferase (ALT) activities were determined using spectrophotometric method according to the method of Reitman and Frankel [32].

Statistical analysis

This was done with the aid of Windows Microsoft Excel and SPSS for windows: SPSS Inc, Chicago, standard version 15.0 to determine differences between mean using analysis of variance (ANOVA). Data were reported as Mean ± Standard deviation.

RESULTS

Data from Fig. 1 and 2 showed no significant difference ($p>0.05$) in plasma and hepatic SOD activities in CFECA, ETECA and ACT treated groups compared with control and other treatment groups. Fig. 3 indicated that ACT significantly decreased ($p<0.05$) plasma catalase activity compared with control. CFECA (40 mg/kg) alone increased plasma catalase activity while combined treatments at 20 mg/kg CFECA and 1 g/kg ACT significantly decreased ($p<0.05$) plasma CAT activity compared with ACT treated group. Conversely, combined 60 mg/kg CFECA and 1 g/kg ACT had increased CAT activity. ETECA 40 mg/kg and 20 mg/kg + 1g/kg groups had a significant rise in plasma CAT activities. Catalase activity in the liver of the treatment groups were significantly ($p<0.05$) elevated when compared with the ACT treated group with the exception of 40 mg/kg CFECA and ETECA and co-administered 20 mg/kg and 1 g/kg ACT group (Fig. 4).

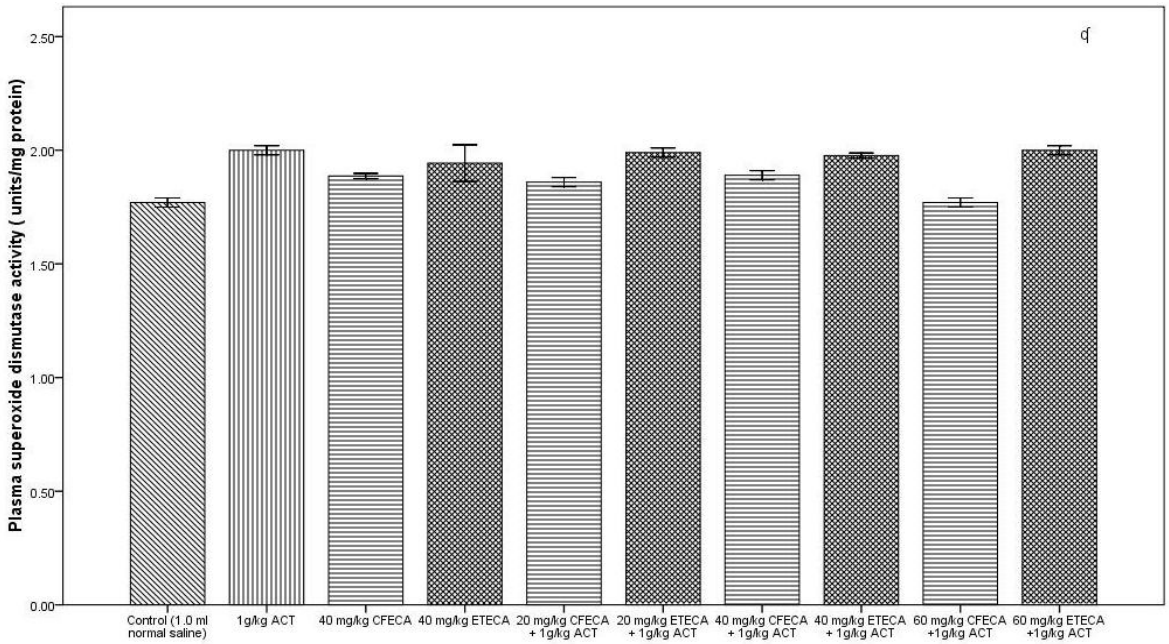

Figure 1. Effect of chloroform and ethanol leaf extracts of Costus afer on plasma superoxide dismutase activity in rats treated for 7 days

#indicates significantly different from control at p<0.05
¥ indicates significantly different from 1g/kg ACT at p<0.05

