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Research Article

Investigating the Preventive and Therapeutic Role of *Costus afer* in Acetaminophen Induced Liver Toxicity

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Abstract:
The objective of this study was to determine the prophylatic and therapeutic role of *Costus afer* in acetaminophen poisoning in rats. The rats were equally divided into two groups; pre-treatment group and post-treatment group with each group have five sub-groups (negative control (NC), positive control (PC), A, B and C) of 5 rats each. NC was without 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*. In the pre-treatment group, the PC was induced with acetaminophen 28 days after administration of distilled water, and "A", "B" and "C" were induced with acetaminophen 28 days after *Costus afer* treatment while 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 induced with acetaminophen before commencing on 28 days *Costus afer* treatment. After the treatment period, the rats were sacrificed and sample and liver organ were collected for laboratory analysis for liver enzymes and histological studies. The results showed that there was no significant change ($p>0.05$) in liver enzymes in the A, B, C subgroups of the pre-treatment group after acetaminophen induction. In the post-treatment group, there was a significant decrease in the liver enzymes in A, B and C subgroups after *Costus afer* treatment. This study has proven that *Costus afer* has the potential to not only prevent acetaminophen liver toxicity but to also treat hepatotoxicity inflicted by acetaminophen in rats.

Keywords: acetaminophen, enzymes, liver, treatment

1. Introduction

The use of herbs and plant extracts in the treatment of various disease conditions in our society today is on the increase. Many prefers these extracts and herbs to the usual orthodox medicine. In most cases these alternative therapies prove very effective hence the use in treatment of a wide range of illnesses including organ damage. *Costus afer* (*C. afer*) is one of 150 species of stout, perennial and rhizomatous herbs of the genus *Costus* and family

Costaceae. It can be found in the forest belt of Senegal, South Africa, Guinea, Niger, Sierra Leone and Nigeria. *C. afer* also known as Ginger Lily or Bush-cane, "Irekeomode" (in Yoruba), "opete" (in Igbo) and "ting"(in khana language). The plant bears white and yellow flowers. The stem, seeds and rhizomes are harvested from the wild plant and they contain several bioactive metabolites (Godswill *et al.*, 2010). This plant contains some active ingredients (phytochemical constituents) as well as vitamins and minerals, which contributes to its therapeutic effects. The presence of major phytochemical components of Costus plant shows a wide range of biological effects leading to their protective and disease preventive attributes. The plant exhibits the following actions: antioxidant, hormonal action, and stimulation of enzymes, interference with DNA replication, anti-microbial effect, and physical action (Godswill *et al.*, 2010). Extracts from the roots, barks, seeds and fruits of these plants are used in the preparation of syrups in traditional medicine such as cough suppressant and in the treatment of oxidative related diseases (Okwu, 2005).

The liver is a very essential organ in the body, and it is usual referred to as the center of metabolism because of its wide role in biochemical metabolism and drug metabolism (Okolonkwo et al., 2022a; Okolonkwo et al., 2022b). Studies have shown the effective of *Costus afer* in the treatment of ailments such as diabetes and other organ-related disorders due to the identifiable phytochemical composition. many researchers have also focused at the treatment effect of this plant in ameliorative liver damage with little concern in the preventive role it has on the liver. Therefore, investigating the preventive and treatment role of *Costus afer* in acetaminophen poisoning in rats will provide also the prophylatic role of the plant. This is designed to rather provide proactive approach to the liver health than taking a reactive step.

2. Materials and Methods

The research designs

The empirical study was an experimental design conducted on 50 rats. The study was divided into two main groups; pre-treatment group and post-treatment group. Two major interventions occurred in this study; acetaminophen poisoning and *Costus afer* treatment. In the pre-treatment group, the rats were treated orally with *Costus afer* for 28 days before acetaminophen poisoning intraperitoneally while in the post-treatment group, acetaminophen poisoning was initiated intraperitoneally before *Costus afer* oral treatment for 28 days. This is intended to determine the preventive and therapeutic role of *Costus afer* in the event of acetaminophen poisoning. After the interventions, the rats were sacrificed, blood samples were collected and liver organs were harvested for laboratory assessment of liver integrity.

