SJIF Impact Factor: 5.464

WORLD JOURNAL OF ADVANCE HEALTHCARE RESEARCH

ISSN: 2457-0400
Volume: 7.
Issue: 12
Page N. 31-37
Year: 2023

Original Article www.wjahr.com

COSTUS AFER KER GAWL RHIZOME ETHYL ACETATE FRACTION MITIGATED DICLOFENAC-INDUCED RENAL TOXICITY IN MALE WISTAR RATS BY SUPPRESSING OXIDATIVE STRESS

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Received date: 26 August 2023 **Revised date:** 17 September 2023 **Accepted date:** 07 November 2023

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ABSTRACT

This study aims to evaluate the preventive effect of *Costus afer ker* Gawl rhizome ethyl acetate fraction (CAREAF) on diclofenac-induced renal toxicity. *In vitro* antioxidant activity was evaluated using TAC and DPPH assays. *In vivo* study, thirty-six (36) male Wistar rats were randomly divided into six groups (n=6), namely normal, induced not treated, reference drug quercetin (10 mg/kg), extract treated groups (100, 300, and 500 mg/kg *b.w.*) for 14 days. The animals were induced using diclofenac sodium (200 mg/kg *b.w.*) after treatment and sacrificed 24 hours after induction. The result showed that C. *afer* rhizome is high in antioxidant capacity from DPPH and TAC assay activity as compared to that of standard (gallic acid) and *in vivo* antioxidant activity also increased in SOD and CAT with reduced MDA. The renal function test showed decreased levels of urea and creatinine in the groups treated with 100, 300, and 500 mg/kg *b.w.* in a dose-dependent manner. These findings suggest that the ethyl acetate fraction of *Costus afer* rhizome may have therapeutic potential in mitigating renal damage caused by diclofenac sodium.

KEYWORDS: *Costus afer*, diclofenac sodium, antioxidant, Renal damage.

INTRODUCTION

The optimization of renal functionality is of utmost importance for the survival of the human body, considering the crucial role the kidney plays in maintaining homeostasis.[1,2] The renal compartments are typically responsible for the execution of essential metabolic activities, including but not limited to acid-base regulation, hormone synthesis, and excretion of toxic waste.[3] Consequently, alterations in these physiological processes may serve as indicators of impaired renal function, which could ultimately result in negative health consequences.[4] Based on contemporary assessments, it is postulated that chronic kidney disorders (CKD) have a prevalence rate of roughly 13.3% among the worldwide populace.[5] A considerable segment of the populace, comprising more than four million individuals, requires renal replacement therapy due to the advanced stage of kidney disease known as end-stage kidney disease (ESKD).[6] Chronic non-communicable conditions, namely diabetes mellitus and hypertension, possess the capacity to advance into the state of chronic kidney disease (CKD).[7] Furthermore, the development of chronic kidney disease (CKD) might be influenced by the nephrotoxic effects of specific conventional drugs.[8] Natural products have been explored as a non-pharmacological approach for managing chronic ailments.[9] Diclofenac sodium is a nonsteroidal anti-inflammatory drug (NSAID) commonly used to treat pain and inflammation.[10] However, long-term use of diclofenac sodium has been associated with various adverse side effects, including chronic kidney damage.[11] The formation of reactive oxygen radicals has been observed as the primary mechanism by which diclofenac induces unfavorable drug reactions, as established in previous studies.[12] *Costus afer ker* Gawl is an indigenous plant, mostly called a ginger lily, spiral ginger, or bush cane.[13] The plant has been reported to modulate various pathological pathways in multiple organs.[14] Studies have investigated

that different parts of *C. afer* plant possess various therapeutic effects. *C. afer* rhizome extracts, however, more research is needed on the rhizome of the plant.

MATERIALS AND METHODS

Costus afer Ker Gawl rhizome were locally obtained from farms in Irolu, Ikenne LGA, Ogun State, South-Western Nigeria, Diclofenac sodium was purchased from Hovid Pharmaceuticals, Nigeria. Trichloroacetic acid (TCA), and glutathione were purchased from Sigma chemical Co. (St. Louis, MO, USA). All other chemicals and reagents used were of analytical grade.

