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Full Length Research Paper

Inhibition of paracetamol-induced oxidative stress in rats by extracts of lemongrass (*Cymbropogon citratus*) and green tea (*Camellia sinensis*) in rats

Ojo, O. O.[1]*, Kabutu, F.R[1], Bello, M.[2] and Babayo, U.[3]

[1]Department of Integrated Science, Federal College of Education, PMB 2042, Yola, Nigeria.
[2]Department of Biology, Federal College of Education, PMB 2042, Yola, Nigeria.
[3]Department of Chemistry, Federal College of Education, PMB 2042, Yola, Nigeria.

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The water extract of green tea and lemongrass were investigated for their antioxidant effects in Wistar albino rats. Control and Test groups of rats were administered with paracetamol (2 g/kg) on the 10th day of the experiment while the test groups were pre-treated with 100 mg/kg body weight of green tea and lemongrass for 10 days. The effect of the extracts on serum levels of malondialdehyde, catalase activity and vitamin C were measured in paracetamol-induced hepatotoxicity in rats. Further, the effects of the extract on cholesterol and phospholipids were estimated. Cholesterol/phospholipids ratio was computed. The extracts of green tea and lemongrass produced significant (P<0.05) antioxidative effect by inhibiting the elevation of serum levels of malondialdehyde and catalase. The depletion of vitamin C was also prevented significantly (P<0.05). Moreover, the extracts were able to prevent alteration to membrane lipids by preventing the increase in cholesterol/phospholipid ratio by paracetamol. From these results, it was suggested that extracts of green tea and lemongrass could protect from paracetamol-induced lipids peroxidation perhaps by its antioxidative effects hence eliminating the deleterious effects of toxic metabolites from paracetamol.

Key words: Lipid peroxidation, paracetamol, catalase, malondialdehyde, cholesterol, phospholipids, membrane fluidity, lemongrass, green tea.

INTRODUCTION

Free radicals or oxidative injury now appears the fundamental mechanism underlying a number of human neurologic and other disorders (Atawodi, 2005). For instance in diabetes, increased oxidative stress which co-exist with reduction in the antioxidant status has been postulated: oxygen free-radical can initiate peroxidation of lipids, which in turn stimulates glycation of protein, inactivation of enzymes and alteration in the structure and function of collagen basement and other membranes, and play a role in the long term complication of diabetes (Sabu and Kuttan, 2002; Boynes, 1991; Collier et al., 1990).

*Corresponding authors E-mail cecejyde@yahoo.com.

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).

The evidence for a role of free radicals in disease is of several types. For example, many human diseases are present with increased production of activated species or with increased level of radical generating substances. Examples include granulocyte activation in inflammation or copper in Wilson's disease. Free radicals have also been implicated in the aetiology of diseases which include cancer, cataract, coronary heart disease, stroke, arthritis, Alzheimer's disease, and aging process (Gieseg, 1999, Maxwell, 2000).

Prime targets for free radical reactions are the unsaturated fatty acids which have a role to play in mem-

brane fluidity and receptor alignment and potentially, in cellular lyses. Free radical damage to sulphur-containing enzymes and other proteins culminates in inactivation, cross lining and denaturation. Damage to DNA can cause mutation that may be carcinogenic. Oxidative damage to carbohydrates can alter any of the cellular receptor functions including those associated with hormonal and neurotransmitter responses (Sies, 1995). Oxygen species such as hydroxyl radicals, superoxide anion radicals and singlet oxygen are agents that attack polyunsaturated fatty acids in cell membranes and give rise to lipid peroxidation in living systems. Lipid peroxidation is also strongly associated with aging and carcinogenesis (Yagi, 1987). Under normal physiological conditions low concentrations of lipid peroxidation products are found in tissues and cells. In the presence of oxidative stress more lipid peroxidation products are formed and released into the serum due to cell damage.

However, living systems are protected from active oxygen species and oxidative stress by cellular antioxidant enzymes such as superoxide dismutase, glutathione peroxidase and catalase. These living systems have also been reported to receive non-enzymatic protection by endogenous antioxidants such as α-tocopherol, ascorbic acid, β-carotene, and uric acid (Ames et al., 1981). Expanding body of evidences from epidemiological and laboratory studies have demonstrated that some edible plants as a whole or their identified ingredients with antioxidant properties have substantial protective effects on human carcinogenesis (Surh and Fergusson, 2003; Park and Pezzuto, 2002; Wattenberg, 1996; Greenwald, 2002; IARC, 1996; Fujiki, 1999; Tsao et al., 2004; Kinghorn et al., 2004; Mehta and Pezzuto, 2002).

Similar evidences also exist to demonstrate the chemopreventive capacities of ethnobotanicals and components of vegetable diets with free-radical scavenging potential on ulcers (Borreli and Izzo, 2000), diabetes (Sabu and Kuttan, 2002), memory and cognitive function (Howes and Houghton, 2003), Alzheimer's disease (Howes et al., 2003; Perry et al., 1998), age-related neurological dysfunction (Youdim and Joseph, 2001; Delanty and Dichter, 2000), cardiovascular and renal disorders (Anderson et al., 1999; Miller, 1998) and several other human ailments (Scartezzini and Speroni, 2000; Borek, 2001; Craig, 1999; Galvano et al., 2001; Lampe, 2003; Surh, 1999).

