Original Articles
Toxicology International, *Sept-Dec 2015 / Vol-22 / Issue-3, 83-91*
ISSN (Print) : 0971-6580
ISSN (Online) : 0976-5131
DOI: 10.22506/ti/2015/v22/i3/137629

Asessment of the Hepatoprotective and Antioxidant Effect of Aqueous Leaf Extract of *Costus after* "Ker Gawl" on Cyclosporine A Induced Hepatotoxicity

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Abstract

Objective: *Costus afer* has long been used in traditional African setting in several instances though lack a prove of evidence for its numerous acclaimed potentials, however, this study is designed to investigate the hepatoprotective and antioxidant properties of aqueous leaf extract of *C. afer* against cyclosporine A (CsA) induced hepatotoxicity. **Methods:** Rats of similar weight were divided into six groups of five rats each. Groups 1 and 2 served as normal and toxic control received olive oil and 50 mg/kg CsA p.o., respectively. Groups 3, 4, 5, and 6 were given 375, 750, 1125 mg/kg p.o of the extract, and 100 mg/kg silymarin an hour prior to 50 mg/kg CsA, respectively, for 5 days. Physical, biochemical, and histological parameters estimated by reported methods serves as scientific evidence in the study. **Results:** *C. afer* significantly restored catalase ($P < 0.005$), superoxide dismutase ($P < 0.005$), malondialdehyde ($P < 0.005$), and glutathione ($P < 0.05$) levels dose dependently. In all cases as mentioned previously, *C. afer* at all dose level promoted significant ($P < 0.05$) restoration of serum liver parameters such as aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, conjugated bilirubin, total bilirubin, total protein, and albumin. The biochemical observations were supplemented with histopathological reports, which showed attenuation of hepatocellular necrosis. **Conclusion:** Aqueous leaf extract of *C. afer* dose dependently prevented the significant changes on the physical, biochemical, and histological changes induced by CsA in the liver. Therefore, aqueous leaf extract of *C. afer* can be employed as an economic agent to treat liver diseases related to CsA administration and possibly other hepatotoxic drugs.

Keywords: *Costus afer*, Hepatocellular Necrosis, Liver Enzyme, Oxidative Stress, Silymarin

Received: 10-07-2015 **Revised:** 10-08-2015 **Accepted:** 17-08-2015

1. Introduction

Liver toxicity from drugs, and also from alternative medicine products such as herbalist's remedies and dietary supplements, is currently an increasingly relevant health issue (Lozano-Lanagrán *et al.*, 2011). Drug induced hepatotoxicity accounts for approximately 2% of cases of inpatient jaundice, and liver is involved in 3-10% of all adverse drug reaction (Lee, 2003; Farell, 1997). Elevations of liver toxic markers in the serum of different cytosolic, mitochondrial and membrane-bound enzymes, and other biomolecules are seen in different forms of liver disease. The most commonly used ones are aspartate Aminotransferase (AST), alanine Aminotransferase (ALT), bilirubin, and Alkaline Phosphatase (ALP) (Pagani *et al.*, 2001; Whitfield, 2001). Drug-induced liver injury poses major clinical problem and is the leading cause of acute liver failure and transplantation in the Western countries (Lee, 2003; Ostapowicz *et al.*, 2002). Free radicals are one of the frequent products of normal cellular metabolism. Imbalance in antioxidants defense mechanism and overproduction of free radicals due to environmental stress is ultimately responsible for neurodegeneration and accompany health challenges (Uttara *et al.*, 2009). Free-radicals-mediated Lipid Peroxidation (LP) is known to change cell membrane structure and its activity. Increase in production of free radicals stimulates LP which leads to increased levels of

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Malondialdehyde (MDA) (Senthilkumar *et al.,* Oboh and Rocha, 2007).

Disparity of metabolism and excessive generation of free radicals predisposes to numerous disorders including liver and kidney diseases. In biological system, oxidative stress can result from; 1. Decrease in endogenous antioxidant levels such as antioxidant defense enzymes such as Superoxide Dismutase (SOD), Catalase (CAT), or Glutathione Peroxidase (GPx); 2. Depletion of dietary antioxidants and other essential dietary constituents. The roles of antioxidants are to neutralize the excess free radicals, to protect the cells against their toxic effects and to contribute to disease prevention (Halliwell, 2007). Plant sources could be considered as novel source of phytochemicals out of which flavonoids and polyphenols are of great importance that may be helpful to cope up with such oxidative stress (Ramchandani *et al.,* 2014).

Plant sources could be considered as novel source of phytochemicals out of which flavonoids and polyphenols are of great importance that may be helpful to cope up with such oxidative stress (Ramchandani *et al.,* 2014; Olalye and Rocha, 2007). *Costus afer* also known as ginger lily or bush cane (Iwu, 1993) is common in many parts of the world and also to the people of Nigeria. The succulent stem is chewed as a remedy for cough. The root decoction is administered for the treatment of sleeping sickness and stomach ache (Iwu, 1993). Most important to this work is the use of this plant in the treatment of diabetes mellitus in folklore medicine (Udem and Ezeasor, 2010) and so many other acclaimed medicinal properties. Cyclosporine also called cyclosporine A (CsA), is a calcineurin inhibitor and potent immunosuppressive agent used largely as a means of prophylaxis against cellular rejection after organ transplantation. However, its clinical and experimental use is limited by several side effects such as, nephrotoxicity, hepatotoxicity, hypertention, and cardiotoxicity (Maryam *et al.,* 2012). In animal models, cyclosporine decrease bile flow which may account for the mild hyperbilirubinemia that occurs with high doses.