In vitro antioxidant assays

1, 1-diphenyl-1-2 picryl-hydrazyl (DPPH) activity

DPPH radical scavenging activity of the fractions was compared by adopting the procedure reported by McCune and Johns.[15] The reaction mixture (3.0 mL) consists of 1.0 mL of DPPH in methanol (0.3 mM), 1.0 mL of the *costus afer* rhizome ethyl acetate fraction, and 1.0 mL of methanol. This were then be incubated for 10 minutes in the dark after which the absorbance was measured at 517 nm. The percentage antioxidant potential was calculated using:

$$\%\ Antioxidant\ Potential = \frac{(OD\ control - OD\ sample)}{(OD\ control)} \times 100$$

The IC50 values were calculated for the samples which denote the concentration of fractions required to scavenge 50% of DPPH radicals.

Total antioxidant capacity (TAC)

The total antioxidant activity of the ethyl acetate fraction of *Costus afer* rhizome was determined using the phosphomolybdenum (PM) test developed by.[16] This test is based on the reduction of phosphate-Mo (VI) to phosphate (Mo) (V) by the sample, resulting in the formation of a bluish-green phosphate/Mo (V) complex at acidic pH. In this study, test tubes containing 3 ml of distilled water and 1 ml of the Molybdate reagent solution were prepared in various concentrations (ranging from 12.5 to 200 mg/ml) of the extract. The tubes were then incubated at 95 °C for 90 minutes. After incubation, the tubes were cooled to room temperature for 20-30 minutes, and the absorbance of the reaction mixture was measured at 695 nm. The absorbance of each concentration was compared to the equivalence of the ascorbic acid standard to determine the total antioxidant activity.

Animal model

A total of thirty-six (36) male Wister rats, weighing between 130-150g were obtained from Babcock University animal facility. The rats were housed in plastic cages and acclimatized for one week in a 12-hour light/12-hour dark cycle, fed with commercial feed and water *ad libithum*. Animals were randomly divided into six (6) groups with six (n = 6) animals each.

Administration of the ethyl acetate fraction of *C. afer* rhizome was done orally at different doses

Group 1: (control): Normal
Group 2: Induced, not treated
Group 3: Treated with 10 mg/kg *b.w.* Quercetin
Group 4, 5 and 6: Treated with 100, 300 and 500 mg/kg *b.w* of *C. afer* rhizome ethyl acetate fraction. The animals were treated with *C. afer* extract for 14 days before the induction of kidney damage using 200mg/kg *b.w* diclofenac sodium on the 15th day and the animals were sacrificed 24 hours after.

Animal sacrifice and Sample collection

The animals were sacrificed 24 hours after the diclofenac sodium dose, blood was collected from retro-orbital venous plexus using lithium heparin bottles, before cervical dislocation to excise the two kidneys. Blood samples were centrifuged at 3000rpm for 10 mins to obtain plasma subsequently used for kidney function tests.

Sample Preparation

The kidneys of the rats were homogenized in 50 mM Tris-HCL buffer (pH 7.4) with 1.15% potassium chloride. The homogenate was centrifuged at 12,000g per 15mins at 4℃, then the supernatant was used for biochemical assays.

Kidney function test

The plasma collected was used for the kidney function test. Urea concentration was determined using Randox laboratories assay kit.[17] Whereas, creatinine concentration was determined using alkaline picrate method.

Measurement of Superoxide Dismutase (SOD)

The SOD activity was measured according to the protocol of Assady et al.[18] The enzymatic activity was evaluated by measuring the enzyme's capacity to inhibit the photochemical reduction of nitro-blue tetrazolium (NBT).

Determination of Catalase (CAT) enzyme activity

Catalase (CAT) enzyme activity was measured by.[19] Briefly, the reaction mixture contained 0.5 mL of the tissue homogenate and 2 mL of 0.1 M sodium phosphate buffer (pH 6.8) and the reaction began with the addition of 0.5 mL of 10mM hydrogen peroxide. The absorbance was measured at 240 nm and the decrease in absorbance was recorded every 15 seconds, for 3 minutes.
Catalase activity was expressed as U/ min/ mg of protein.

