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**Effect of aqueous leaf extract of *Cymbopogon citratus*
(Lemongrass- *Achara tea*) on liver function in
paracetamol-induced hepatotoxicity in albino rats**

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Abstract

The effect of aqueous leaf extract of *Cymbopogon citratus* (lemongrass) on liver function in paracetamol-induced hepatotoxicity in albino rats was investigated using these biochemical markers: total and conjugated bilirubin, alanine aminotransferase (ALT), Aspartate aminotransferase (AST), and alkaline phosphatase. A total of forty eight adult albino rats (male/female) weighing between 150g to 200g were used for the study. The animals were randomly divided into six groups of eight animals each. Group I served as healthy control and was fed with normal animal feed and water throughout the experiment. Animals in groups II to VI were administered with paracetamol (1g/ kg) in distilled water solution per oral. After three days of this challenge, animals in groups III, IV, V and VI were treated with 100mg/kg, 200mg/kg, 300mg/kg and 400mg/kg body weight of aqueous lemongrass leaf extract respectively daily for 14 days. Animals in group II (disease control) however did not receive any treatment with lemongrass extract and were instead given sterile water. All the animals were allowed unlimited access to tap water and grower's mash. There were significant decreases ($p < 0.05$) in final body weight ($161.3 \pm 11.3g$), liver weight ($3.3 \pm 0.2g$) with corresponding increases ($P < 0.05$) in ALT ($86. \pm 7.2$ U/L), AST (41.4 ± 3.9 U/L), ALP (21.8 ± 2.1 U/L) total bilirubin (1.50 ± 0.19 mg/dl), and conjugated bilirubin (1.06 ± 0.18 mg/dl) of group II when compared with those of group 1: final body weight ($182.5 \pm 15.8g$), liver weight ($5.9 \pm 0.6g$), ALT (10.9 ± 2.0 U/L), AST (11.3 ± 2.3 U/L), ALP (17.4 ± 1.3 U/L), total bilirubin (0.53 ± 0.16 mg/dl) and conjugated bilirubin (0.28 ± 0.13 mg/dl). However, post-treatment of the diseased animals of groups III to VI with different concentrations of aqueous leaf extract of *Cymbopogon citratus* alleviated most of those changes though not strictly in a dose dependent manner. The results of this study indicate that aqueous leaf extract of *Cymbopogon citratus* has anti-hepatotoxic action against paracetamol- induced hepatic toxicity in rats.

Keywords: aqueous leaf extract, *cymbopogon citratus* (lemongrass- achara tea), liver function, paracetamol-induced hepatotoxicity in albino rats

Introduction

Organic chemistry and the emergence of medicinal chemistry as a major route for the discovery of novel and more effective therapeutic agents (Roja and Rao, 2000).

Cymbopogon citratus of the Poaceae family is a tall aromatic coarse grass of 1.5m high. It is a monocotyledonous hypogean perennial plant with slender sharp edged leaves with a pointed apex. The entire plant is attached to the soil by fibrous root (Burkill, 1996). Lemongrass stalks are commonly used in the cuisines of Africa, the Middle East and Southeast Asia. It is native to Sri Lanka and South India and is currently widely cultivated in the tropical areas of America and Asia.

In the folk medicine of Brazil, *Cymbopogon* is believed to have anxiolytic, hypnotic and anticonvulsant properties (Blanco *et al*, 2009; Rodriguez *et al.*, 2006). In addition *Cymbopogon citratus* has been used as an inexpensive remedy for the treatment of oral thrush in HIV/AIDS patients (Wright *et al.*, 2009). It has been most frequently used as a remedy for gastrointestinal disorders, e.g stomachache, acid indigestion, abdominal cramps, diarrhoea and dyspepsia (Duke and Vasquez, 1997). *Cymbopogon citratus* has been shown to possess antioxidant properties (Koh *et al.*, 2012). However, there is relative insufficient report on the effect of aqueous leaf extract from the local lemongrass on liver function in paracetamol-hepatotoxicity. The present study on the effect of aqueous leaf extract of lemongrass on liver function employing the biochemical markers is premised on the reported antioxidant properties of this plant. The rationale for the choice of paracetamol is due to its relative commonness, use and misuse by human population, and it is also a common liver hazard.