The present research was undertaken to evaluate the hepatoprotective and antioxidant potentials of aqueous leaf extract of *C. afer* against CsA induced hepatotoxicity.

2. Experimental

2.1 Sample Collection

The leaves of *C. afer* were collected from Pharmacognosy garden of Faculty of Pharmaceutical Science, University of Portharcourt, in Ikwere Local Government Area of Rivers State, Nigeria in February, 2015.

2.2 Sample Identification

The plant was identified and authenticated by A.O. Ozioko, International Center for Ethnomedicine and Drug Development (INTERCEDD), University of Nigeria Nsukka, Enugu State and the voucher Number is INTERCEDD/033 and Professor M.E Bassey of Botany Department, University of Uyo, Akwa Ibom State, Nigeria.

2.3 Sample Processing and Extraction

After identification of the plant, the samples collected were washed with tap water to remove dirt and unwanted particles from the leaf. The leaf were kept in a under shade for the water to dry off and also to preserve the active constituents.

2.4 Extraction

Two hundred fifty grams of the leaf were washed, shade dried, weighed, and cut into smaller pieces with a stainless steel knife, then transferred into a grinding machine to pulverize it into the smallest particle that could be macerated. The grounded leaf were then transferred into a macerating jar containing about 500 ml of distilled water and allowed to stand for 24 h with constant agitation. After 24 h, the mixture was strained, the marc was pressed and the liquid was filtered and stored in a refrigerator at 4°C. The solution was discarded every 3 days and the process repeated till the end of the treatment.

2.5 Animal Care and Handling

After 2 weeks of acclimatization, 30 male Wistar albino rats weight range (70-150 g) obtained from the Department of Pharmacology animal house, University of Port Harcourt, Choba, Rivers state, Nigeria were used in the study. These animals were grouped according to their body weight into six groups of five animals each (n = 5), designated as Group 1 - Normal Control [NC]; Group 2 - Toxic Control [TC]; Group 3-375 mg/kg CA; Group 4-750 mg/kg CA; Group 5-1125 mg/kg CA; and Group 6-100 mg/kg silymarin (SLM). They were housed in a standard cage and maintained at standard laboratory conditions of ambient temperature with relative humidity (55-64 %) and light and dark conditions (12/12 h). They were fed with standard diet (Sander Nigeria Ltd.) and water *ad-libitum*. Animal ethics and proper handling

methods were strictly adhered to. The bedding of the cages made with sawdust was changed daily and the cages washed and disinfected daily.

2.6 Dose Levels Selection

The dose used for the current work was selected based on LD_{50} of 1500 mg/kg from the previous work by Ezejiofor *et al.* (2013). The low, medium, and highest dose used for this work was taken to represent 25, 50, and 75% of the LD_{50}.

2.7 Cyclosporine A Induced Hepatotoxicity in Rat

This research conducted in two phases involved thirty male Wistar albino rats assigned into six groups of five animals each ($n = 5$) and dosed as follows:

- Group 1 - NC: Single dose of distil water (1 ml/kg, p.o.) daily for first 5 days and olive oil (1 ml/kg, p. o.) for another 5 days.
- Group 2 - TC: Distil water (1 ml/kg, p.o.) once daily for 5 days followed by CsA: Olive oil (1:1, 50 mg/kg, p. o.) for another 5 days.
- Group 3: *C. afer* (375 mg/kg, p.o.) once daily for 5 days and same dose of extract 1-h prior to 50 mg/kg CsA: olive oil p.o.) for another 5 days.
- Group 4: *C. afer* (750 mg/kg, p.o.) once daily for 5 days and same dose of extract 1-h prior to 50 mg/kg CsA: Olive oil p.o.) for another 5 days.
- Group 5: *C. afer* (1125 mg/kg, p.o.) once daily for 5 days and same dose of extract 1-h prior to 50 mg/kg CsA: Olive oil p.o.) for another 5 days.
- Group 6: SLM (100 mg/kg, p.o.) once daily for 5 days and same dose of SLM 1-h prior to 50 mg/kg CsA: Olive oil p.o.) for another 5 days.

The experimental period was 10 days. Distilled water was used as the vehicle for SLM, while olive oil was the vehicle for cyclosporine (50 mg/20 ml). The dose of the extract administered was based on the LD_{50} (that is, 1500 mg/kg) of the extract (Ezejiofor *et al.,* 2013). *C. afer* leaf extract and SLM was administered 1-h prior to CsA administration on phase two. The fluid and feed intake of the rats were monitored daily in both the control and treated groups for a period of 10 days; body weight was taken on day 0, 5 and 10. Animals were sacrificed 24 h after the last treatment. Blood was collected by retero orbital sinus puncture and serum separated by centrifugation. Rats were sacrificed; liver were excised, rinsed clean in saline, weighed and preserved in 10% formalin for antioxidant assay and histopathological study.