Determination of lipid peroxidation

The kidney homogenate was precipitated with 0.1 mL of 25% trichloroacetic acid, and centrifuged at 4000g, the homogenate was removed and used for lipid peroxidation assay by measuring the level of malondialdehyde (MDA) using the TBA reaction by.[17]

Statistical analysis

The statistical tool used was GraphPad Prism® 7.0. Data comparison was done using one-way analysis of variance (ANOVA) followed by *Tukey's post hoc* test. The data were expressed as mean ± SEM (n=6). Value of $p<0.05$ was considered statistically significant.

RESULTS

In Vitro Antioxidant Assay

Figure 1 illustrates the antioxidant activity of CAREAF. In comparison to ascorbic acid, which exhibits an inhibition rate of 84% on DPPH activity, CAREAF has a notably greater inhibition rate of 80%. Figure 2 illustrates the noteworthy TAC of 1035.69 GAE/mg achieved by CAREAF.

Effects of CAREAF on Protein Concentration

Figure 3 illustrates a significant reduction in protein concentration in the untreated group. Nevertheless, both CAREAF and the standard medication demonstrated effective protein concentration maintenance at values close to normal throughout all fraction doses. This finding suggests that CAREAF exerts a beneficial influence on the maintenance of protein homeostasis.

Effects of CAREAF on Antioxidant Enzymes

The results depicted in Figures 4 and 5 demonstrate the efficacy of CAREAF in mitigating renal damage caused by diclofenac sodium. Furthermore, CAREAF was found to restore antioxidant activity in a dosage-dependent manner. The group that received treatment demonstrated a statistically significant elevation in the activities of superoxide dismutase (SOD) and catalase (CAT), when compared to the group that did not receive any treatment. The observed augmentation in enzymatic activity played a significant role in the restoration of renal function to a state closely resembling normalcy. A post-hoc analysis was conducted using the Tukey test, which indicated statistically significant ($p<0.05$) elevations in the activities of superoxide dismutase (SOD) and catalase (CAT) in the group that received CAREAF treatment. The observed increases in the quercetin treatment group and the normal group were found to be similar, suggesting significant reno-protective benefits.

Effects of CAREAF on Lipid Peroxidation

Figure 6 illustrates a statistically significant decrease in the concentration of MDA among the group that received treatment with CAREAF, as indicated by a p-value of less than 0.05. The levels of MDA in this group exhibited a tendency towards normalcy when compared to the untreated group. The decrease in malondialdehyde (MDA) content detected suggests a decline in oxidative damage inside the renal tissues of rats subjected to CAREAF treatment.

Effects of CAREAF on Urea and Creatinine Concentrations

The assessment of the renal functional state involved the measurement of plasma levels of urea and creatinine, as illustrated in Figures 7 and 8. The findings suggest that the administration of the extract and quercetin has a positive impact on rats that have been experimentally induced with renal injury. The ethyl acetate fraction derived from the rhizome of *C afer*, in combination with quercetin, demonstrated a significant decrease ($p<0.05$) in the levels of urea and creatinine when compared to untreated rats. This resulted in a restoration of these levels to a state closely resembling normal values.

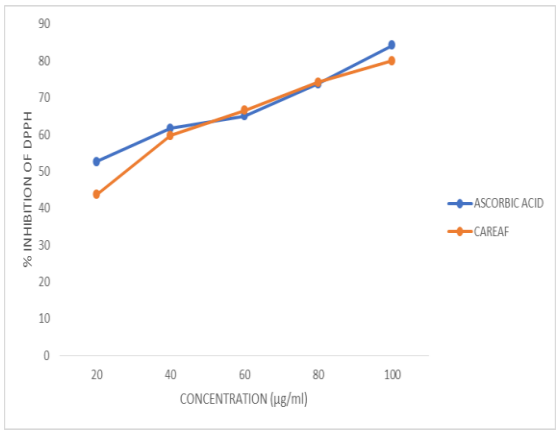

Figure 1: Percentage inhibition of 1'1'-diphenyl-2-picrylhydrazyl radical by ethyl acetate fraction of *C. afer* rhizome and ascorbic acid (standard).