Materials and Methods

Plant Material

Fresh and apparently uninfected leaves of *Cymbopogon citratus* (lemongrass) were collected from plants growing within Ikeduru L. G. A. of Imo state in February, 2013. The botanical identification of the plant was confirmed by Dr. F.N. Mbagwu at the Department of Plant Science and Biotechnology, Imo State University, Owerri, where voucher samples are kept for reference.

Experimental Animals

A total of forty eight (48) adult albino rats of Wister strain (male/ female) weighing between 150g to 200g were purchased from Animal Farm of Michael Okpara University of Agriculture Umudike, Umuahia in Abia State. The animals were housed at the Animal house of

College of Medicine, Imo State University Owerri and were acclimatized for two (2) weeks. The animals were allowed free access to normal animal feed and water before the experiment. In addition they were maintained at twelve (12) hour light and dark cycle.

Drug (Paracetamol) / Chemical

The paracetamol tablets used were purchased from Milan Chemists Douglas road Owerri. Other chemicals were purchased from HI -TECH Diagnostics Ltd, Nigeria and were of analytical grade, AR.

Preparation of the Extract

Fresh leaves of *Cymbopogon citratus* were sun-dried for five days, followed by grinding. Thereafter the ground material was sieved through a 1mm sieve to obtain a fine powder. Exactly 200g of the fine powder was soaked in 1000ml (1L) of distilled water in a conical flask, the mixture allowed to stand on the laboratory bench for 30mins, thereafter shaken and boiled for 1hour. It was then cooled and filtered. The filtrate was evaporated in a hot air oven to yield a dry weight of 60g. The following weights of the residue: 1g, 2g, 3g, and 4g were prepared in 10ml of distilled water corresponding to 100mg/ml, 200mg/ml, 300mg/ml and 400mg/ml concentrations respectively to be given to the animals per kilogram body weight.

Experimental Design

After two weeks of acclimatizing the animals, they were grouped into six groups of eight animals each and their initial body weights taken. Group I served as negative control (control 1) and was fed with normal animal feed and water only until the end of the experiments. Hepatotoxicity was induced in animals in groups II to VI using a relatively high dose of paracetamol (1g/kg body weight) in distilled water solution per oral. Group II served as positive control (control 2), which did not receive treatment with aqueous extract of *Cymbopogon citratus*. Group III, IV, V and VI received treatment with 100mg/kg body weight, 200mg/kg body weight, 300mg/kg body weight, and 400mg/kg body weight of aqueous extract of lemongrass in 1ml volume daily respectively for four weeks through the oral route. During this period however, animals were allowed free access to normal animal feed and water.

Twelve hours after the last treatment and the last meal, the animals were re-weighted and sacrificed. Using a 5ml syringe, about 4ml of blood were collected by cardiac puncture. The blood was collected into plain containers.

Serum was obtained from the non-coagulated blood after clotting, centrifugation and stored at -20°C prior to use. The serum was used for bilirubin, and liver enzymes (ALT, AST and ALP) estimations.

Laboratory Procedures

All reagents were commercially purchased and the manufacturer's standard operating procedure (SOP) strictly followed. Biosystems kit, Barcelona, Spain which is a modification of that used by Pearlman and Lee, (1974) was used for the determinations of total and conjugated bilirubin. RANDOX of United Kingdom kit which is a modification of that used by Reitman and Frankel, (1957) was used for the estimation of AST. Also RANDOX of United Kingdom kit which is a modification of that used by Reitman and Frankel. (1957) was used for ALT estimation. TECO DIAGNOSTICS of USA kit which is a modification of

that described by Tietz, (1976) was used for the estimation of alkaline phosphatase, ALP.