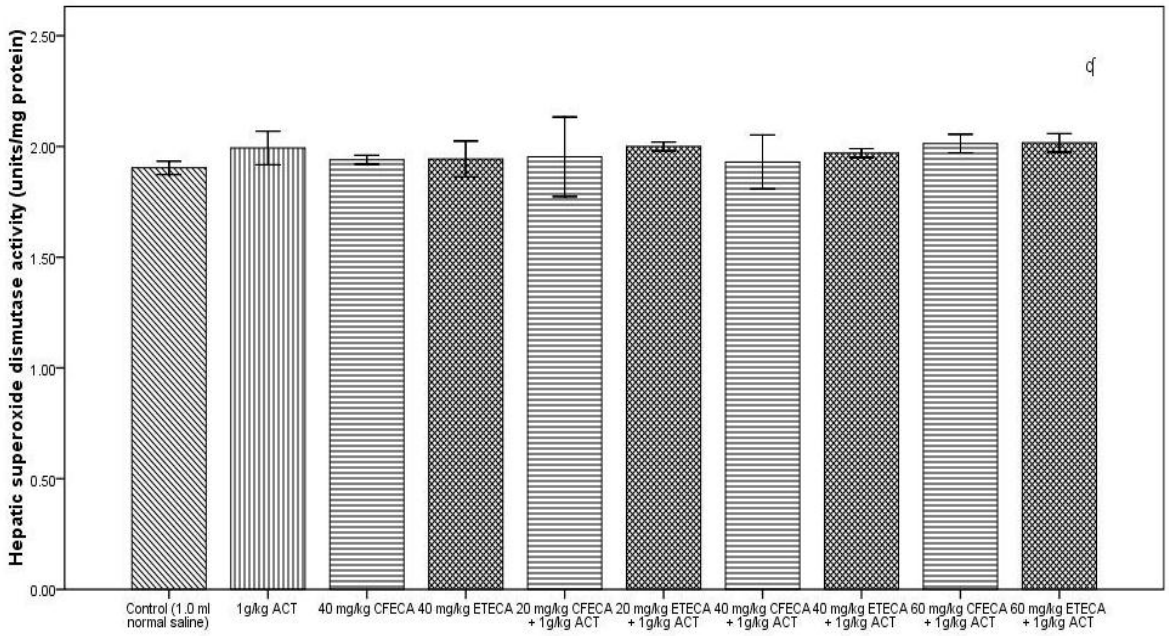

Figure 2. Effect of chloroform and ethanol leaf extracts of Costus afer on hepatic superoxide dismutase activity in rats treated for 7 days

#indicates significantly different from control at p<0.05
¥ indicates significantly different from 1g/kg ACT at p<0.05

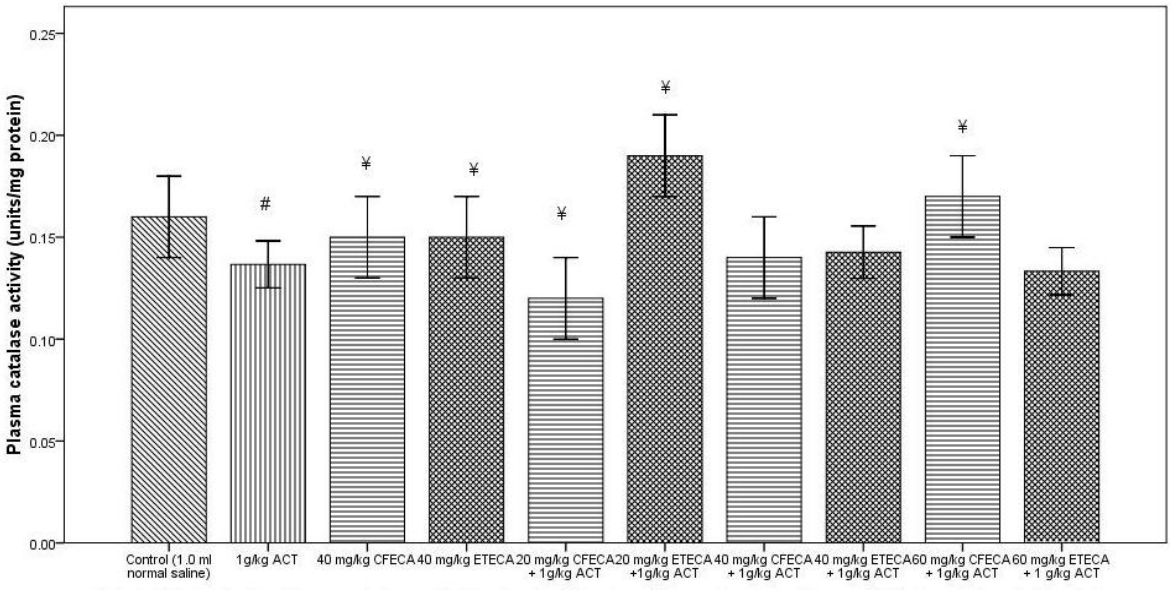

Figure 3. Effect of chloroform and ethanol leaf extracts of Costus afer on plasma catalase activity in rats treated for 7 days