2.8 Preparation of Tissues and Biochemical Assays

At the end of the treatment (10 days), the rats were anesthetized with thiopental (50 mg/kg body weight i.p., Cristália, Brazil) and sacrificed by exsanguinations (blood was collected by cardiac puncture). The liver was quickly removed, weighed, and homogenized in ice-cold 50 mM phosphate buffer, pH 7. After centrifugation of the homogenates (3000 rpm, 15 min), the supernatants were used for biochemical assays related to drug-induced liver function damage, described below.

2.9 Plasma Enzymes

For biochemical analysis, heparinized blood was centrifuged at 3000 rpm for 15 min. Serum transaminases: Serum alanine transaminase, aspartate transaminase levels were estimated by Reitman and Frankel's method (Reitman and Frankel, 1957), alkaline phosphate level was estimated by colorimetric end point method, Total Bilirubin (TB), and Conjugated Bilirubin (CB) levels were estimated according to Jendrassik and Grof method (Jendrassik and Grof, 1938). Albumin (ALB) level was estimated using Bromocresol Green method, and the level of Total Protein (TP) was estimated.

3. Antioxidant Assays

3.1 Thiobarbituric Acid Reactive Substances Levels

The rationale behind MDA estimation is based on the fact that under acidic condition, MDA produced from the peroxidation of fatty acid membranes and food products reacts with the chromogenic reagent, 2-Thiobarbituric Acid (TBA), to yield a pink colored complex with maximum absorbance at 532 nm. LP in the liver tissue was measured by estimating the formation of TBA reactive substances, in accordance with Ohkawa *et al.* (1979). Tissue supernatant (10%, w/v) was boiled (100°C) in Trichloroacetic Acid (TCA) (10%) and TBA (0.67%) for 60 min, cooled and centrifuged. The absorbance of the supernatant was read at 535 nm. Results were expressed as nmol MDA/g tissue.

3.2 Hepatic Glutathione Content

An aliquot of the sample (0.2 ml) was added to 1.8 ml of distilled water and 3 ml of the precipitating solution and mixed. The mixture was then allowed to stand for approximately 5 min and then centrifuged at 1200 g for 10 min (4°C). 1 ml of filtrate was added to 4 ml of 0.1 M phosphate buffer, DTNB (0.5 ml) was finally added. A blank was prepared with 4 ml of 0.1 M phosphate buffer, 1 ml of diluted precipitated solution and 0.5 ml DTNB. The solution was kept at room temperature for 15 min and read at 412 nm on a spectrophotometer. Glutathione (GSH) was proportional to the absorbance at that wavelength and the estimate was obtained from the GSH standard. The results were expressed in µg GSH/min/mg protein (Khynriam and Prasad, 2003).

3.3 Catalase Activity Assay

This method is based on the fact that CAT in the sample preparation split hydrogen peroxide which can be measured spectrophotometrically at 240 nm. For determination of CAT activity, 0.2 ml of sample (equivalent to 50–100 µg protein) was added to 50 mM of phosphate buffer (pH 7.4) containing 100 mM (v/v) of H_2O_2 (Sigma, St. Louis, USA) in total of 1 ml. The reaction mixture was incubated for 2 min at 37°C and the rate of absorbance change at 240 nm was recorded, which indicated the decomposition of H_2O_2. Activities were calculated using the molar extinction coefficient of H_2O_2 at 240 nm, 43.59 L/mol cm. One unit of CAT activity equals the amount of protein that converts 1 µmol H_2O_2/min. All samples were measured in quadruplicates (Gornall *et al.,* 1949).

3.4 Superoxide Dismutase Activity Assay

The ability of SOD to inhibit the autoxidation of epinephrine at pH 10.2 makes this reaction a basis for a simple assay for this dismutase. An aliquot of supernatant from 12,000 g centrifugation of tissues homogenates was diluted in distilled water to make a 1:10 dilution. Two hundred microliter of diluted sample was added to 2.5 ml of 0.05 M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer and the reaction started by the addition of 0.3 ml of freshly prepared 0.3 mM epinephrine to the mixture which was quickly mixed by inversion. The reference cuvette contained 2.5 ml buffer, 0.3 ml of substrate (epinephrine) and 0.2 ml of distilled water. The increase in absorbance at 480 nm was monitored every 30 s for 25 min (Liu *et al.,* 1977).

3.5 Glutathione Peroxidase Activity Assay

The assay is based on the measurement of the residual GSH remaining after the action of GPx. The assay mixture containing 0.5 ml of sodium phosphate buffer, 0.1 ml sodium azide, 0.2 ml of reduced GSH, 0.1 ml of H_2O_2, and 0.5 ml 1:10 cell extract was taken and the total volume was made up to 2.0 ml with distilled water. The tubes were incubated at 37°C for 3 min and the reaction was terminated by the addition of 0.5 ml 10% TCA. To determine the residual GSH content, the supernatant was removed after centrifugation and to this, 4.0 ml of disodium hydrogen phosphate (0.3 M) solution, and 1 ml of the DTNB (Rekka *et al.,* 1996).

