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Studies on lipid peroxidative and antioxidant activities of aqueous leaf extract of *Cymbopogon citratus* (Lemon grass) on paracetamol-induced hepatoticity in albino rats.

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Keywords

Lipid Peroxidative, Antioxidant ,*Cymbopogon citratus* (Lemon Grass), Paracetamol-Induced Hepatoticity

Abstract

Studies on lipid peroxidative and antioxidative activities of aqueous leaf extract of *Cymbopogon citratus* (lemongrass) on paracetamol-induced hepatotoxicity in albino rats were evaluated employing the following biochemical parameters: malondialdehyde (MDA), Catalase (CAT), Superoxide dismutase (SOD), and total Glutathione (GSH). 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 lOOmg/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 ±11.3g), liver weight (3.3 ± 0.2g), catalase (13.50 ± 2.88 nmol/min/ml), SOD (0.03 ± 0.01 U/ml), and total glutathione (6.51 ± 0.90 nM) with corresponding increase ($P< 0.05$) in MDA (1.60 ± 0.17 nmol/ml) of group II when compared with those of group 1: final body weight (182. 5 ± 15.8g), liver weight (5.9 ± 0.6g), catalase (25. 25 ± 1.91 nmol/min/ml), SOD (0.20 ± 0.02 U/ml), total glutathione (12.26 ± 1.27 uM), MDA (0.45 ± 0.09 nmol/ml). However, post- treatment of the diseased animal 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 result of this study indicate that aqueous leaf extract of *Cymbopogon citratus* anti-lipid peroxidative and antioxidant action against paracetamol- induced hepatic toxicity in rats.

Introduction

Aerobic organs such as the liver generate reactive oxygen species that induce oxidative tissue damage. These reactive oxygen species otherwise known as free radicals react with cell membranes and thus induce lipid peroxidation or cause inflammation, have been implicated as important pathological mediators in many clinical disorders such as heart disease, diabetes, gout and cancer (Slatter, 1984). Similarly, in carcinogenesis, reactive oxygen species are responsible for initiating the multistage carcinogenesis process starting with DNA damage and accumulation of genetic events in one or

few cell lines which leads to progressively dysplastic cellular appearance, deregulated cell growth, and finally carcinoma (Tsao *et al.,* 2004). However, the antioxidant enzymes which form a major defence mechanism convert active oxygen molecules into non-toxic compounds.

The detoxification of reactive oxygen species (ROS) is *a* major prerequisite of aerobic life which is accomplished via several enzymatic and non- enzymatic antioxidant mechanisms that are available in different cell compartment (Luis, 2009). Secondary mechanisms, restoring used cofactors and repairing altered biomolecules are also required; in addition to those triggering the expression of proteins damaged by ROS or needed to attain cell survival (Droge, 2002). These mechanisms need to be coupled to the intermediary metabolism for ATP, NADPH, and precursors supply, and depend on the dietary replenishment of essential components to maintain pro- oxidant reaction and cellular damage at a minimum level under basal conditions.

Because of its unique metabolism and relationship to the gastro intestinal tract, the liver is an important target of the toxicity of drugs, xenobiotics, and oxidative stress. Several mechanisms initiate liver cell damage and aggravate ongoing injury processes. Mitochondria are prominent target for the hepatotoxicity of many drugs including acetaminophen.

Dysfunction of these vital cell organelles therefore results in impairment of energy metabolism and an intracellular oxidant stress with excessive formation of reactive oxygen species and peroxynitrite. In addition to mitochondria, induction of cytochrome P450 isoenzymes such as CYP2EI also promotes oxidant stress and cell injury (Hartmut *et al.,* 2001). Once hepatocellular function is impaired, accumulation of bile acids causes additional stress and cytotoxicity. Cell injury, gut-derived endotoxin or a combination of both also activate Kupffer cells and recruit neutrophils into the liver. Although responsible for removal of cell debris and part of the host - defense system under certain circumstances these inflammatory cells initiate additional liver injury. However, cell injury and death is not only determined by the nature and dose of a particular drug but also by factor such as an individual's gene expression profile, antioxidant status, and capacity for regeneration (Hartmut *et al.,* 2001). Because of the many direct and indirect mechanisms of drug- induced liver cell injury, hepatotoxicity remains a major reason for drug withdrawal from pharmaceutical development and clinical use (Hartmut *et al.,* 2001).