Statistical Analysis

All values were expressed as means \pm SD. Statistical significance was determined by ANOVA and then the student's t-test using SPSS Version 16 windows 8 and the individual comparisons were obtained by the Least Significant Difference (LSD) and Turkey method.

Differences between groups were considered significant at $P < 0.05$ and highly significant at $P < 0.01$.

Results

Table 1: Effect of aqueous leaf extract of *Cymbopogon citratus* (lemongrass) on body and liver weights of albino rats in paracetamol-induced hepatotoxicity

Groups/treatments	Initial body weight(g)	Final body weight(g)	Liver weight(g)
I Healthy control (n=8)	173.8 \pm 16.9 ^a	182.5 \pm 15.8 ^a	5.9 \pm 0.6 ^a
II Disease control (n=8)	170.9 \pm 13.5 ^a	161.3 \pm 11.3 ^b	3.3 \pm 0.2 ^b
III IOOmg lemongrass/kg body weight (n=8)	171.9 \pm 12.5 ^a	172.5 \pm 7.5 ^C	5.0 \pm 0.2 ^C
IV 200mg lemongrass/kg b.w.	171. 3 \pm 14.1 ^a	173.8 \pm 11.9 ^{a,c}	5.2 \pm 0.2 ^C (n=8)
V 300mg lemongrass/kg b.w. (n=8)	173. 1 \pm 14.1 ^a	175.6 \pm 14.5 ^{a,c}	5.4 \pm 0.4 ^{a,c}
VI 400mg lemongrass/kg b.w (n=8)	172.5 \pm 11. 3 ^a	175.0 \pm 13.9 ^{a,c}	5.3 \pm 0.3 ^C
ANOVA			
F - Value	0.057	3.348	50 .942
P - Value	P >0.05	F <0.05	P <0.05

Values are means \pm S.D. Means in the same column with different superscript letter(s) are significantly different, $P < 0.05$ (One-way ANOVA followed by post-hoc LSD and Turkey)

Table 2: Effect of aqueous leaf extract of *Cymbopogon citratus* (lemongrass) on hepatic function markers.

Groups/Treatments	Total	Conjugated	ALT	AST	ALP
	Bilirubin	Bilirubin(mg/dl)	(U/L)	(U/L)	(U/L) (mg/dl)
I Healthy control	0.53 \pm 0.16 ^a	0.28 \pm 0.13 ^a	10.9 \pm 2.0 ^a	11.3 \pm 2.3 ^a	17.4 \pm 1.3 ^a (n=8)
II Disease control	1.50 \pm 0.19 ^b	1.06 \pm 0.18 ^b	86.1 \pm 7.2 ^b	41.4 \pm 3.9 ^b	21.8 \pm 2.1 ^b (n=8)
III IOOmg lemongrass/kg b.w.	0.96 \pm 0.14 ^C	0.61 \pm 0.20 ^C	72.4 \pm 5.1 ^C	30.8 \pm 3.6 ^C	19.5 \pm 1.9 ^o
IV200mg lemongrass/kg b.w. (n=8)	0.91 \pm 0.16 ^C	0.51 \pm 0.16 ^C	68.5 \pm 3.5 ^o	24.8 \pm 3.4 ^d	18.9 \pm 1.4 ^{a,o}
V300mg lemongrass /kg b.w. (n=8)	0.69 \pm 0.08 ^d	0.30 \pm 0.11 ^a	25.6 \pm 2.1 ^d	16.0 \pm 2.7 ^e	18.3 \pm 2.1 ^{a,c}
VI400mg lemongrass/kg b.w (n=8). ANOVA	0.66 \pm 0.13 ^{a, d}	0.31 \pm 0.08 ^a	23.0 \pm 3.3 ^d	15.3 \pm 3.1 ^e	18.5 \pm 2.1 ^{a,c}
F-Value	44.492	33.903	113.749	99.838	5.269
P-Value	P <0.05	P <0.05	P <0.05	P <0.05	P <0.05