Animal grouping

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

Group 1 pretreatment 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)
- 3 A2 rats that were given 200mg/kg b/w of *Costus afer* stem extract for 28 days after induction
- 4 B2 rats that were given 400mg/kg b/w of *Costus afer* stem extract for 28 days after induction
- 5 C2 rats that were given 800mg/kg b/w of *Costus afer* stem extract for 28 days after induction. (Momoh *et al.*, 2011).

Animal Care and Handling

The albino rats were weighed and grouped based on their body weight and allowed free access to feed and water *ad libitium* for a period of ten days to acclimatize to the new housing condition. Then different concentrations of the stem extract were given to the rats orally.They were housed in standard cage and maintained in standard laboratory condition at ambient temperature (25±2°C) with relative humidity (55-64%) and light and dark conditions (12/12h). They were fed with standard diet Top Feed Premier Feeds (Broiler finisher) manufactured by Premier Feed Mills Co. Ltd. (A subsidiary of Flour Mills Nig. Plc., Lagos State) was purchased for proper nutrition at Choba, Port Harcourt. Animal ethics and proper handling method were strictly adhered to. The bedding of the cage (sawdust) was always changed daily and the cage also washed and disinfected weekly (Ochei & Kolhakar, 2007).

Preparation of Costus afer extract

After identification, the samples were washed and shade dried in a well ventilated place for 24hrs to allow the water to drain off and to avoid contamination with dust. The stems were then cut with a sharp stainless steel knife into small bits and then crushed using a mechanical blender (NAKAI, Model no; 462, 230V-, 50Hz, 300W). One thousand grams (1000g) of the grounded stem was weighed with a weighing balance (JT3003D, Shandhai Science Instrument, Co, Ltd Zhejiang, China), strained and marc pressed and the liquid was allowed to stand for about 12 hours the liquid was

then filtered and the sediment concentrated into syrup using a rotary evaporator (OE-RX20-EA model # 700-041-006) at 4000rpm at 40°c.

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) /1000g X 200 = 24mg

Therefore, 24mg of the stock solution was measured and dissolved in 1.2ml of normal saline and administered to the rat orally (Erhirhie *et al.*, 2014).

Blood sample collection

At the end of the treatment period, (28days) rats in all the groups were anaesthetized with chloroform and dissected, and blood samples collected by jugular puncture into plain bottles, spun using a centrifuge at 4000 rpm and serum transferred into other plain bottles and stored in the laboratory freezer frozen (0-4°C)until time for analysis.

Laboratory assessment

Blood samples collected were assayed for liver enzymes (AST, ALT and ALP) using Reitman and Frankel method as described by Okolonkwo *et al*. (2022b).

Histological Examination

After dissection, the liver tissues were excised and collected in a sterile universal container containing 10% neutral formalin. They were processed and embedded in paraffin wax to provide a hard support for sectioning. The sections were mounted on glass slides to make a smear, and stained with hematoxylin and eosin stain (Ogenyi *et al*., 2022).

Statistical analysis

The statistical analysis was carried out using graph pad prism 5.3 software for descriptive and inferential statistics. ANOVA and Tukey's multiple comparison tests were done at a significance level of 0.05.

3. Results

The results presented in table 1 and 2 shows that there was no significant change ($p>0.05$) in the liver enzymes of rats induced with acetaminophen after 28 days treatment with *Costus afer*.

The results presented in table 3 and 4 shows that there was a significant decrease ($p<0.05$) in the liver enzymes of A, B and C subgroup after *Costus afer* treatment after a previous acetaminophen induced liver enzyme rise.