3.6 Histopathological Studies

The thirty Wistar albino rats used for the study were sacrificed under thiopental and the liver harvested, weighed, and fixed in 10% formalin. After 72 h, the organs were dehydrated in graded alcohol, cleared in xylene, and embedded in paraffin. Tissue processing was carried out by an autotechnicians and the prepared 5 µ thick section were mounted on slides and stained with hematoxylin and eosin. Stained sections were morphologically evaluated and the pictures of the slides were taken for comparison.

3.7 Statistical Analysis

All the data obtained were statistically analyzed and expressed as a mean ± standard deviation using one-way ANOVA and values of $P < 0.05$ was considered significant.

4. Results

This study revealed that, there was a marked increase in liver enzymes and reduction in plasma proteins (TP and ALB) upon CsA administration. Treatment with aqueous leaf extract of *C. afer* significantly ($P < 0.05$) reduces an increased AST, ALT, AST, TB, and CB with significant ($P < 0.05$) increase in TP and ALB. The restoration in the liver parameters seen with treated groups was significant when compared with the standard drug (SLM) with $P < 0.05$, this was shown in Table 1.

In CsA induced hepatotoxicity, a reduction in the levels of CAT, GSH-s-transferase (GST), GPx, GSH and SOD and an increase in MDA was seen in the toxic group (CsA alone). On administration of the extract, there was a significant ($P < 0.05$) reversal in the level of antioxidant parameters measured as shown in Table 2.

Table 1. Effect of aqueous leaf extract of *C. afer* on different liver enzymes in cyclosporine induced hepatotoxic rats

Groups	AST (μ/L)	ALT (μ/L)	ALP (μ/L)	TP (g/L)	ALB (g/L)	TB (μmol/L)	CB (μmol/L)
NC	25.67±2.30	12.33±4.50	48.33±2.51	77.67±3.05	36.67±2.08	3.70±0.00	2.40±0.00
TC (CsA alone)	38.00±6.24^{ac}	24.33±2.30^{ac}	80.67±10.06^{ac}	67.00±29.00^{a}	24.67±7.50^{a}	6.80±1.03^{a}	4.40±0.69^{c}
CsA±375 mg/kg *C. afer*	25.67±2.30^{bc}	16.33±1.15^{bc}	55.33±5.83^{bc}	71.00±2.00^{c}	30.00±8.88	8.67±1.09	4.40±0.69
CsA±750 mg/kg *C. afer*	24.33±2.30^{b}	12.00±0.00^{bc}	49.67±2.08^{bc}	72.00±10.81	32.67±3.21	6.20±1.03	4.00±0.69
CsA±1125 mg/kg *C. afer*	20.33±2.31^{b}	8.33±3.51^{b}	48.00±5.28^{b}	79.00±3.46^{b}	32.33±4.50	4.33±1.09^{b}	2.80±0.69^{b}
CsA±SLM	16.00±3.00^{b}	5.33±2.30^{b}	38.00±8.18^{b}	94.33±6.65^{b}	36.33±1.52^{b}	3.70±0.00^{b}	2.40±0.00^{b}

All values represent a mean±SD (n=5); $^{a}P<0.05$ versus NC, $^{b}P<0.05$ versus TC, $^{c}P<0.05$ versus SLM. TC=Toxic control, NC=Normal control, SLM=Silymarin, AST=Aspartate aminotransferase, ALT=Alanine aminotransferase, ALP=Alkaline phosphatase, TP=Total protein, ALB=Albumin, TB=Total bilirubin, CB=Conjugated bilirubin, CsA=Cyclosporine A, *C. afer=Costus afer*, SD=Standard deviation

Table 2. Effect of aqueous leaf extract of *C. afer* on antioxidant parameters in CsA induced hepatotoxic rats

Group	GSH (μg/mg)	CAT (μmol)	MDA (μmol/mg)	GPx (μg/mg)	GST (μg/mg)	SOD (μg/mg)
NC	0.077±0.001	0.117±0.0032	0.017±0.001	0.142±0.002	0.750±0.005	0.062±0.000
TC	0.050±0.001^{b}	0.093±0.001^{bc}	0.040±0.001^{bc}	0.130±0.003^{b}	0.721±0.008^{bc}	0.044±0.001^{bc}
CsA±375 mg/kg *C. afer*	0.062±0.001^{ac}	0.159±0.001^{ac}	0.036±0.004^{ac}	0.157±0.002^{ac}	0.796±0.004^{a}	0.089±0.002^{ac}
CsA±750 mg/kg *C. afer*	0.065±0.001^{a}	0.207±0.004^{a}	0.027±0.002^{a}	0.164±0.012^{ac}	0.852±0.123^{a}	0.084±0.002
CsA±1125 mg/kg *C. afer*	0.081±0.003^{a}	0.221±0.0031^{a}	0.022±0.003^{a}	0.181±0.012	0.872±0.004^{a}	0.086±0.000
CsA±SLM	0.056±0.001^{a}	0.248±0.002^{a}	0.028±0.001^{a}	0.121±0.002	0.854±0.022^{a}	0.087±0.001^{a}

All value represent a mean±SD (n=5); $^{a}P<0.05$ versus TC; $^{b}P<0.05$ versus NC; $^{c}P\leq0.05$ versus SLM. TC=Toxic control, NC=Normal control, SLM=Silymarin, GSH=Glutathione, CAT=Catalase, MDA=Malondialdehyde, GPx=Glutathione peroxidase, GST=Glutathione-s-transferase, SOD=Superoxide dismutase, CsA=Cyclosporine A, *C. afer=Costus afer*, SD=Standard deviation

The restoration potential of the aqueous leaf extracts of *C. afer* on the antioxidant parameters parallel that seen with the standard drug (SLM).