According to Pryor et al.(2000), over the past 25 years, epidemiological studies have shown a diminished risk of chronic diseases in populations consuming diets high in fruits and vegetables. It has been suggested that antioxidants found in large quantities in fruits and vegetables may be responsible for this protective effect. (Halliwell,1994). Food antioxidants generally act as reducing agents, reversing oxidation by donating electrons.

Interestingly, recent studies of some tropical African plants have shown that many of such plants used in traditional medical practices have antioxidant properties (Ojo et al., 2005). This present paper reports the antioxidant and anti-lipid peroxidative properties of aqueous leaf extract of Cymbopogon citratus (lemongrass) in rats challenged with acetaminophen. Lemongrass is a coarse grass with a strong lemon taste used for cooking, medicinal teas and poypourri (Ojo et al., 2005). Lemongrass stocks are commonly used in the cuisines of Africa, the Middle East and Southeast 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).

The choice of lemongrass is premised on its widely acclaimed antioxidant properties. In addition it appears; relatively insufficient work has been done on the local lemongrass. This present study is therefore aimed at exploiting and exploring the acclaimed antioxidant properties of the plant. The choice of paracetamol is due to its commonness and because it is 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 Ihour. 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: Ig, 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 (Ig/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 was collected by cardiac

puncture. The blood was collected into EDTA bottle. The EDTA anticoagulated blood was centrifuged at 4,000 rpm for Smins and plasma obtained for malondialdehyde (MDA) estimation. The cells after thorough washing with normal saline and constitution of cell lysate using ice- cold distilled water were used for SOD, catalase and total glutathione (GSH) estimations.

Laboratory Procedures.

All reagents were commercially purchased and the manufacturer's standard operating procedure (SOP) strictly followed. Plasma malondialdehyde, MDA was determined using the TBARS Assay kit of Zepto Metrix Corporation USA which is a modification of that used by Lef` ever (1998). Cayman's Superoxide Dismutase Assay kit, USA, which is a modification of that used by Malstrom et al., (1975) was used for SOD determination. Cayman's catalase Assay kit, USA which is a modification of that used by Johansson and Borg (1988) was used for the determination of red cell catalase activity. Total glutathione was estimated using the Cayman's Total Glutathione Assay kit, USA which is a modification of that used by Eyer and Podhradsky (1986).

Statistical Analysis

All values were expressed as means ±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 Healthv control (n=8)	173.8 ± 16.9a	182.5 ±15.8a	5.9±0.6a
II Disease control (n=8)	170.9 ±13.5a	161.3 ±I1.3b	3.3±0.2b
III lOOmg lemongrass/kg" body weight (n=8)	171.9 ±12.5*	172.5 ± 7. 5C	5.0±0.2C
IV 200mg lemongrass/kg b.w. (n=8)	171. 3± 14.1a	173.8±11.9a,c	5.2±0.2C
V 300mg lemongrass/kg b.w. (n^8)	173. l±14.la	175.6 ±14.5a,c	5.4±0.4a'c
VI 400mg lemongrass/kg b.w (n=8)	172.5 ±11. 3*	175.0 ±13.9a,c	5.3±0.3C
ANOVA			
F- Value	0.057	3.348	50.942
P - Value	P>0.05	P < 0.05	P < 0.05

Values are means ± S.D. Means in the same column with different superscript letters) 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 oxidative and antioxidative parameters in paracetamol - induced hepatotoxicity.