Table 1. Mean activity of liver enzymes (Aspartate Amino Transferase (AST), Alanine Amino Transferase (ALT) and alkaline phosphatase (ALP) in pre-treatment phase (group) (mean±SD)

Groups	Aspartate amino transferase (AST)IU/L	Alanine amino transferase (ALT)IU/L	Alkaline phosphatase (ALP)IU/L
Negative control (CN)	86.9±8.7	49.8±6.2	146.2±7.7
Positive control (PC)	143.4±11.3	110.4±8.5	187.2±3.8
200mg/kg extract (group A)	79.4±6.3	70.2±6.7	156.2±6.5
400mg/kg extract (group B)	62.4±4.8	47.2±6.3	151.2±4.1
800mg/kg extract (group C)	65.2±4.1	49.9±1.4	65.2±8.7s
F-value	19.1	18.3	12.2
P-value	<0.0001	<0.0001	<0.0001

Table 2. Tukey's test of multiple comparison between groups for Aspartate amino transferase (AST), Alanine amino transferase (ALT) and Alkaline phosphatase pre-treatment

Tukey's test of multiple comparison	AST Significant? p<0.05	ALT significant? p<0.05	ALP significant? p<0.05
A Vs B	No	No	No
A Vs C	No	No	No
A Vs PC	Yes	Yes	Yes
A Vs NC	No	No	No
B Vs C	No	No	No
B Vs PC	Yes	Yes	Yes
B Vs NC	No	No	No
C Vs PC	Yes	Yes	Yes
C Vs NC	No	No	No
PC Vs NC	Yes	Yes	Yes

Key; A= 200mg/kg extract. B=400mg/kg extract, C=800mg/kg extract, PC= Positive and CN= Negative control

Table 3. Mean activity of liver enzymes AST, ALT and ALP in post-treatment phase (group 2) (mean±SD)

Groups	Aspartate amino transferase (AST) IU/L	Alanine amino transferase (ALT) IU/L	Alkaline phosphatase (ALP) IU/L
Negative control (CN)	86.9±8.7	49.8±6.2	146.2±7.4
Positive control (PC)	129.8±7.0	101.9±8.8	134.0±6.4
200mg/kg extract (group A)	76.2±3.0	56.7±7.7	158.8±4.0
400mg/kg extract (group B)	77.8±8.0	56.4±5.8	137.4±3.1
800mg/kg extract (group C)	68.6±6.2	49.3±3.4	143.6±7.4
F-value	12.7	11.2	2.5
P-value	<0.0001	<0.0001	0.0722

Table 4. Tukey's test of multiple comparison between groups for AST) and ALT post-treatment

Tukey's of multiple comparison	AST Significant p<0.05	ALT Significant p<0.05
A Vs B	No	No
A Vs C	No	No
A Vs PC	Yes	Yes
A Vs NC	No	No
B Vs C	No	No
B Vs PC	Yes	Yes
B Vs NC	No	No
C Vs PC	Yes	Yes
C Vs NC	No	No
PC Vs NC	Yes	Yes

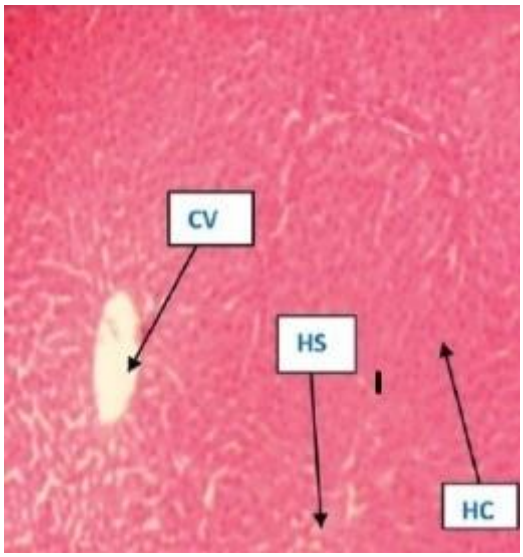

Figure 1. Histology of Rat Liver Obtained from Negative Control

CV- central vein, HS-hepatic sinusiod and HC- hepatocyte showing normal histology of negative contol rat.

The negative control group shows normal hepatocytes, central vein and hepatic sinosoid with no hepatic distortion of tissue clearly seen in fig 1.

