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LIVER FUNCTION OF WISTAR RATS FED THE COMBINED ETHANOLIC LEAF EXTRACT OF *ALCHORNEA CORDIFOLIA* AND *COSTUS AFER* IN PARACETAMOL-INDUCED TOXICITY

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ABSTRACT

The effect of combined ethanolic leaf extract of *Alchornea cordifolia* (AC) and *Costus afer* (CA) orally administered at two doses (50mg/kg and 100mg/kg) on some biochemical parameters of rats with paracetamol induced hepatotoxicity was evaluated. The sixteen rats used for the experiment were of the wistar strain and were divided into four groups of four rats each ; group A(control) received standard feed and water, while tests groups C and D in addition to standard feed and water received 50mg/kg and 100mg/kg body weight of the extract respectively once daily for 28 days and paracetamol on the twenty fifth day, while group B received paracetamol on the twenty fifth day only. The results obtained showed that paracetamol treated group (group B) showed a significant reduction in weight compared to group A, C and D. Analysis of hepatic enzymes also showed an increase ($p < 0.05$) in serum marker enzymes of hepatic damage AST, ALT, and ALP, after paracetamol administration in group B. The combined ethanolic leaf extracts of *Alchornea cordifolia* and *Costus afer* brought the serum levels of these enzymes in group C and D respectively close to the control. In conclusion combined ethanolic leaf extract of *Alchornea cordifolia* and *Costus afer* has hepatoprotective effects on paracetamol induced hepatoxicity in Wistar rats.

KEYWORDS: *Costus afer*, *Alchornea cordifolia* biochemical parameters, Paracetamol-induced toxicity.

INTRODUCTION

Medicinal plants are of particular importance because they contain useful secondary products with high potency in the management of human ailments. it is generally assumed that the active constituents contributing to the efficacy of the medicinal plants are the phytochemicals, minerals and vitamins. Plants have provided mankind with herbal remedies for many diseases for many centuries till date. They continue to play a major role in primary healthcare as therapeutic remedies in developing countries. The role of plants in folklore medicine is attributed to the presence of phytochemicals; which are non nutritive plant chemicals that have disease preventing or curative properties.[1] Medicinal plants have various effects on living systems.Some are sedatives, analgesics, antipyretics, cardioprotectives,antibacterials, antivirals and antiprotozoals. However, this study focuses on remedies for liver damage;there is an alarming increase in the incidence of alcohol and drug related liver damage. Conversely, a number of plants have been found to offer some hepatoprotection. These include *Trichilia roka*[2], *Hemidesmus indicus* [3], *Cassia fistula* leaf extract[4], legumes[5] and *Acanthus ilicifolius.*[6] *Vernonia amydalina del*[7] *Ocimum gratissimum linn* [8] *Cymbopogon citratu.s.*[9]

Alchornea Cordifolia per (Schum and thorn) mull argo (euphoriaceae), known as Christmas bush is found abundantly along the coast in West African sub-region known traditionally as "Gyamma". *Alchornea* is used for a variety of disease by traditional medical practitioners in Ghana and Nigeria.[10] *Alchornea Cordifolia* is used to treat diarrhea, wound, sores, cuts[11] and applied topically as an anti inflammatory agent[12] Alchornea is also reported to possess a multiplicity of biological effects: it is an antibacterial[13,14] Spasmolytic.[15] Antiinflammatory[16,17] Hepatoprotective[18], anitdiarrhoeal[19] antioxidant[20] and antimicrobial.[21] *C. afer,* which is commonly called bush sugar cane or monkey sugar cane.[22]

C. afer which belongs to the family *Zingiberaceae* is a monocot and a relatively tall, herbaceous, unbranched tropical plant with creeping rhizome. It is commonly found in moist or shady forest of West and Tropical Africa.[23] *C. afer* is a useful medicinal plant that is highly valued for its anti-diabetic, anti-inflammatory and anti-anthritic properties in South-East and South-West Nigeria.[24] Its Alleviation of Carbon Tetrachloride – induced Hepatic

Oxidative Stress and Toxicity has also been reported.[25,26] also reported that stem extracts of *C. afer* ameliorates paracetamol induced tissue injury in rats.