#indicates significantly different from control at p<0.05
¥ indicates significantly different from 1g/kg ACT at p<0.05

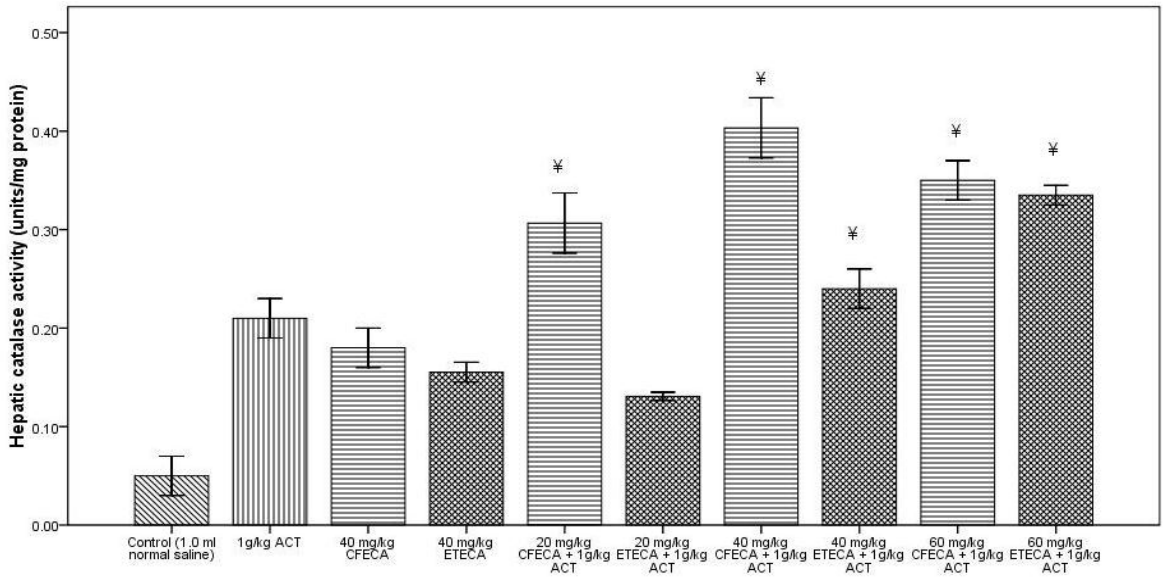

Figure 4. Effect of chloroform and ethanol leaf extracts of Costus afer on hepatic catalase activity in rats treated for 7 days

#indicates significantly different from control at p<0.05
¥ indicates significantly different from 1g/kg ACT at p<0.05

Plasma and liver GSH levels in ACT treated group were depleted compared with control group and was elevated significantly (p<0.05) in the CFECA and ETECA treated groups (Table 1).

The plasma and liver GST activity in the CLE treated groups were significantly (p<0.05) reduced when compared with acetaminophen treated group. Plasma and hepatic GST activity in showed no significance difference (p>0.05) in all ETECA groups compared

with ACT group except plasma GST in 20 mg/kg ETECA and 1 g/kg ACT (Table 1).

The plasma and liver MDA concentrations in the CFECA and ETECA treated groups were reduced compared with acetaminophen treated group. The MDA levels in ETECA treated groups were lower than CFECA with the exception of 60 mg/kg ETECA and 1 g/kg group (Table 1).

The plasma and hepatic AST and ALT activities in the CFECA and ELECA treated groups were significantly ($p<0.05$) reduced when compared with the acetaminophen treated group. However, the ACT induced elevation of AST and ALT levels was significantly reduced by ELECA treated groups than CFECA treated groups (Table 1).

Table 1. Effect of varying doses of chloroform and ethanol leaf extracts of Costus afer on plasma and liver antioxidant and hepatotoxic biomarkers (n=5)