CsA induced hepatotoxicity induced a significant ($P < 0.05$) increase in the relative and absolute weight of the liver. Treatment with the extract exhibit a significant ($P < 0.05$) decrease in the absolute and relative weight of the liver compared with the TC, also the decreased in both the absolute and relative weight of the liver exhibited by the extract parallel that of the SLM, this was shown in Table 3. The effect of the *C. afer* leaf extract on the feed and fluid intake was also studied on the rats. It was observed that the NC group (Group 1) had the highest feed and fluid intake, while there was a significant ($P < 0.05$) reduction in both feed and food intake in toxic group (Table 3).

In CsA induced nephrotoxicity, the NC group (Group 1) showed 12.7% and 22.5% increase in body weight on the 5th and 10th day, respectively. Also, the body weight of the TC group showed about 7.1% increase on the 5th day then drastically reduced increment of 0.89% on the 10th day. However, the remaining groups (Groups 3–6) showed marked increase on the 5th and moderate increase on the 10th day, respectively, with $P < 0.05$ as shown in Figure 1.

Histopathological examination on the liver showed marked inflammation, cytoplasmic vacuolation, and necrotic hepatocytes on the TC group indicating severe toxicity which was restored dose dependently in extract treated group, as well as the standard drug SLM as seen in Figure 2.

Table 3. Effect of aqueous leaf extract of *Costus afer* on MBW, ALW, RLW, feed and fluid intake of CsA induced hepatotoxic rats

Group	MBW (g)	ALW (g)	RLW	Feed (g)	Fluid (ml)
NC	92.80±4.025	3.00±0.00	3.29	71.80±9.46	182.80±6.88
CsA alone (TC)	134.80±3.98	5.00±0.53	4.08	64.74±13.24^{a}	152.80±34.65^{a}
CsA±375 mg/kg *C. afer*	125.00±6.33	4.12±0.03	3.29	68.00±9.97^{bc}	170.00±9.72^{bc}
CsA±750 mg/kg *C. afer*	121.80±5.81	3.96±0.08	3.25	60.70±14.18^{c}	158.50±22.74
CsA±1125 mg/kg *C. afer*	111.00±10.77	3.50±0.76	3.15	68.15±5.518^{bc}	178.20±7.24^{b}
CsA±SLM	162.20±8.07	4.30±0.30	2.65	54.30±16.13	161.50±24.90^{b}

All value represent a mean±SD (n=5); $^{a}P<0.05$ versus TC; $^{b}P<0.05$ versus TC; $^{c}P\leq0.05$ versus SLM. MBW=Mean body weight, ALW=Absolute liver weight, RLW=Relative liver weight, TC=Toxic control, NC=Normal control, SLM=Silymarin, CsA=Cyclosporine A, *C. afer=Costus afer*, SD=Standard deviation

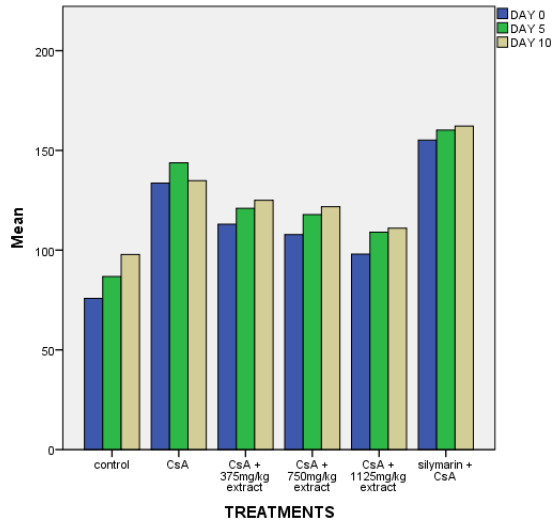


Figure 1. Average body weight of cyclosporine induced hepatotoxic rats treated with the extract.

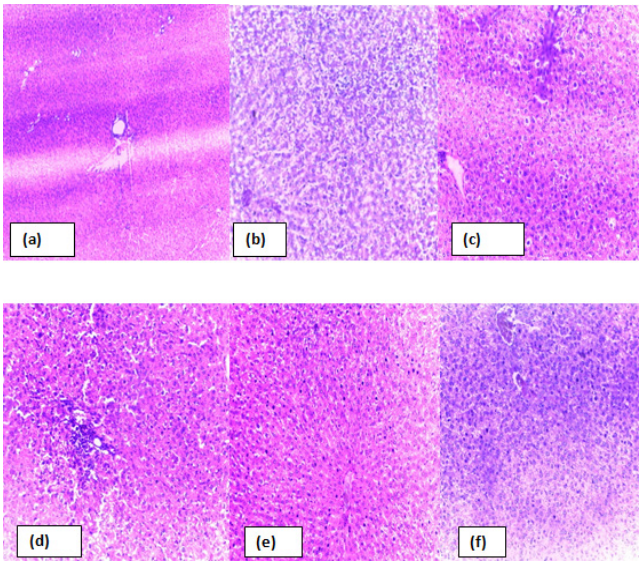


Figure 2. Photomicrograph of the liver of cyclosporine A induced hepatotoxic rats treated with the extract. (a) Normal control. (b) Toxic control. (c) Cyclosporine A ± 25% LD_{50} *Costus afer*. (d) Cyclosporine A ± 50% LD_{50} *Costus afer*. (e) cyclosporine A ± 75% LD_{50} *Costus afer*. (f) Cyclosporine A ± silymarin.