Polyherbal therapy which is the use of a combination of various agents from different plant sources for therapeutic purposes is a current pharmacological principle and has the advantage of producing maximum therapeutic efficacy with minimum side effects.[27]

According to[28] polyherbal therapies have the synergistic, potentiative, agonistic/antagonistic pharmacological agents within themselves,that work together in a dynamic way to produce therapeutic efficacy with minimum side effects. It is in this light that this work was designed to investigate the effects of combined extracts from two widely used plants *Alchornea cordifolia* and *Costus afer* employed traditionally in the management of some maladies. Studies have however been conducted independently on the anti hepatotoxic effects of both *Alchornea cordifolia* and *Costus afer*.

Consequently, the aim of this study is to investigate the efficacy of the extracts of *Alchornea cordifolia* and *Costus afer i*n combination on Paracetamol-induced toxicity in Wistar Rats

MATERIALS AND METHODS

Animals

Sixteen (16) adult healthy male albino rats, weighing between 180 and 260g were used in this study. The rats were obtained from the animal house of the Niger Delta University, College of Health Sciences, Bayelsa State and housed in standard cages. They were then allowed free access to standard feed (growers mash) and water for a period of two weeks to acclimatize to the cage environment prior to the commencement of the experiment. All the protocols were performed in accordance with the Institutional Animal Ethical committee (IAEC) as per the directions of the Committee for the purpose of control and supervision of experiments on animals (CPCSEA).

Chemicals

All chemicals used were of analytical standard.

Preparation of extracts

Fresh leaf of *Alchornea cordifolia* and *Costus afer* were collected from a residential farmyard in Niger Delta University (N.D.U),Amassoma, Wilberforce Island, Bayelsa State,

Nigeria and was botanically identified and deposited at the Herbarium of department of biological science, in Niger Delta University (N.D.U), Amassoma, Wilberforce Island, Bayelsa State, Nigeria. The leaves of *Alchornea cordifolia* and *Costus afer* were thoroughly washed with distilled water to remove debris and contaminants, the air dried leaves were grounded and later milled into powder form using an electric blender (Blender, 462 Nakai Japan). 200g of the powdered mixture (i.e 100g each of *Alchornea cordifolia* and *Costus afer* was extracted in 600ml of absolute ethanol for 24 hours at room temperature with constant shaking using a flask shaker (Model, Denly A-500). The extract was filtered with Whatman No 1 filter paper and the resulting filtrate evaporated to dryness using a rotatory evaporator at 40 ℃, the resultant concentrate was then reconstituted in distilled water to give the required doses used in the study.

Experimental design and procedures

Experimental design

Sixteen rats of the wistar strain weighing between 180-260g were distributed into four groups of four (4) animals each.

The animals were fed with standard pellet chow and drinking water, and they received the following treatment schedule.

Group A: Normal Control (received only distilled water).

Group B: Paracetamol (3g/kg, body weight, orally).

Group C: Combined ethanolic leaf extract of *Alchornea cordifolia* and *Costus afer* (50 *mg/kg)* body weight orally + Paracetamol *(3g/kg* b.w*)* orally) on the twenty-fifth day of the twenty-eight days experiment.

Group D: combined ethanolic leaf extract of *Alchornea cordifolia* and *Costus afer (100mg/kg)* body weight, orally + paracetamol *(3g/kg* b.w*)* orally) given on the twenty-fifth day of the twenty-eight days experiment.

Biochemical Analysis

Sample Collection

After the experimental period, animals in different groups were sacrificed. By the end of each experimental period, the rats were reweighed, starved for 24 hours and sacrificed under chloroform anesthesia. 5ml of blood was collected from each animal by cardiac puncture using sterile needle and syringe. Part of the blood sample was put into test tubes and allowed

to clot for 30 minutes before centrifuging at 800g for 5 minutes. The supernatant was used for the biochemical analysis.

Biochemical estimation

The following liver function test were conducted to investigate derangement in the liver of the animals used for the study, aspartate aminotransferase (AST), alanine aminotransferase (ALT) were determined by the colorimetric method of[29] using a commercial assay kit from Randox Laboratories Ltd, Co. Antrim, United Kingdom. Alkaline phosphatase (ALP) was extimated by the colorimetric method of[30] using assay kits from Randox Laboratories Ltd. Serum protein and serum albumin were estimated by Biuret method and Bromocresol Green (BCG) binding method respectively using a commercial assay kit from Randox Laboratories Ltd.

