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Research Article

Unraveling the Preventive and Therapeutic Roles of Costus Afer in Oxidative Stress Management in Acetaminophen-poisoned Rats

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Abstract: The goal of this study was to evaluate the prophylatic and therapeutic role of *Costus afer* in managing oxidative stress markers in acetaminophen poisoning in rats. The rats were similarly isolated into two groups; pre-treatment groug and post-treatment group with each group having 5 sub-groups (negative control (NC), positive control (PC), A, B and C) of 5 rats each. NC had not received any treatment; PC was treated with 800mg/kg of acetaminophen; A was treated with 200mg/kg of *Costus afer*; B was treated with 400mg/kg of *Costus afer*; C was treated with 800mg/kg of *Costus afer*. The PC was administered distilled water for 28 days before being induced with acetaminophen in the pre-treatment group, and "A," "B," and "C" were treated with *Costus afer for 28 days* before being induced with acetaminophen in the pre-treatment group. In the post-treatment group, the PC was induced with acetaminophen before commencing on 28 days administration of distilled water, and "A," "B," and C" were poisoned with acetaminophen before being treated with *Costus afer*. After the treatment period, the rats were sacrificed and blood samples were collected for oxidative stress marker assessment. The results showed that while there was no significant difference in oxidative stress markers levels between NC and the test groups (A, B and C) in the pre-treatment group, there was a significant improvement in superoxide dismutase (SOD) in subgroup A and B. In the post-treatment group, there was no significant difference in the level of total antioxidant capacity (TAC) between NC and test groups (A, B and C), and there were improvements in SOD level and malondialdehide (MDA) level. This study has shown that Costus afer prevents and therapeutically manages oxidative stress markers in acetaminophen poisoning in rats.

Keywords: acetaminophen, Costus afer, oxidative stress, prevention, rat, treatment

Introduction

The application of plant extracts and micronutrients like vitamins and minerals in the treatment of different disease conditions in our general public today is on the rise [1-3]. Many people prefer these herbs and extracts to conventional medicine. These alternative treatments are used to treat a wide range of diseases,

including organ damage, because they have been found to be effective [3]. *Costus afer* (*C. afer*) is one of 150 types of strong, lasting and rhizomatous herb of the genus Costus and family Costaceae. It is found in Senegal, South Africa, Guinea, Niger, Sierra Leone, and Nigeria's forest belt. C. also known as Bush-cane or Ginger Lily, "Irekeomode" in Yoruba, "opete" in Igbo, and "ting" in Khana. The plant bears white and yellow blossoms. The stem, seeds and rhizomes are collected from the wild plant and they contain a few bioactive metabolites [4]. This plant contains a few phytochemical constituents as well as nutrients and minerals, which add to its restorative impacts. The Costus plant's major phytochemical components have a wide range of biological effects that contribute to their disease prevention and protection properties. The plant shows the accompanying activities: antioxidation, hormonal action, and promotion of enzyme function, interference with DNA replication, anti-microbial activity [4]. In traditional medicine, extracts from these plants' roots, barks, seeds, and fruits are used to make syrups that are used to treat oxidative-related diseases and cough suppressants [5].

Oxidative stress has been known to be a major contributor to organ failure and development of many chronic diseases today [6,7]. Many studies of *C afer* have focused on the role of this plant extract in managing disease conditions like the liver but few have considered its role in addressing the root cause of the disease [3], that is, oxidative stress. That is why this study, is focused on assessing the preventive and treatment roles of *C afer* in acetaminophen poisoning in rats. This will provide valuable insight on the anti-dotal therapeutic role of the plant extract in the event of acetaminophen poisoning and the effectiveness of the plant extract to prevent oxidative damage or increased in oxidative stress markers in acetaminophen poisoning.

Materials and Methods

Experimental design

A total of 50 rats were used in the study. The rats were divided equally between two groups; pre-treatment group and post-treatment group. This study involved two significant interventions: poisoning with acetaminophen and *Costus afer* treatment. In the pre-treatment group, *Costus afer* was administered for 28 days before the acetaminophen poisoning. This represented the preventive assessment of *Costus afer* in avoiding acetaminophen toxicity. In the post-treatment group, acetaminophen toxicity was first inflicted before *Costus afer* treatment was administered for 28 days. This represented the therapeutic role of Costus afer in treating acetaminphen toxicity when rats are already exposed to the toxicant. Blood samples were collected after the study period and laboratory analysis was performed to assess oxidative marker levels.

Animal grouping

The animal was divided into two mean treatment phases 1 and 2

Group 1 pre-treatment phase was further subdivided into 5 groups of 5 rats each

- NC rats that were given distilled water alone (negative control)
- PC rats that were given distilled water for 28 days before induction with 800mg/kg acetaminophen (positive control)
- A1 rats that were given 200mg/kg b/w of *Costus afer* stem extract for 28 days before induction
- B1 rats that were given 400mg/kg b/w of *Costus afer* stem extract for 28days before induction
- C1 rats that were given 800mg/kg b/w of *Costus afer* stem extract for 28 days before induction

Group 2 post-treatment phase was also subdivided into 5 groups of 5 rats each.