5. Discussion

The liver is the largest and one of the most vital organs in the body which is found to be more susceptible to disease and infection. Drug-induced injury is a potential complication of several medicines. Liver injury induced by various hepatotoxins has been recognized as a major toxicological problem for years (Akanitapichat *et al.*, 2010). Due to lack of reliable liver protection drugs in modern medicine, a large number of medicinal preparations are recommended for the treatment of liver disorders (Rahate and Rajasekaran, 2015). Hence, it is essential to unfold the scientific basis of the antihepatotoxic nature of the traditionally useful herbal drugs. It is reported that hepatoprotective and antioxidant activity by scavenging free radicals are probably due to phenolic/flavonoid compounds present in the plant (Okwu, 2005; Uruquiaga and Leighton, 2000).

The major functions of the liver are carbohydrate, protein and fat metabolism, detoxification, secretion of bile, and storage of vitamins. Thus, maintaining a healthy liver is a crucial factor for the overall health and well-being, but it is continuously and variedly exposed to environmental toxins and abused by poor drug habits, alcohol, and prescribed over-the-counter drugs which can eventually lead to various liver ailments such as hepatitis, cirrhosis, and alcoholic liver disease (subramonium).

In Nigeria, many indigenous plants are used as spices, food or medicine; these plants often exhibit a wide range of biological and pharmacological activities. Extracts from the roots, barks, seed, and fruits of these plants are used in the preparation of syrups in traditional medicine as cough suppressant and in the treatment of oxidative related diseases (Akpan *et al.,* 2012). Generally, the active constituents contributing to the protective effects are phytochemical constituents, vitamins, and minerals (Akpan *et al.,* 2012). Phytochemicals exhibit a wide range of biological effects resulting in their protective or disease preventive properties. There possible actions include; antioxidant, hormonal action, stimulation of enzymes, and interference with DNA replication, antimicrobial effect, and physical actions (Ukana *et al.,* 2012; Momoh *et al.,* 2011).

Following the recent studies carried out on the genus *Costus,* it was revealed that *C. afer* Ker Gawl contains potent biological active compounds. Ukana *et al.*, (2004) evaluated the phytochemical, proximate, and mineral element composition of stem of *C. afer* (Bush cane). Evaluation of the phytochemical composition and hypoglycaemic activity of methanolic leaf extract of *C. afer* by Momoh *et al.*, (2011) showed the presence of alkaloids, saponins, flavonoids, tannins, phenols, cardiac glycosides, anthraquinone, and terpenoids. These phytochemicals present in the leaves account for the various biological activities of *C. afer*, thus, its anti-inflammatory, antifungal, and antimicrobial effects could

be due to its alkaloid content (Ayansor *et al.,* 2010), as well as its antihypertensive activity (Sinclair *et al.,* 1991). Flavonoids, saponins, and phenols are potent water soluble antioxidants which prevent oxidizing cell damage, thus, also suggesting anti-inflammatory properties. The therapeutic potential of antioxidants in controlling degenerative diseases with marked oxidative damage from reactive oxygen species or free radicals have been reported (Fridovich, 1975). Acute toxicity testing carried out on the aqueous extract of *C. afer* leaf indicated that the LD_{50} is more than 2000 mg/kg (Ezejiofor *et al.,* 2013). This implies that the aqueous extract of *C. afer* leaf has a higher safety margin; however, in this study the LD_{50} of 1500 mg/kg was used.

The moderate increase observed on the 10th day might be due to the protective ability of the plant on the liver. The effect of the *C. afer* leaf extract on the feed and fluid intake studied on the rats showed that the NC group (Group 1) had the highest feed and fluid intake, while there was a significant reduction in both feed and food intake on the CsA alone treated group (Group 2). The feed and fluid intake gradually increased in Group 3 (375 mg/kg *C. afer* ± CsA) and Group 5 (1125 mg/kg *C. afer* ± CsA). It could be assumed that the gradual increase in feed and fluid intake is as a result of induction of appetite of the extract on the rats.

The liver being the major organ of metabolism of drugs, when examined, it was observed that CsA induced a significant percentage increase in the liver weight ($P < 0.05$) in relation to the mean body weight of the rat. Also, when compared with the control and the treated groups there was significant increase in liver weight $P < 0.05$. The blockage of bile flow and inflammation of hepatocytes by CsA could account for the increase in liver weight. During liver injury, elevation of serum cystolic, mitochondrial, and membrane bound enzymes are highly marked. The commonly present ones are AST, ALT, ALP, and bilirubin (Pagani and Panteghini, 2001; Whitfield, 2001). AST is predominantly found in the mitochondrial of hepatocytes, alanine transaminase is more specific to liver, thus, a better indicator of liver injury (Drotman and Lawhan, 1978). Albumin and TPs are also biomarkers of hepatotoxicity thereby showing marked decrease during liver injury.