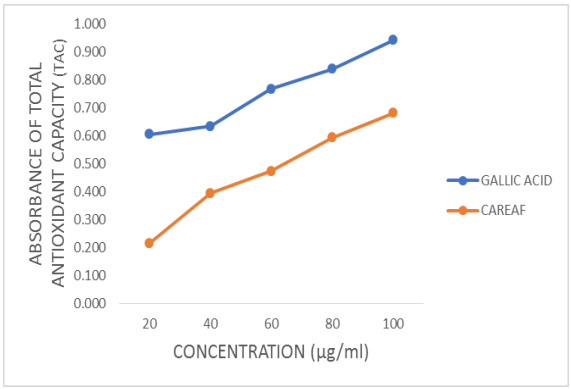

Figure 2: Total antioxidant capacities of ethyl acetate fraction of *C. afer* rhizome and gallic acid at different concentrations.

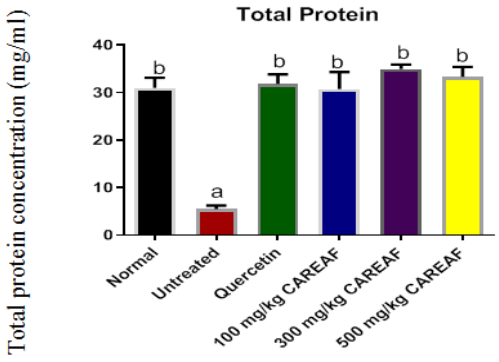

Figure 3: The total protein concentration in the kidneys of the diclofenac-induced rats treated with *C.*

afer **rhizome ethyl acetate fraction. CAREAF: *C. afer* rhizome ethyl acetate fraction. Each bar represents the mean ± SEM of 6 rats per group. a: p<0.05 against normal, b: p<0.05 against the untreated group.**

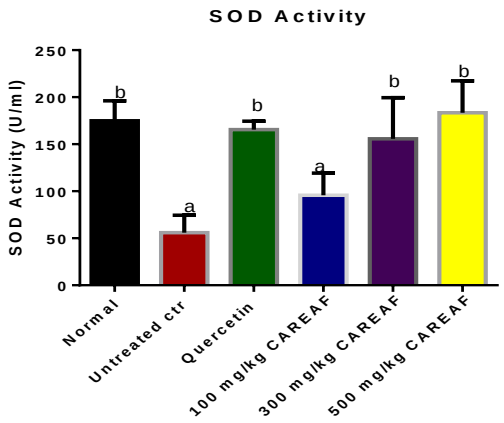

Figure 4: Effect of *C. afer* rhizome ethyl acetate fraction on the SOD activity in kidney of diclofenac sodium-induced rats. CAREAF: *C. afer* rhizome ethyl acetate fraction. Each bar represents mean ± SEM of 6 rats per group. a: p<0.05 against normal, b: p<0.05 against the untreated group.

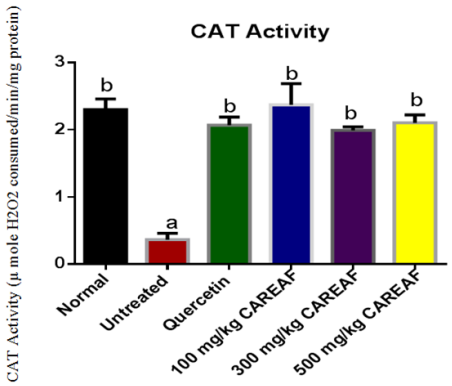

Figure 5: The effect of C. afer rhizome ethyl acetate fraction on the catalase activity in kidneys of diclofenac-induced rats. CAREAF: *C. afer* rhizome ethyl acetate fraction. Each bar represents mean ± SEM of 6 rats per group. a: p<0.05 against normal, b: p<0.05 against the untreated group.