Total and conjugated bilirubin was determined using commercial kits from Randox Laboratories Ltd, using colorimetric method described by.[31]

Statistical Analysis

The result obtained from the study were analyzed using the statistical package for social science (SPSS) version 16.0 for windows. One-way ANOVA followed by Post-hoc Turkey was used to compare mean ± S.D, and values were considered significant at $p< 0.05$.

RESULTS

Table 1: Effect of *Alchornea cordifolia* and *Costus afer* on liver enzymes in paracetamol induced hepatotoxicity in rats

GROUPS	AST (U/L)	ALT (U/L)	ALP (U/L)
Group A	36.98 ± 0.46^{a}	16.38 ± 0.46^{a}	40.17 ± 0.73^{a}
Group B	85.88 ± 0.57^{b}	56.85 ± 0.31^{b}	89.13 ± 0.53^{b}
Group C	64.50 ± 0.55^{c}	29.00 ± 0.34^{c}	65.78 ± 0.78^{c}
Group D	52.90 ± 0.63^{d}	19.65 ± 0.82^{d}	49.78 ± 0.75^{d}

Data mean±SD (n = 4). Mean in the same column with different superscript letter(s) are significantly different $p<0.05$ (one way ANOVA followed by Post-hoc Turkey)

Table 2: Effect of *Alchornea cordifolia* and *Costus afer* on Alb, TP, TB and CB in paracetamol induced hepatotoxicity in rats.

GROUPS	ALB (g/dl)	TP (g/dl)	TB(umol/l)	CB(umol/l)
Group A	36.95 ± 1.05^{a}	63.27 ± 0.59^{a}	5.30 ± 0.43^{a}	1.05 ± 0.12^{a}
Group B	24.15 ± 0.44^{b}	45.50 ± 0.50^{b}	12.30 ± 0.62^{b}	1.95 ± 0.12^{b}
Group C	27.75 ± 0.31^{c}	50.45 ± 0.59^{c}	7.77 ± 1.72^{c}	1.65 ± 0.058^{c}
Group D	33.87 ± 0.26^{d}	$57.53\pm O.56^{d}$	6.60 ± 0.18^{d}	$1.32\pm O.96^{d}$

Data mean±SD (n = 4). Mean in the same column with different superscript letter(s) are significantly different $p<0.05$ (one way ANOVA followed by Post-hoc Turkey)

The results for the effects of combined ethanolic leaf extract of *Alchornea cordifolia* and *Costus afer* on serum levels of ALB, TP, TB, CB, AST, ALT and ALP of rats administered with paracetamol are presented (Table 1 and 2). Treatment of rats with paracetamol alone resulted to a significant difference ($p <0.05$).

There was a significant increase ($p < 0.05$) in serum levels of AST, ALT, ALP of the rats administered with paracetamol alone when compared to the normal control, while there was a significant decrease ($p < 0.05$) in the serum levels of, AST ALT, ALP of the rats administered with both paracetamol and the extracts when compared with the rats given paracetamol only.(Table 1) There was also a significant decrease ($p < 0.05$) in the serum levels of ALB and TP of the rats administered with paracetamol alone when compared to the normal control, while those rats administered with both paracetamol and the extract had a significant increase ($p < 0.05$) when compared with the rats administered with paracetamol only (Table 2). There was also a significant increase ($p < 0.05$) in serum levels TB and CB of the rats administered with paracetamol alone when compared to the normal control, while there was a significant decrease ($p < 0.05$) in the serum levels of TB and CB of the rats administered with both paracetamol and the extracts when compared with the rats given paracetamol only.(Table 2).

DISCUSSION

Many research efforts have been directed towards the provision of empirical proofs to back up the use of many tropical plants for trado-medical practices.[32,33,34,35] Atawodi (2005)[36] reported the antioxidant potentials of many African plants. However, there still exist a large number of tropical

plants/trees with tremendous potential yet to be investigated.