GSH is an important endogenous antioxidant system that is found in particular high concentration in the liver and it is known to have key functions in the protective processes. The reduced form of GSH is readily oxidized to oxidized GSH on interaction with free radicals (Gaetani *et al.,* 1989). GSH content decreased in CsA alone treated group compared to the normal group with $P < 0.05$. However, pretreatment with the aqueous leaf extract of *C. afer* elevated the hepatic GSH levels. The increased MDA levels in the liver suggest enhanced LP leading to tissue damage and failure of antioxidant defense mechanism to prevent the excessive free radicals. *C. afer* was found to circumvent oxidative damage by enhancing the levels of antioxidants and producing a decline in the levels of MDA in the *C. afer* treated groups ($P < 0.05$). SOD safeguards cells against deleterious effects of superperoxide anion derived from the peroxidative process in the liver tissues (Brauce *et al.,* 1982). Excessive production of free radicals may result in alteration in the biological activity of cellular macromolecules. Therefore, the reduction in the activity of these enzymes may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide. CAT, GPx, and GST are considered as the most important H_2O_2 removing enzymes and also a key component of oxidative defense system (Gaetani *et al.,* 1989). GPx is a selenium containing enzyme present in significant concentrations, detoxifies H_20_2 to H_20 through the oxidation of reduced GSH (Brauce *et al.,* 1982). In this study, it was observed that, there was a reduction in the levels of CAT, GPx, and SOD in the group treated with CsA alone and gradual increase follows down the other groups treated with *C. afer* leaf extract with a $P < 0.05$ when compared with both the control and SLM treated group. GPx, GST, and SOD levels were also high in the CsA alone (TC) treated group and gradual increase follows down the group with $P < 0.05$ when the groups were compared with the CsA alone treated group. The increase in this liver antioxidant could account for the hepatoprotective activity of *C. afer* in the rat.

Histopathological examination on the liver showed marked inflammation, cytoplamic vacuolation, and necrotic hepatocytes on the CsA alone (Group 2) treated group indicating severe toxicity which is in agreement with previous work by Arshed *et al*. and Kingstone and Swamamoni (Lahon and Das, 2011; Arshed *et al.,* 2012). The photomicrogragh of the control group (Group 1) showed normal liver histology with the absence of inflammation or distortion of tissue, the central vein, hepatocytes, and portal tracts were clearly seen. The *C. afer* treated groups showed mild restoration of the liver hepatocytes and reduced inflammation. However, treatment with the *C. afer* aqueous leaf extract could

not completely reverse the hepatic injury caused by the CsA within the period of treatment. There was complete restoration of tissue hepatocytes on the SLM treated group (Group 6).

6. Conclusion

Treatment with aqueous leaf extract of *C. afer* dose dependently prevented the significant changes on the physical, biochemical, and histological changes induced by CsA in the liver. Therefore, aqueous leaf extract of *C. afer* Ker Gawl may be an economic agent to treat liver diseases related to CsA administration and possibly other hepatotoxic drugs.