PARAMETERS	Control (0.9% normal saline)	1 g/kg ACT	40 mg/kg CFECA	40 mg/kg ETECA	20 mg/kg CFECA + 1 g/kg ACT	20 mg/kg ETECA + 1 g/kg ACT	40 mg/kg CFECA + 1 g/kg ACT	40 mg/kg ETECA + 1 g/kg ACT	60 mg/kg CFECA + 1 g/kg ACT	60 mg/kg ETECA + 1 g/kg ACT
PLASMA										
GSH (µg/ml)	1.16 ± 1.2	1.09 ± 0.31	1.15 ± 0.25¥§	1.15 ± 1.28¥	1.17 ± 0.11¥§	1.23 ± 1.20¥	1.19 ± 0.12¥§	1.20 ± 0.22¥	1.20 ± 0.14¥§	1.25 ± 1.12¥
GST (µmol/min/mg)	0.16 ± 0.21	0.25 ± 0.14	0.18 ± 0.11¥	0.21 ± 1.22□	0.15 ± 0.12¥*	0.44 ± 0.13¥	0.17 ± 0.22*	0.38 ± 1.41□	0.14 ± 0.37¥*	0.37 ± 0.28□
MDA (nmol/dl)	0.04 ± 2.4	0.05 ± 0.02	0.040 ± 0.15¥	0.02 ± 0.01¥	0.03 ± 0.19¥	0.02 ± 1.23¥	0.03 ± 0.14¥	0.03 ± 0.08¥	0.02 ± 1.32¥	0.03 ± 0.03¥
AST (U/L)	11.50 ± 1.12	20.0 ± 0.26	12.12 ± 3.11¥	7.6 ± 4.125¥	15.16 ± 2.29¥	10.39 ± 1.20¥	10.25 ± 0.97¥	10.05 ± 1.11¥	10.22 ± 2.5¥	10.21 ± 1.65¥
ALT (U/L)	18.02 ± 0.01	45.0 ± 0.20	19.54 ± 3.56¥	30.18 ± 4.51¥	19.30 ± 0.02¥	36.0 ± 21.02¥	18.24 ± 1.66¥	24.16 ± 1.39¥	19.11 ± 1.12¥	24.10 ± 1.20¥
LIVER										
GSH (µg/ml)	1.52 ± 1.34	1.30 ± 0.02	1.40 ± 0.08¥§	1.40 ± 2.20¥	1.47 ± 0.22¥	1.49 ± 0.12¥	1.51 ± 2.11¥§	1.42 ± 2.98¥	1.50 ± 4.52¥§	1.49 ± 0.33¥
GST (µmol/min/mg)	0.45 ± 0.07	0.51 ± 2.12	0.38 ± 2.12¥	0.49 ± 0.32□	0.41 ± 1.14§	0.52 ± 1.07□	0.41 ± 1.55¥	0.39 ± 0.52□	0.34 ± 2.73¥	0.55 ± 4.10□
MDA (nmol/dl)	0.04 ± 2.44	0.08 ± 0.24	0.05 ± 3.01¥	0.02 ± 1.13¥	0.06 ± 1.05¥	0.01 ± 2.11¥	0.05 ± 1.27¥	0.03 ± 1.14¥	0.02 ± 0.11¥	0.03 ± 2.99¥
AST (U/L)	15.01 ± 0.05	50.06 ± 1.02	26.15 ± 1.13¥	20.98 ± 2.09¥	29.11 ± 2.11¥	25.18 ± 0.01¥	35.12 ± 2.13¥	32.66 ± 1.23¥	32.76 ± 1.20¥	30.42 ± 0.21¥
ALT (U/L)	40.11 ± 0.88	60.21 ± 1.67	45.32 ± 2.54¥	41.11 ± 2.14¥	59.57 ± 0.26¥	54.42 ± 0.02¥	47.38 ± 3.47¥	45.33 ± 1.43¥	41.65 ± 1.11¥	39.12 ± 1.56¥

¥ indicates significantly different from 1 g/kg ACT at $p<0.05$

§ indicates not significantly different from ethanol leaf extract (ETECA) of C. afer at $p<0.05$

* indicates significantly different from ethanol leaf extract (ETECA) of C. afer at $p<0.05$

□ indicates not significantly different from 1 g/kg ACT at $p<0.05$

n = 5, where n indicates number of rats per group

DISCUSSION

The toxic acetaminophen electrophilic metabolite, NAPQI covalently binds to macromolecules and cellular proteins and also oxidizes lipids, alters homeostasis of calcium and endogenous antioxidants [33, 34, 35]. Consequently, serum transaminases AST and ALT from the damaged hepatic tissue leak into the blood stream in conformity with the extent of damage [36].

In this present investigation the plasma and hepatic SOD activities in CFECA and ETECA treated groups did not show any significant difference compared to the acetaminophen treated group. This seems to be in agreement with the claim that SOD might not be involved in the first line of endogenous antioxidant defence against acetaminophen induced toxicity [37]. Superoxide anion ($O_2^{\bullet-}$) is often generated from the reduction of oxygen by semiquinone, and rapidly it is dismutated to hydrogen peroxide by SOD [38; 39].