Values are means \pm S.D. Means in the same column with different superscript letter(s) are significantly different, $P < 0.05$ (One-way ANOVA followed by post-hoc LSD and Turkey),

Table 3: The values of Pearson's correlation coefficients calculated between weights (initial body weight, final body weight and liver weight) and hepatic function parameters

		Total bilirubin	Conjugated Bil	AST	ALT	ALP
Initial body weight	Pearson correlation	0.21	0.064	-0.056	-0.056	-0.057
	Significance	0.885	0.667	0.704	0.703	0.701
	N	48	48	48	48	48
Final body weight	Pearson correlation	-0.392**	-0.364*	-0.470**	-0.417**	-0.299*
	Significance	0.006	0.011	0.001	0.003	0.039
	N	48	48	48	48	48
Liver weight	Pearson correlation	-0.802**	-0.753**	-0.800**	-0.712**	-0.530**
	Significance	0.000	0.000	0.000	0.000	0.000
	N	48	48	48	48	48

** Correlation is significant at the 0.01 level (2-tailed) * Correlation is significant at the 0.05 level (2-tailed).

The results generated from this study show that there was no significant difference ($P > 0.05$) in the initial body weight of the animals in all the groups. However, there was a significant decrease ($P < 0.05$) in the mean final body weight of group II (diseased control) (161.3 ± 11.3 g) when compared with that of group I (healthy control) (182.5 ± 15.8 g). Also the mean value of final body weight of group III (172.5 ± 7.5 g) was significantly ($P < 0.05$) lowered compared with that of group I (182.5 ± 15.8 g). See table 1.

The mean value of the final body weight of groups III, IV, V, and VI (172.5 ± 7.5 g, 173.8 ± 11.9 g, 175.6 ± 14.5 g, and 175.0 ± 13.9 g respectively) did not change significantly ($P > 0.05$) and also did not achieve statistically significant difference except for that of group III (172.5 ± 7.5 g) when compared with group I (healthy control). However that of group II (161.3 ± 11.3 g) was significantly ($P < 0.05$) lowered when compared with those of groups III, IV, V and VI.

The mean value of the liver weight of group II (disease control) (3.3 ± 0.29) was significantly ($P < 0.05$) lowered when compared with those of groups I, III, IV, V and VI (5.9 ± 0.6 g, 5.0 ± 0.2 g, 5.2 ± 0.2 g, 5.4 ± 0.4 g, and 5.3 ± 0.3 g respectively).

In addition the mean values of liver weight of groups III, IV, and VI were significantly ($P < 0.05$) lowered when compared with group I. That of group V did not change statistically ($P > 0.05$) when compared with group I (healthy control).

Table 2 depicts the mean values of liver function markers (Total bilirubin, conjugation bilirubin, ALT,

AST, and ALP) of all the experimental groups. The mean value of total bilirubin of group II (diseased control) (1.50 ± 0.9 mg/dl) was significantly higher ($P < 0.05$) than those of group I, III, IV, V and VI (0.53 ± 0.16 mg/dl, 0.96 ± 0.14 mg/dl, 0.91 ± 0.16 mg/dl, 0.69 ± 0.08 mg/dl, and 0.66 ± 0.13 mg/dl respectively).

The mean values of total bilirubin of groups III and IV were significantly ($P < 0.05$) higher than those of groups I, V, and VI. That of group VI (0.66 ± 0.13 mg/dl) did not change statistically ($P > 0.05$) when compared with that of group I (0.53 ± 0.16 mg/dl). There were no significant differences ($P > 0.05$) between the mean values of conjugated bilirubin of group I, V and VI (0.28 ± 0.13 mg/dl, 0.30 ± 0.11 mg/dl, and 0.31 ± 0.08 mg/dl respectively) but however were significantly ($P < 0.05$) lowered when compared with that of group II (diseased control) (1.06 ± 0.18 mg/dl). The mean values of conjugated bilirubin of groups III and IV (0.61 ± 0.20 mg/dl, and 0.51 ± 0.16 mg/dl respectively) did not achieve any significant ($P > 0.05$) difference.