7. References

1. Lozano-Lanagrán M, Robles M, Lucena MI, Andrade RJ. Hepatotoxicity in 2011- Advancing resolutely. Rev Esp Enferm Dig. 2011; 103:472–9.
2. Lee WM. Drug-induced hepatotoxicity. N Engl J Med. 2003; 349:474–85.
3. Farell GC. Drug induced hepatic injury. J Gastroenterol Hepatol. 1997; 22:455–8.
4. Pagani F, Panteghini M. 5'-Nucleotidase in the detection of increased activity of the liver form of alkaline phosphatase in serum. Clin Chem. 2001; 47:2046–8.
5. Whitfield JB. Gamma glutamyl transferase. Crit Rev Clin Lab Sci. 2001; 38:263–355.
6. Ostapowicz G, Fontana RJ, Schiødt FV, Larson A, Davern TJ, Han SH, *et al.* Results of a prospective study of acute liver failure at 17 tertiary care centers in the United States. Ann Intern Med. 2002; 137:947–54.
7. Uttara B, Singh AV, Zamboni P, Mahajan RT. Oxidative stress and neurodegenerative diseases: A review of upstream and downstream antioxidant therapeutic options. Curr Neuropharmacol. 2009; 7:65–74.
8. Senthilkumar S, Yogeeta SK, Subashini R, Devaki T. Attenuation of cyclophosphamide induced toxicity by squalene in experimental rats. Chem Biol Interact. 2006; 160:252–60.
9. Oboh G, Rocha JB. Polyphenols in red pepper (*Capsicum annuum* var. aviculare) and their protective effect on some pro-oxidants induced lipid peroxidation in brain and liver. Eur Food Res Technol. 2007; 225:239–47.
10. Halliwell B. Dietary polyphenols: Good, bad, or indifferent for your health? Cardiovasc Res. 2007; 73:341–7.
11. Ramchandani D, Ganeshpurkar A, Bansal D, Karchuli MS, Dubey N. Protective effect of curculigo orchioides extract on cyclophosphamide-induced neurotoxicity in murine model. Toxicol Int. 2014; 21:232–5.
12. Olalye MT, Rocha JB. Commonly used tropical medicinal plants exhibit distinct *in vitro* antioxidant activities against hepatotoxins in rat liver. Exp Toxicol Pathol. 2007; 58:433–8.
13. Iwu MM. Handbook of African Medical Plants. London: CRC Press; 1993. p. 161–2.
14. Udem S, Ezeasor C. The acute and subchronic toxicity studies of aqueous leaf and stem bark extract of *Costus afer* ker (costaceae) in mice. Comp Clin Path. 2010; 6:75–80.
15. Maryam M, Babak H, Mohammad HS, Ali K, Mehran N. Prophylactic effect of Vitamin C on cyclosporine A-induced liver toxicity. Stud J Med Sci. 2012; 1:24–6.
16. 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.
17. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol. 1957; 28:56–63.
18. Jendrassik L, Grof P. Colorimetric method for the determination of serum glutamic pyruvate transaminase. Am J Clin Pathol. 1938; 28:56–63.
19. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979; 95:351–8.
20. Khynriam D, Prasad SB. Changes in endogenous tissue glutathione level in relation to murine ascites tumor growth and the anticancer activity of cisplatin. Braz J Med Biol Res. 2003; 36:53–63.
21. Gornall AG, Bardawill CJ, David MM. Determination of serum proteins by means of the biuret reaction. J Biol Chem. 1949; 177:751–66.
22. Liu J, Simon LM, Phillips JR, Robin ED. Superoxide Dismutase (SOD) activity in hypoxic mammalian systems. J Appl Physiol Respir Environ Exerc Physiol. 1977; 42:107–10.
23. Rekka EA, Kourounakis AP, Kourounakis PN. Investigation of the effect of chamazulene on lipid peroxidation and free radical processes. Res Commun Mol Pathol Pharmacol. 1996; 92:361–4.
24. Akanitapichat P, Phraibung K, Nuchklang K, Prompitakkul S. Antioxidant and hepatoprotective activities of five eggplant varieties. Food Chem Toxicol. 2010; 48:3017–21.
25. Rahate KP, Rajasekaran A. Hepatoprotection by active fractions from *Desmostachya bipinnata* stapf (L.) against tamoxifen-induced hepatotoxicity. Indian J Pharmacol. 2015; 47:311–5.
26. Okwu DE. Photochemical vitamins and mineral constituents of ten Nigeria medicinal plant. Int J Mol Adv Sci. 2005; 1:375–81.
27. Uruquiaga I, Leighton F. Plant polyphenol antioxidant and oxidative stress. Biol Res. 2000; 33:159–65.
28. Akpan MM, Odeomena CS, Nwachukwu CN, Danlandi B. Antimicrobial assessment of methanolic extract of *Costus afer* leaves. Asian J Plant Sci Res. 2012; 2:335–41.
29. Ukana DA, Uwemedimo EU, Aniekan EA. Evaluation of phytochemical, proximate and mineral element composition of stem of *Costus afer*. Asian J Plant Sci. 2012; 2:607–12.

30. Momoh S, Yusuf OW, Adamu MM, Agwu CO, Atanu FO. Evaluation of the phytochemical composition and hypoglyceamic activity of methanolic leaves extract of *Costus afer* in albino rats. Br J Pharm Res. 2011; 1:1–8.
31. Okwu DE. Phytochemical and vitamins content of indigenous spices of South Eastern Nigeria. J Sustain Agric Environ. 2004; 6:140–7.
32. Sofowora A. Medicinal plants and traditional medicine in Africa. Rep. edition. Ibadan: Spectrum Books Ltd; 2006. p. 150.
33. Ayansor GN, Ogunwenmo KO, Olatunji AO, Blessing EA. Phytochemical, proximate and mineral element composition of *Costus afer* stem. Asian J Plant Sci Res. 2010; 5:607–12.
34. Sinclair AJ, Barnett AH, Lunie J. Free radicals and auto-oxidant systems in health and disease. J Appl Med. 1991;17:409.
35. Fridovich I. Superoxide dismutases. Annu Rev Biochem. 1975; 44:147–59.
36. Gaetani GF, Galiano S, Canepa L, Ferraris AM, Kirkman HN. Catalase and glutathione peroxidase are equally active in detoxification of hydrogen peroxide in human erythrocytes. Blood. 1989; 73:334–9.
37. Brauce A, Freeman D, James C. Biology of disease free radicals and tissue injury. Can J Physiol Pharmacol. 1982; 47:412–26.
38. Lahon K, Das S. Hepatoprotective activity of *Ocimum sanctum* alcoholic leaf extract against paracetamol-induced liver damage in Albino rats. Pharmacognosy Res. 2011; 3:13–8.
39. Arshed ID, Ramesh CS, Suresh KB. Hepatoprotective: A Hallmark of *Cistrullus colocynthus L.* against paracetamol induced hepatotoxicity in Swiss Albino Rats 2012. Am J of Plant Sc. 2012; 3:1022–7.

Source of Support: Nil. **Conflicts of Interest:** None declared.