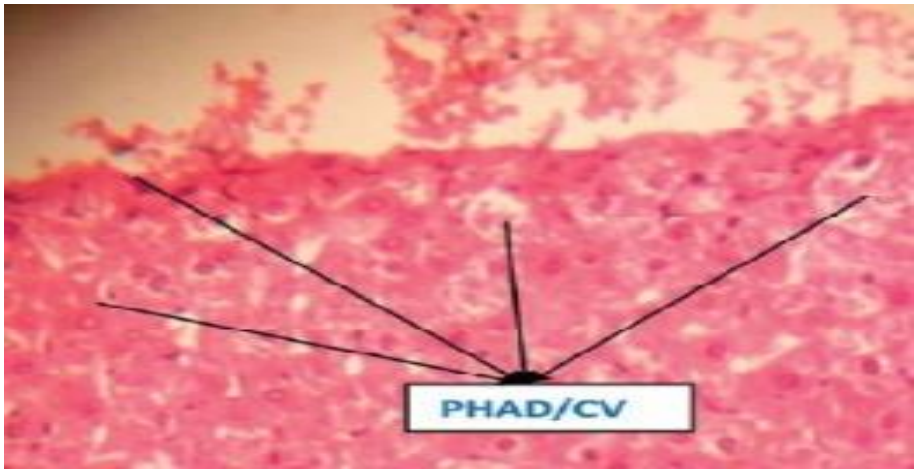

Figure 2. Histology of Rat Liver Obtained from Positive Control

PHAD/CV: Periventricular hepatocyte and central vein showing vascular changes

The positive control group administered with acetaminophen without treatment with *C. afer* extract showed the most prounounced histopathological changes as they present with areas of periventricular hepatocytic histological distortion and central vein with vascular changes as shown in fig 2.

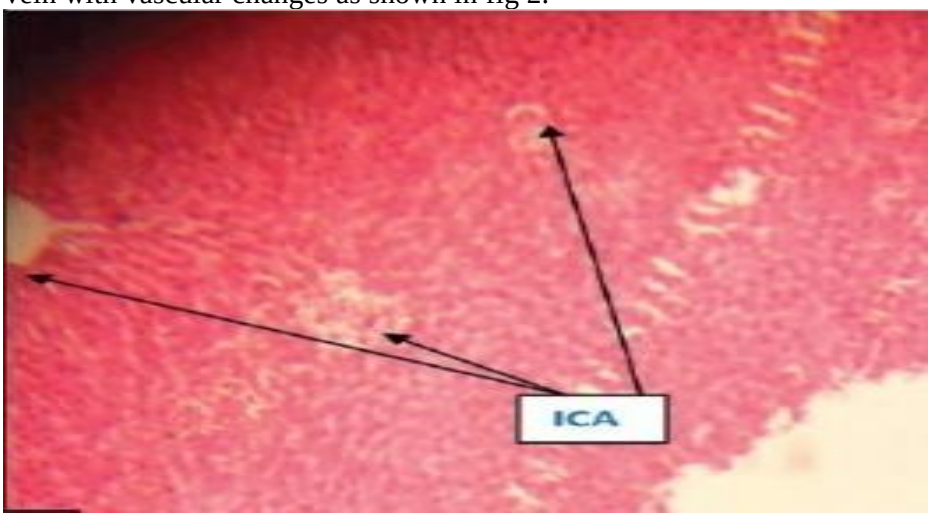

Figure 3. Histology of Rat Liver Obtained From 200mg/Kg Group

ICA- Inflamatory cell aggregate.

The group administered with 200mg/kg b/w presents with inflammatory aggregates shown fig 3.

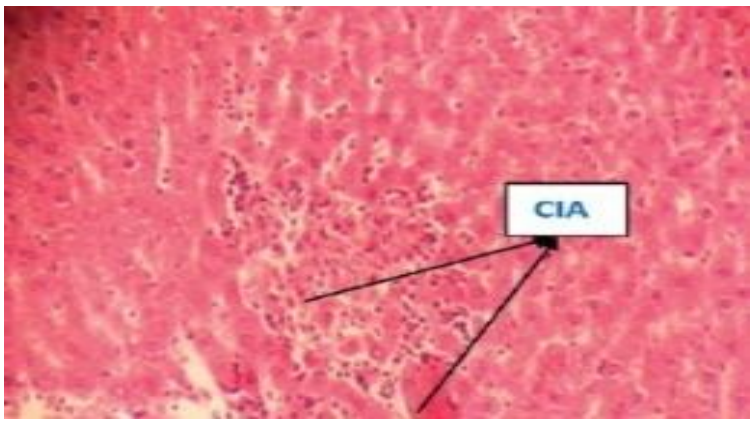

Figure 4. Histology of Rat Liver Obtained From 400mg/Kg Group

ICA- Inflamatory cell aggregate.