The mean value of ALT of group II (disease control) (86.1 ± 7.2 U/L) was significantly ($P < 0.05$) higher than those of groups I, III, IV, V and VI (10.9 ± 2.0 U/L, 72.4 ± 5.1 U/L, 68.5 ± 3.5 U/L, 25.6 ± 2.1 U/L, and 23.0 ± 3.3 U/L respectively). Those of groups III and IV did not achieve any significant ($p > 0.05$) difference. Also those of groups V, VI were not significant ($P > 0.05$) when compared with each other. However, the values for groups III, IV, V, VI were significantly higher ($P < 0.05$) when compared with that of group I (healthy control).

The mean values of AST of groups II, III, IV, V and VI (41.4 ± 3.9 U/L, 30.8 ± 3.6 U/L, 24.8 ± 3.4 U/L, 16.0 ± 2.7 U/L, and 15.3 ± 3.1 U/L respectively) were significantly higher ($P < 0.05$) than that of group I (11.3 ± 2.3 U/L). However the values for groups III, IV, V, and VI were significantly lower ($P < 0.05$) than that of group II. There was no significant difference ($P > 0.05$) between the AST mean values of groups V, VI.

The mean value of ALP of group II (21.8 ± 2.1 U/L) was significantly higher ($p < 0.05$) when compared with those of groups I, III, IV, V and VI (17.4 ± 1.3 U/L, 19.5 ± 1.9 U/L, 18.9 ± 1.4 U/L, 18.3 ± 2.1 U/L, and 18.5 ± 2.1 U/L respectively). There were no significant ($P > 0.05$) differences in the mean values of ALP of groups III, IV, V, and VI. There were also no significant differences ($P > 0.05$) in the mean values of ALP of groups I, IV, V, and VI. Table 3 depicts the values of Pearson's correlation coefficients calculated between weights (initial body weight, final body weight, liver weight) and hepatic function parameters. There were no significant positive associations among initial body weight and both total bilirubin ($r = 0.021$; $P > 0.05$) and conjugated bilirubin ($r = 0.064$; $P > 0.05$) of the animals in both experimental and control groups ($N = 48$). Also there were no significant negative associations among initial body weight and among AST ($r = -0.056$; $P > 0.05$), ALT ($r = -0.056$; $P > 0.05$), and ALP ($r = -0.057$; $P > 0.05$).

However, there was a significant negative association between final body weight and total bilirubin ($r = -0.392$; $P < 0.01$). Association between final body weight and conjugated bilirubin was significantly negative ($r = -0.364$; $P < 0.05$).

However, there were highly significant negative associations among final body weight and both AST ($r = -0.470$; $P < 0.01$) and ALT ($r = -0.417$; $P < 0.01$). There was also a significant negative association between final body weight and ALP ($r = -0.299$; $P < 0.05$). For that of liver weight, there existed highly strong negative associations among liver weight and total bilirubin ($r = -0.802$; $P < 0.01$), conjugated bilirubin ($r = -0.753$; $P < 0.01$), AST ($r = -0.800$; $P < 0.01$) ALT ($r = -0.712$; $P < 0.01$) and ALP ($r = -0.530$; $P < 0.01$) of all the groups ($N = 48$).

Discussion

In the present study it was observed that treatment of animals with acetaminophen (1,000 mg/kg) resulted in significant hepatic damage gauged by weight loss, decreased liver weight, increased total, and conjugated bilirubin, increased ALT, AST, and ALP activities in the serum. These changes in the marker levels will reflect in hepatic structural integrity. The rise in the AST is usually accompanied by an elevation in the levels of ALT, which plays a vital role in the conversion of amino acid to keto acids (Sallie *et al*,

1999). It is widely reported that hepatotoxic doses of paracetamol deplete the normal levels of hepatic glutathione, when its toxic reactive metabolite, N-acetyl-P- benzoquinoneimine (NAPQI) covalently binds to cysteine groups on proteins to form 3-(cysteine-S-yl) acetaminophen adducts. The glutathione protects hepatocytes by combining with the reactive metabolite of paracetamol thus preventing their covalent binding to liver proteins (Vermeulen *et al.*, 1992).