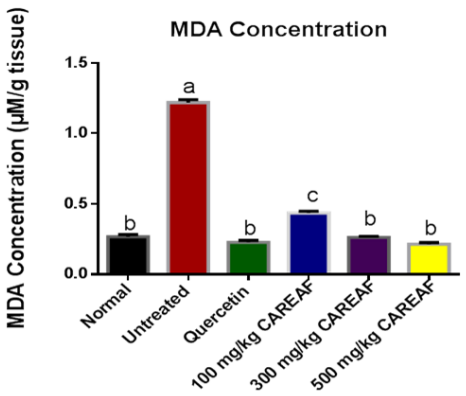

Figure 6: The effect of C. afer rhizome ethyl acetate fraction on the MDA level in kidneys of diclofenac-induced rats. CAREAF: *C. afer* rhizome ethyl acetate fraction. Each bar represents mean ± SEM of 6 rats per group. a: p<0.05 against normal, b: p<0.05 against the untreated group, c: p<0.05 against both the normal and untreated groups.

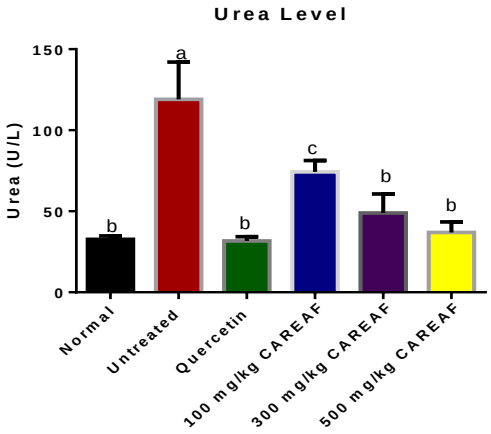

Figure 7: Effect *C.afer* rhizome ethylacetate fraction on the urea concentration in diclofenac sodium-induced rats. Each bar represents mean ± SEM of 6 rats per group. a: p<0.05 against normal, b: p<0.05 against the untreated group, c: p<0.05 against both the normal and untreated groups.

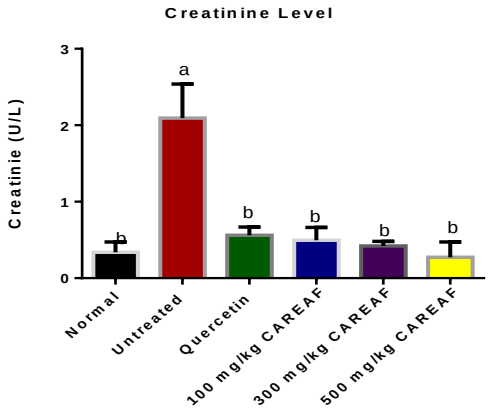

Figure 8: Effect *C.afer* rhizome ethylacetate fraction on the creatinine concentration in diclofenac sodium-

induced renal damage. Each bar represents mean ± SEM of 6 rats per group. a: p<0.05 against normal, b: p<0.05 against the untreated group.

DISCUSSION

One aspect of clinical interest is around the observation that the utilization of nonsteroidal anti-inflammatory medicines (NSAIDs) as a treatment regimen has been linked to significant alterations in various biochemical markers that serve as indicators of distinct organ dysfunction.[20] Regrettably, this possesses the capacity to heighten the probability of life-endangering and irreversible harm to crucial bodily organs, notably the kidney. Diclofenac has been documented to elicit adverse drug reactions predominantly via stimulating the generation of reactive oxygen radicals.[21] In recent times, increasing empirical information have demonstrated that indigenous medicinal plants are remarkable repositories of plant based secondary metabolites which are very potent in modulating various pathological cascades.[17] Importantly, the research findings presented in this study provide valuable insights into the potential therapeutic benefits of the ethyl acetate fraction of *Costus afer* rhizome (CAREAF). Specifically, the study highlights its antioxidant properties and its ability to protect the kidneys in a rat model of renal injury induced by diclofenac.

Interestingly, CAREAF exhibited significant antioxidant activity, as evidenced by the considerable percentage suppression of DPPH activity. The level of inhibition observed with this compound was only marginally less than that observed with the reference compound, ascorbic acid. In addition, it is worth noting that CAREAF exhibits a significant total antioxidant capacity (TAC) value, which highlights its robust antioxidant capabilities. The findings of this study align with the longstanding use of *Costus afer* in traditional herbal medicine and offer substantiation for its effectiveness in treating conditions related to oxidative stress.[22]

A notable discovery in this study was the significant reduction in protein concentration observed in the untreated group, indicating the presence of renal impairment attributed to diclofenac. On the other hand, the administration of CAREAF, along with the standard medication, successfully preserved protein concentrations at levels that closely resembled the normal range across multiple doses of the fraction. The observed impact suggests that CAREAF plays a substantial role in preserving protein homeostasis, a crucial component of maintaining optimal cellular function.