The group administered with 400mg/kg b/w showed collections of inflammatory cell aggregates seen in fig 4.

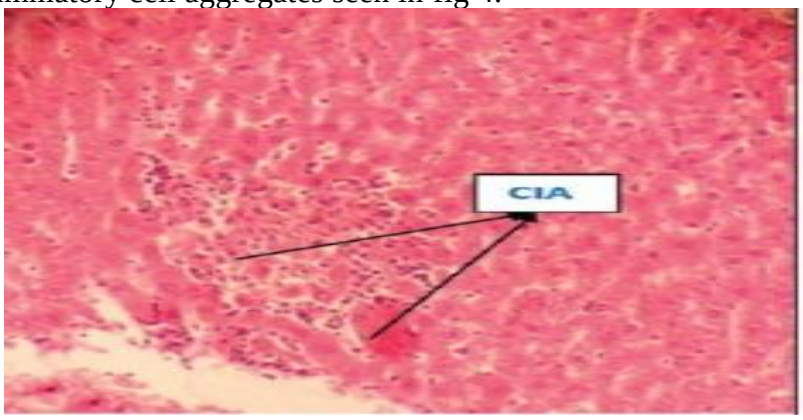

Figure 5. Histology of Rat Liver Obtained From 800mg/Kg Group

Collection of inflammatory cell aggregates.

The group administered 800mg/kg b/w group showed collections of inflammatory cell aggregates as seen in fig 5.

Discussion

This study evaluates the prophylactic and therapeutic effects of aqueous stem extract of *Costus afer* on acetaminophen induced liver toxicity in albino rats. The parameters analyzed were some liver enzymes (aspartate amino transferase (AST), alanine amino transferase (ALT) and alkaline phosphatase (ALP)

From the results presented in table 1 and 2, the sustained level of liver enzymes even after acetaminophen induction is an indication that *C afer* prevented acetaminophen hepatotoxicity in the rats. Recall that when acetaminophen is ingested it is converted by drug metabolizing enzyme to a reactive metabolite that is covalently bound to protein. At a nontoxic dose the metabolite is detoxified by glutathione forming an

acetaminophen-glutathione complex that can be easily eliminated from the body. Whereas in toxicity the metabolite (n-acetyl-benzoquinone imine) depletes the hepatic glutathione and subsequently covalently bound to protein, forming acetaminophen-protein adducts. The amount of covalent bond is relative to the degree of tissue damage that in turn causes an excessive release of transaminase level (Usoh *et al*., 2005). This explains the significant increase ($p<0.05$) in enzyme activity of the positive. The enzyme activities in the pre-treatment groups were comparable with the healthy or control. This has indeed demonstrated that C fer has hepatoprotective effect against future acetaminophen liver toxicity in rats.

In the previous paragraph, investigation on the prophylactic potential of *C afer* against acetaminophen on liver was studied, however, efforts were equally made to investigate the therapeutic role of *C afer* in ameliorating existing acetaminophen induced toxicity in the rats. The results revealed that *C afer* at all studied doses significantly reduced the effect of the inflicted hepatocellular injury posed by acetaminophen induction. When damage occurs in the liver cell due to overdose of acetaminophen, AST and ALT leak from the cell in the blood stream to the extent to which the damage has occurred. This increase in enzymes levels has been found to be reduced in *Costus afer* treated rat (Usoh *et al.*, 2005). In this study, there was a significant drop in the liver enzymes to levels that were comparable to the control group. This also correlates with work conducted by Godswill *et al.* (2014) that explain that *Costus afer* contains some anti-inflammatory substances that can protect the liver from being damage.

4. Conclusion

This study has demonstrated that *C afer* is not only effective in preventing acetaminophen exposure toxicity but it has also shown that it can as well treatment rats when they suffer from acetaminophen induced hepatotoxicity. The reported prophylatic and therapeutic achievements similar in doses between 200mg of *C afer* per kg of rats to 800mg of *C afer* per kg of rats.

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