The reactive hydrogen peroxide produced by SOD activity is neutralized by peroxidases such as catalase into water and molecular oxygen to protect tissues damages resulting from peroxidation or accumulation of highly reactive hydroxyl radicals [40]. CAT, a major antioxidant enzyme with hematin as the prosthetic group is ubiquitously present in all aerobic cells

containing a cytochrome system [41]. The reduction in plasma and hepatic CAT activities in the ACT treated group is an indication of hightened oxidative stress caused by accumulation of highly toxic hydroxyl radical and H_2O_2 [42]. In the co-treated groups, an appreciable increase in CAT activity indicates that *C. afer* might be able to protect liver tissues from reactive oxygen species damages accumulated due to ACT induced lipid peroxidation [43]. The CFECA treated group showed an enhanced CAT activity compared to the ETECA treated groups, suggesting the CFECA might contain bioactive agents that could either augment or enhance CAT enzyme activity. The attenuation, suppression or inhibition of free radicals or reactive oxygen specie activity by antioxidant is important in providing protection against liver damage [44].

Studies have also shown that reduced glutathione (GSH), a non-enzymatic antioxidant serves as a sulphydryl buffer which protects the –SH groups of protein from the damaging effects of reactive oxygen species [45]. It also serve as a critical determinant of tissue susceptibility to oxidative damage and depletion of hepatic GSH has been associated to predispose liver cells to drug toxicity [46]. The reduction in plasma and hepatic GSH levels in the ACT treated group supports the assertion that GSH participate in the first line of defense during acetaminophen induced hepatotoxicity [3; 4]. The reactive ACT metabolite NAPQI react with GSH causing 90% depletion of hepatic GSH concentration which can result in hepatocellular death and mitochondrial dysfunction [47]. Increased GSH concentration observed in the CFECA and ETECA treated groups could have resulted from the repleting effect of *C. afer* extracts on GSH, protecting the cells from NAPQI-induced free radical hepatotoxicity. Previous studies showed that *C. afer* possess bioactive compounds such as flavonoids, phenols, anthraquinones, cardiac glycosides, terpenoids, alkaloids and tannins with antioxidants property [21; 22]. It is supposed that these plant antioxidants could have augumented and/or complemented the endogenous antioxidant defence system conferring protection to the liver. This also could account for the reason why no significant difference was obseved in the plasma and hepatic SOD activities among the treatment groups [48].

Glutathione *S*-transferase (GST) catalyzes the initial reaction involving the conjugation of xenobiotics having electrophilic constituent with glutathione thereby removing reactive electrophiles and hence protecting vital nucleophilic groups in macromolecules such as proteins and nucleic acids [49]. The result of this study indicated increased GST activity in the acetaminophen treated group which implied an increased response of GST to detoxify accumulating reactive species caused by paracetamol induced lipid peroxidation. However, treatment with *C. afer* extracts counteracted the rise in GST activity. This suggests that *C. afer* may prevent the peroxidation of lipids induced by acetaminophen metabolism [50].

Furthermore, ACT treated group showed elevated plasma and hepatic MDA levels compared with other treated groups. MDA is a biomarker for lipid peroxidation which is one of the major outcomes of free radical mediated injury to tissue [51]. Increased MDA level indicates an increased lipid peroxidation due to heightened oxidative stress during acetaminophen metabolism [52]. Peroxidation of fatty acyl groups occurs mostly in membrane phospholipids and can greatly alter the physicochemical properties of membrane lipid bilayers including decrease in membrane fluidity and inactivation of membrane bound enzymes, resulting in severe cellular dysfunction [53]. This effect was reversed in CFECA and ETECA treated groups indicating hepatoprotective effect of *C. afer*. The observation was more pronounced in ETECA treated groups than CFECA.

Further investigation also showed elevated levels of plasma and hepatic AST and ALT activities in the acetaminophen treated animals. This observed increase was reversed in the presence of the CFECA and ETECA, although the effect was more pronounced in the ETECA treated groups than CFECA treated groups. This suggests that the hepatoprotective potentials of the *C. afer* leaf extract could be polar metabolite(s).

Therefore, we conclude that chloroform leaf extract of *C. afer* could serve as a potential source of biopharmaceutical agents with antioxidative property while the ethanol leaf extract could serve as a source of hepatoprotective agents against acetaminophen induced tissue injuries. More so, the antioxidative property of *C. afer* leaf may have contributed to its hepatoprotective potentials. Further studies are in progress to better understand the mechanism of action of *C. afer* that is responsible for the hepatoprotective and antioxidant activity.

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CONFLICT OF INTEREST

The authors declare no conflict of interest and are solely responsible for the writing and content of this work.

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