Also in recent years, attention has been focused on the role of biotransformation of chemicals to highly reactive metabolites that initiate cellular toxicity. Many compounds including clinically useful drugs can cause damage through metabolic activation of the chemical to highly reactive compound such as free radicals, carbenes and nitrenes.[37]

Moreover, the hepatotoxicity of Paracetamol, a widely used antipyretic-analgesic drug, produces acute hepatic damage on accidental overdosage. It is established that, a fraction of paracetamol is converted via the cytochrome P450 pathway to a highly toxic metabolite, N–acetyl–p–benzoquinamine (NAPQI)[38] which is normally conjugated with glutathione and excreted in urine. Overdose of paracetamol depletes glutathione stores, leading to accumulation of NAPQI, mitochondrial dysfunction[39] and development of acute hepatic necrosis. Several P450 enzymes are known to play an important role in N-acetyl-p-aminophenol (APAP) bioactivation to NAPQI. P450 2E1 (CYP2E1) have been suggested to be primary enzymes for paracetamol bioactivation in liver microsomes (Parmar *et al*.,1998)[40] Studies demonstrated that paracetamol induced hepatotoxicity can be modulated by substances that influence P450 activity (Raucy *et al*.,1989).[41]

Paracetamol has been attributed to the formation of toxic metabolites when part of the drug is activated by hepatic cytochrome P450 (Savides and Oehme, 1983)[42] to highly reactive metabolites N-acetyl P-benzoquinoneimine (NAPQI) (Vermeulen *et al.*, 1992).[43] NAPQI is initially detoxified by conjugation with reduced gluthathione (GSH) to form mercapturic acid (Moore *et al.*, 1985).[44] However when the rate of NAPQI formation exceeds the rate the detoxification by gluthathione, it oxidizes tissue macro-molecules such as lipid or thiol groups of protein and even alters the homeostasis of calcium after depleting GSH.

In the assessment of liver damage by paracematol or any other hepatotoxin, the determination of enzyme activities such as ALT and AST is largely inevitable (Dobbs *et al.*, 2003).[45] Necrosis or membrane damage releases these enzymes into coronary circulation therefore it can be measured in the blood. High levels of AST indicate liver damage. ALT catalyses the conversion of alanine to

pyruvate and glutamate, and is released in a similar manner. ALT is more specific to the liver thus is a better parameter for detecting liver injury.

In this study, the administration of Paracetamol alone cause a significant elevation ($p < 0.05$) in the serum levels of AST, ALT, ALP, CB and TB, while there was a significant decrease ($p < 0.05$) in the serum levels of ALB and TP in the animals (Table 1 and 2).

The result showed that combined ethanolic leaf extract of *Alchornea cordifolia* and costus afer caused significant reduction in serum AST, ALT, ALP, CB and TB while there was significant increase in ALB and TP levels when administered with paracetamol (Table1 and 2). This observation indicates that *Costus afer* and *Alchomea cordifolia* ameliorate or offer protection to the liver against paracetamol induced hepatotoxicity. Confirming these findings, Ukpabi *et al*.(2012)[25] indicated the hepatoprotective effect of C. *afer* on carbon tetrachloride- induced hepatic stress and toxicity.

This study confirms the result on the hepaprotective and invivo anti-oxidant activity of *Costus afer* leaf extract against acetaminophen induced hepatotoxicity in rats by.[46] The result obtained from this research showed that the combined ethanolic leaf extract of *Alchornea cordifolia* and *Costus afer* has protective effect against paracetamol induced hepatic damage which is in agreement with the work of[47] on inhibition of paracetamol induced oxidative stress in rats by extracts of alchornea cordifolia and camellia sinensis. It is also in agreement with work on some medicinal plants which include *Trichilia roka* [2], *Hemidesmus indicus*[3], *Cassia fistula* leaf extract[4], legumes[5] and *Acanthus ilicifolius.*[6] *Vernonia amydalina del*[7] *Ocimum gratissimum linn*[8] *Cymbopogon citratus.*[9][1] reported the presence of alkaloids, flavonoids, tannins, saponins, steroids and glycosides in the leaves of *Costus afer* .These are potent water soluble antioxidants which prevent oxidizing cell damage and may be responsible for the protective activity observed with the combined ethanolic leaf extracts. The therapeutic potential of antioxidants in controlling degenerative disease with marked oxidative damage from reactive oxygen species or free radicals have been reported.[48,39,49]

CONCLUSION

This study concluded that high dose of paracetamol predisposes to hepatic damage. It could be inferred from the result that the combined Ethanolic leaf extract of *Alchornea cordifoilia* and *Costus afer* reduced the effect of paracetamol induced hepatic damage in rats.

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