The observed impact of the CAREAF intervention on renal function demonstrated notable protective advantages. In the experimental model of renal damage induced by diclofenac, the ethyl acetate fraction demonstrated a dual effect by attenuating renal damage and restoring the activity of two important antioxidant enzymes, specifically superoxide dismutase (SOD) and catalase (CAT), in a dosage-dependent manner. The observed increases in superoxide dismutase (SOD) and catalase (CAT) activity exhibited a substantial renoprotective effect. A post-hoc comparison was performed using the Tukey test to evaluate the impact of CAREAF on enzymatic activities. The findings suggest that the impact of CAREAF was similar to that of the reference drug quercetin and approached the levels observed in the control group. Similarly,[23] reported that an aqueous extract of *C. afer* leaf improved SOD and catalase activities in a rat model of lead-induced nephrotoxicity. The collective results indicate that CAREAF has the potential to prevent kidney damage by improving antioxidant defence.

Furthermore, the study findings indicate that the administration of CAREAF led to a significant reduction in the concentration of malondialdehyde (MDA) in the renal tissues of the treated rats, as compared to the untreated group. The measurement of malondialdehyde (MDA) serves as a reliable indicator for assessing both lipid peroxidation and oxidative stress levels. The observed decrease in MDA concentration suggests that the administration of CAREAF effectively mitigated oxidative damage and lipid peroxidation in the renal tissues. This observation is in tandem with those of previous investigators. Comparatively,[24] reported that hydroethanolic extract of *C. afer* significantly normalizes renal antioxidant status in a rat model of Streptozotocin induced toxicity. This discovery offers additional evidence to substantiate the protective role of CAREAF in mitigating renal damage induced by oxidative stress.

Meanwhile, data obtained from figures 7 and 8 offer further insight into the preventive effects of the ethyl acetate fraction of *Costus afer* rhizome (CAREAF) on diclofenac-induced renal injury. The study findings suggest that CAREAF consistently exhibits reno-protective properties, as evidenced by improvements in urea and creatinine levels. Both the CAREAF-treated group and the quercetin-treated group exhibited a significant decrease in urea and creatinine levels compared to the untreated group of rats. The observed decrease indicates a positive trend in the recovery of these biomarkers towards their initial levels. These findings are consistent with earlier observations that depicted the positive impact of CAREAF on antioxidant parameters. Distinctively, this suggests that CAREAF can effectively reduce oxidative stress and offer kidney protection. Additionally, this study presents evidence supporting the potential effectiveness of the intervention in restoring renal function, as demonstrated by improvements in urea and creatinine levels. These findings contribute significantly to our understanding of the potential therapeutic advantages associated with CAREAF. Intriguingly, this outcome showed that natural products from plants possess remarkable bioactive mechanisms for alleviating organ dysfunctions.[17] The decrease in urea and creatinine levels suggests an

improvement in glomerular filtration and a reduction in renal dysfunction.[25] The aforementioned factor plays a crucial role in maintaining renal health. CAREAF's efficient regulation of these parameters, resulting in optimal levels, highlights its potential as a reno-protective medication.

CONCLUSION

The results suggest that the ethyl acetate fraction derived from the rhizome of *Costus afer* (CAREAF) demonstrates significant promise as an antioxidant and reno-protective drug in a model of diclofenac-induced kidney injury. The findings indicate that CAREAF exhibits potential as a therapeutic intervention for the prevention and alleviation of renal damage caused by oxidative stress, as well as the maintenance of renal function. Additional research is necessary to explore the specific mechanisms that underlie the antioxidant and reno-protective properties of CAREAF. Furthermore, further investigation is warranted to evaluate the potential clinical applicability of this intervention in the management of renal diseases associated with oxidative stress.

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