- NC rat that were given distilled water alone (negative control)
- PC rats that were given distilled water for 28 days after induction (positive control)
- A2 rats that were given 200mg/kg b/w of Costus afer stem extract for 28 days after induction
- B2 rats that were given 400mg/kg b/w of Costus afer stem extract for 28 days after induction
- C2 rats that were given 800mg/kg b/w of Costus afer stem extract for 28 days after induction. [3,8].

Animal care and Handling

The rats were obtained from the Animal House of Biology Department of Rivers State University and transported to the site for the experimental study. They were allowed to acclimatize for two weeks before the commencement of the study. After the acclimatization, the study protocol was followed according to the design [1,2].

Preparation of Costus afer extract

Costus afer stems were identified and obtained. The stems were washed and the water was allowed to drain-off in a well-ventilated space for 24 hours. The stems were then cut with a sharp knife into little pieces and afterward crushed with a mechanical blender. 1,000 grams (1000g) of the grounded stem was weighed with a weighing balance, strained, marc pressed, and the fluid was allowed to stand for 12 hours, the fluid was then filtered and the residue packed into syrup using a revolving evaporator at 4000rpm at 40^{o}C [3].

Calculation of Concentration of Extract (mg)

Dosage in mg= (weight of rat in gram)/(1000gram) X mg
For example, 200mg/kg of plant extract and 120g rat = (120g X 200)/ 1000g = 24mg
Therefore, 24mg of the stock solution was measured and dissolved in 1.2ml of normal saline and administered to the rat orally [9].

Blood collection and Laboratory analysis

After the treatment duration (28days), the rats were anesthetized with chloroform, sacrificed, and blood samples were collected by jugular cut, the samples were spun at 4000 rpm and the serum samples were transported it the research center where

they were refrigerated at temperatures between 0-4^{o}C until the time for examination [1-3].
The samples were eventually assayed using ELISA technique for oxidative stress markers such as SOD, TAC and MDA.

Statistical analysis
Descriptive statistics expressed as mean and standard deviation, while ANOVA and Post-hoc analysis were performed to test the null hypotheses, claiming there were no differences in oxidative stress marker levels between the *C afer* treatment groups and acetaminophen induced groups in the pre-treatment and post-treatment phases. The tests were considered significant if the p-value is less than 0.05.

Results

The analysis of mean concentration of SOD, TAC and MDA are shown in Table 1. The analysis shows that there were significant differences (p-value < 0.05) in SOD, TAC and MDA levels among the groups of the pre-treatment phase.

Table 1. Mean Concentration of Oxidative Stress Markers (Superoxide dismutase (SOD), Total antioxidant capacity (TAC) and Malondialdehyde (MDA) in pre-treatment phase (group A). (mean±SD)

Groups	Superoxide dismutase (SOD) ng/ml	Total antioxidant capacity (TAC)mmol/l	Malondehaldehyde (MDA) ng/ml
Negative control (CN)	2.6±0.2	0.9±0.1	48.6±4.5
Positive control (PC)	1.7±0.3	0.3±0.1	137.6±10.8
200mg/kg extract (group A)	1.6±0.1	0.7±0.1	72.1±6.3
400mg/kg extract (group B)	1.6±0.1	0.8±0.2	54.5±5.4
800mg/kg extract (group C)	2.5±0.2	0.9±0.1	65.2±8.8
F-value	7.2	5.9	22.7
P-value	0.0118	0.0059	<0.0001

Table 2. Turkey's test of multiple comparison between groups for Superoxide dismutase, Total Antioxidant Capacity and Malondialdehyde for Pre-treatment

Tukey's test of multiple comparison	SOD significant? p<0.05	TAC significant? p<0.05	MDA significant? p<0.05
A Vs B	no	no	no
A Vs C	yes	no	no
A Vs PC	no	no	yes
A Vs NC	yes	no	no
B Vs C	yes	no	no
B Vs PC	no	yes	yes
B Vs NC	yes	no	no
C Vs PC	no	yes	yes
C Vs NC	no	no	no
PC Vs NC	yes	yes	yes

The analysis of mean concentration of SOD, TAC and MDA are shown in Table 2. The analysis shows that there were significant differences (p-value < 0.05) in SOD, TAC and MDA levels among the groups of the post-treatment phase.