Groups/Treatments	MDA (nmol/ml)	Catalase (nmol /min/ml)	SOD (U/ml)	Total Glutathione (MM)
I Healthy control (n=8)	0.45 ± 0.09a	25.25±1.91d	0.20±0.02a	12.26 ±1.27"
II Disease control (n=8)	1.60±0.17b	13.50±2.88b	0.03±0.0ib	6.51 ±0.90b
III 100mg lemongrass/kg	1.45 ± 0.20°	20.25±2.60C	0.05±0.01C	7.23 ± 0.58b b.w. (n=8)
IV 200mg lemongrass /kg	1.13 ± 0.14d	21.50±2.27C	,0.10±0.01d	9.64 ± 0.55C b.w. (n=8)
V 300mg lemongrass/kg	0.94±0.07e	22.88±3.31a'c	0.12±0.01e	9.82±0.88C b.w.(n=8)
VI 400mg lemongrass/kg	0.75 ±0.09*	23.00±2.88a>c	O.igiO.Ol1	9.98±0.54C b.w. (n=8) ANOVA
F - Value 80.615	18.391	342.959	50.381	
P-Value	P<0.05	P<0.05	P<0.05	P<0.05

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

Table 3: The values of Pearson's correlation coefficients calculated between MDA and the antioxidants (SOD, catalase and GSH)

		SOD	Catalase	GSH
MDA	Pearson correlation	- 0.924**	-0.699**	-0.837**
	Significance	0.000	0.000	0.000
	N	48	48	48

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

Discussion

Paracetamol is a commonly and widely used analgesic and antipyretic agent. Toxic dose of paracetamol especially to the hepatocyte leads to depletion of hepatic glutathione, when its toxic metabolite, N-acetyl-p- bebzoquinoneimine (NAPQI) covalently binds to cysteine groups on proteins to form 3-(cysteine-S-yl) acetaminophen adducts (Tirmenstein and Nelson, 1989). The glutathione protects hepatocytes by combining with the reactive metabolite of acetaminophen thus preventing their covalent binding to liver proteins. Lipid peroxidation is an autocatalytic process, which is a common consequence of cell death. This process may cause peroxidative tissue damage in inflammation, cancer and toxicity of xenobiotics. Malondialdehyde (MDA) is one of the end products of lipid peroxidation process.

This present study has demonstrated that toxic dose of acetaminophen caused increased lipid peroxidation- mediated hepatotoxicity in the rats by virtue of significantly increased plasnma level of malondialdehyde in the disease animals that did not receive treatment with lemongrass leaf extract. This finding is in agreement with those of Ojo *et* al.(2005) who did similar research.The significant elevation of malondialdehyde {MDA), an end product of lipid peroxidation in the plasma of paracetamol- treated rats suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals. However, treatment of the disease rats (Groups III-VI) with different concentrations of aqueous leaf extract of lemongrass resulted in significant decreases in the plasma level of MDA dose-dependently with maximum effect at the 400mg/kg concentration. It may be possible that the mechanism of hepatoprotection by aqueous leaf extract of *Cymbopogon citratus* is due to its antioxidant effect. This point is buttressed further by the finding of significant (P<0.05) elevations in the levels of red cell SOD, catalase and total glutathione following administration of the aqueous leaf extract to the animals with paracetamol-induced hepatotoxicity. The increases in the levels of red cell catalase , SOD activities and red cell total glutathione levels among the lemongrass treated rats suggests that this leaf extract possesses antioxidant properties which could be responsible for the hepatotoxicity ameliorating effects.

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, activity (Ramanathan *el 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. Correlation study among MDA and the antioxidants measured revealed highly significant ($P<0.01$) negative association, explaining the fact that increased oxidative stress is associated with diminished or impaired activities of the antioxidants and vice versa.

Conclusion

This present study indicates the anti-lipid peroxidative and antioxidant properties of aqueous leaf extract of *Cymbopogon citratus* on albino rats exposed to toxic dose of paracetamol. Maximum antioxidant protective role was demonstrated at 400mg/k.g body weight of extract. This has therefore established the ability of this plant leaf product to ameliorate oxidative hepatotoxicity due to toxic dose of acetaminophen.

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