In living systems, the liver is considered to be highly sensitive to toxic substances. Thus the study of the activities of the different liver marker enzymes such as ALT, AST, ALP and serum bilirubin (total and conjugated) including serum proteins have been found to be of immense value in the overall assessment of clinical, subclinical and experimental liver damage.

In the present study, post- administration of aqueous leaf extract of *Cymbopogon citratus* to animals with paracetamol induced liver disease improved the alterations in the liver cell markers such as bilirubin, ALT, AST, and ALP. In addition to this was the improvement in body and liver weights following treatment with aqueous leaf extract of *Cymbopogon citratus*. The rationale for administering *Cymbopogon citratus* extract after induction of hepatotoxicity is because most therapeutic agents are usually administered after the expression of clinical diseases. Therefore it was hypothesized that lemongrass might affect the course of hepatic repair after the onset of paracetamol - induced hepatotoxicity and thus accelerate recovery in the rats. In the present study also there were significant reductions ($P < 0.05$) in the hepatic function markers such as total and conjugated bilirubin, ALT, AST, and ALP levels following treatment with various concentrations of aqueous leaf extract of lemongrass though not in a strongly dose - dependent manner. Arhoghro *et al.*, (2012) also reported a marked decrease in the activities of the three marker enzymes ALT, AST and ALP with administration of aqueous leaf extracts of *Cymbopogon citratus* in cisplatin induced hepatotoxicity.

The work of Koh *et al*, (2012) who also reported significant decrease in the activities of the marker enzymes ALT, AST, ALP, GOT with administration of aqueous leaf extracts of *Cymbopogon citratus* in carbon- tetrachloride- induced oxidative stress in rats corroborated the present findings.

The improvement on the levels of these liver function markers following the administration of the aqueous leaf extract of lemongrass to the rats suggests that the leaf extract was able to condition the hepatocytes so as to protect the membrane integrity against acetaminophen -induced leakage of marker enzymes into the circulation. The above changes can be considered as an expression of the functional

improvement of hepatocytes, which may be caused by an enhanced regeneration of parenchyma cells. It is likely that the hepatoprotective role of *Cymbopogon citratus* which stems from its antioxidant properties as reported by Koh *et al.*, (2012) may be traceable to its chemical composition. Several research works have been done aiming at enlarging the knowledge of the chemical composition of the essential oil of *Cymbopogon* leaves (Chisowa *et al.*, 1998). These studies have been revealing that although the chemical composition of the essential oil of *Cymbopogon citratus* varies according to the geographical origin, the compounds as hydrocarbon terpenes, alcohols, ketones, esters and mainly aldehydes, have constantly been registered (Costa, 1986; Trease, 1996). According to Omotade, (2009) the leaves of *Cymbopogon citratus* contains saponins, sesquiterpenes, lactones, steroids, flavonoids. Flavonoids are reported to exhibit antioxidant activity (Ramanathan *et al.*, 1989) and are effective scavengers of superoxide anions (Robak and Grygleuski, 1988). The aqueous leaf extract of lemongrass may have exhibited hepatoprotection due to its possible antioxidant content attributable to flavonoids. According to Singh *et al.*, (1991) saponins especially terpene glycosides enhance natural resistance and recuperative powers of the body.

Conclusion

The present study indicates that aqueous leaf extract of *Cymbopogon citratus* exerts significant protection against paracetamol- induced toxicity by its ability to improve the alterations of the hepatic function markers that were measured following paracetamol intoxication. Hepatoprotection was at maximum at the concentration of 400mg /kg b.w extract. This study has therefore established the effectiveness of this plant material in the treatment of liver disease especially drug induced type.

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