Table 3. Mean Concentration of Oxidative Stress Markers (Superoxide dismutase (SOD), Total antioxidant capacity (TAC) and Malondialdehyde (MDA) in post-treatment phase (group A). (mean±SD)

Groups	Superoxide dismutase (SOD) ng/ml	Total Capacity (TAC) mmol/l	Antioxidant Malondialdehyde (MDA) ng/ml
Negative control (CN)	2.6±0.2	0.9±0.1	48.6±4.5
Positive control (PC)	1.2±0.1	0.3±0.04	128.8±5.2
200mg/kg extract (group A)	1.8±0.1	0.8±1.1	60.7±5.1
400mg/kg extract (group B)	1.8±0.1	0.9±0.1	56.3±7.8
800mg/kg extract (group C)	2.4±0.2	0.8±0.1	48.6±4.5
F-value	18.1	1.3	29.2
P-value	<0.0001	0.296	<0.0001

Table 4. Turkey's test of multiple comparison between groups for Superoxide dismutase and Malondialdehyde for post treatment

Tukey's test of multiple comparison	SOD significant? $p<0.05$	MDA significant? $p<0.05$
A Vs B	No	No
A Vs C	Yes	No
A Vs PC	No	Yes
A Vs NC	Yes	No
B Vs C	Yes	No
B Vs PC	No	Yes
B Vs NC	Yes	No
C Vs PC	No	Yes
C Vs NC	No	No
PC Vs NC	Yes	Yes

The comparison between the Oxidative stress parameters of the pre-treatment and the post-treatment phases was done using the student t-test the data is shown in table 5. From the result obtained for oxidative stress markers no significant ($p>0.05$) difference was seen between the both phases of treatment.

Table 5. T-Test Superoxide dismutase (SOD), Total antioxidant capacity (TAC) and Malondialdehyde (MDA) comparing the two phases

	GROUPS	t-values	p-values	Remark
SOD	A1 Vs A2	1.0	0.2	NS
	B1 Vs B2	1.2	0.1	NS
	C1 Vs C2	0.3	0.4	NS
TAC	A1 Vs A2	1.0	0.1	NS
	B1 Vs B2	0.8	0.2	NS
	C1 Vs C2	0.8	0.2	NS
MDA	A1 Vs A2	1.4	0.1	NS
	B1 Vs B2	1.0	0.2	NS
	C1 Vs C2	0.8	0.2	NS

Key; A1=200mg/kg, B1=400mg/kg, and C1=800mg/kg Pretreatment group, A2=200mg/kg, B2=400mg/kg and C2= 800mg/kg for post-treatment phase respectively.

Discussion

This study focused on assessing both the preventive and therapeutic role of *C afer* in managing acetaminophen poisoning in rats. This was such designed to know if taking *Costus afer* stem extract before acetaminophen poison exposure can prevent acetaminophen toxicity if rats are exposed to acetaminophen overdose. At the same time, the study was designed to know if *Costus afer* can equally heal or ameliorate the toxicity on a post-toxicant exposure case in rat.

The findings from this study revealed that *Costus afer* could prevent oxidative stress damage inflicted by acetaminophen in rats. This was revealed by the results presented in Table 2 where it was shown that there was a noble difference between the positive control group, and A, B and C groups. Since A, B and C represented the treatment groups, it meant that after the treatment there was an improvement

in the oxidative stress markers, particularly TAC and MDA when compared with positive control group which represented the acetaminophen-poisoned group without *C afer* treatment intervention. This finding agrees with the work conducted by other authors who reported improvement after *C afer* treatment [3,10]. From previous studies, it was found that *Costus afer* has strong antioxidants like total phenolic compounds, tannins, flavonoids etc. The presence of these phytochemicals in the extract may be responsible for the significant decrease in MDA and notable increase in TAC activities in rats treated with Costus afer extract [11].

On the other hand, when assessing the post-treatment effect on oxidative stress markers, it was revealed that treatment with the plant extract amelioration the oxidative effect of acetaminophen induced toxicity in rats which determined my the noble decrease in MDA level in all treatment groups (A, B and C) when compared to the positive control (PC) group. The increase in MDA levels is in line with the findings of Sen and Chakraborty in 2013 where they reported that acetaminophen is capable of inducing an increase in levels of MDA and causes a decrease in SOD due to an increase in reactive oxygen species in the liver [12]. It is supposed that this *C afer* antioxidant could be augmenting the endogenous antioxidant and defense system [3]. Anyasor in 2010 also suggested that *C. afer* might be able to protect the liver tissue from reactive oxygen species damage accumulation that is caused by lipid perioxidation that occurs in acetaminophen induced liver toxicity [13,14].

The revelation of Table 5 clearly demonstrated that the effect of *Costus afer* on oxidative stress markers are similar irrespective of whether it was administered before the toxicity was inflicted or it was administered after the toxicity has been inflicted. The result outcome is the same.

Conclusion

In this study, the preventive and the therapeutic potential of *Costus afer* were evaluated in rats poisoned with acetaminophen. The study revealed that *Costus afer* has both preventive and therapeutic effects in cases of acetaminophen poisoning. This was demonstrated by the determination of their oxidative stress status before and after the interventions.

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