Over the past 25 years, epidemiological studies have shown a diminished risk of chronic diseases in populations consuming diets high in fruits and vegetables (Pryor et al., 2000). It has been suggested that antioxidants found in large quantities in fruits and vegetables may be responsible for this protective effect (Halliwell, 1994). Generally, food antioxidants act as reducing agents, reversing oxidation by donating and some antioxidants isolated from natural sources with high activity have been reported (Parasakthy et al., 1996; Kutsuzaki et al., 1993; Okamura et al., 1993).

Moreover, 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 paper reports the results of the investigation of the antioxidant potentials of lemongrass and green tea extracts in rats challenged with paracetamol.

Lemongrass, *Cymbopogon citratus* (DC. Ex Nees) stapf. and other *Cymbopogon* species (Fam. Gramineae), is a tall, coarse grass with a strong lemon taste used for cooking, medicinal teas and potpourri. Lemongrass stalks are commonly used in the cuisines of Africa, the Middle East and Southeast Asia. *C. citratus*, otherwise known as West Indian lemongrass, is native to Sri Lanka and South India and is now widely cultivated in the tropical areas of America and Asia. Its oil is used as a culinary flavouring, a scent, and medicine. A tea made from the leaves of West Indian lemongrass has been used to treat fevers, colds and upset stomachs (Hatch, 1995).

Lemongrass is also a folk remedy for coughs, consumption, elephantiasis, flu, gingivitis, headache, leprosy, malaria, ophthalmia, pneumonia and vascular disorders. Lemongrass is principally taken as a tea to remedy digestive problems; diarrhoea and stomach ache (Ozer et al., 1995). As a medicinal plant, lemongrass has been considered a carminative and insect repellent (Patrick, 1999).

Tea plant (*Camellia sinensis*) belongs to the Family Theaceae. The Family contains about 520 species and are placed in 28 genera. The Family is distributed through tropical and sub-tropical areas, but most species occur in Eastern Asia and South America (Ojo and Ladeji, 2005). Green tea leaf has been reported to contain polyphenols, most importantly flavonoids (catechin, epicatechin, epicatechin gallate, epigallocatechin gallate and proanthocyanins). Epigallocatechin gallate is considered the most active compound (Murray, 1995). Green tea is produced by withering the tea leaves. The leaves are then dried, steamed, rolled and further dried, so that when brewed in hot water, the complete leaf often unfolds.

MATERIALS AND METHODS

Plant materials

Lemongrass (whole plant) was collected in Sangere Village near the Federal University of Technology, Yola. Taxonomy of the species was determined in the Herbarium of the Department of Forestry and Wildlife Management of the University. The plant was dried at room temperature and ground to powder. A 10% aqueous suspension of the ground sample was prepared by mixing 10 g of the powdered plant with 100 ml of distilled water. The mixture was left for 12 h and then filtered with Whatman's No 1 filter paper. The filtrate was stored in refrigerator until used. Commercially available green tea of Highland Tea brand was purchased from Jimeta market. A stock tea solution was prepared by extracting 50 g of green tea in 1000 ml of distilled water overnight. The stock tea extr-

Table 1. Effects of extracts of green tea and lemongrass on serum levels of malondialdehyde, reduced ascorbic acid and catalase activity.

Groups	Malondialdehyde (nm/h)	Vitamin C (mg/100 ml)	Catalase (% activity)
Normal (No treatment)	10.24 ± 2.15[a]	89.23±3.25[a]	2.78±0.56[a]
Control (Paracetamol alone)	47.23±2.87[b]	21.02±2.28[b]	10.95±1.52[b]
Test 1 (Green Tea + Paracetamol)	27.59 ± 3.56[c]	65.35±1.04[c]	4.23 ± 0.36[c]
Test 2 (Lemongrass + Paracetamol)	15.23 ± 2.10[d]	75.81±2.78[d]	5.42±1.04[c]

Values are means ± standard deviation for five rats per group. Means in the same column having different superscript are significantly different (P <0.05).

act was diluted to give the desired concentration to be fed into different groups of rats. Administrations of tea and lemongrass extracts were through the oral route.

Experimental animals

Matured, male albino rats of Winstar strain of average weight of 130 g were used for this study. They were purchased from the animal house of the University of Jos. Experimental animals were kept under standard laboratory condition and were allowed to feed of commercial rat pellets and water *ad libitum*.

Chemicals

All reagents were purchased from Sigma Aldrich Chemical Co. (U.K). Commercially available paracetamol produced by Dana Pharmaceutical, Limited Nigeria was used to induce lipid peroxidation.

Treatment of animals

Rats were divided randomly into groups namely normal (no treatment), control (paracetamol alone), and test (extract of plants + paracetamol) with 5 rats in each group. Rats in normal group received an oral dose of 2 g/kg of sucrose on the 10th day of the experiment. Control rats were administered toxic dose (2 g/kg body weight) of paracetamol on the 10th day of the experiment orally and rats in Test 1 and Test 2 groups received a daily oral dose of 100 mg/kg body weight of the extracts of green tea and lemongrass respectively for 10 days and a single dose of 2 g/kg body weight of paracetamol on the 10th day. All rats were killed 12 h after administration of paracetamol under mild ether anaesthesia. Blood were collected, serum separated immediately and stored in the refrigerator. All serum samples were used within 12 h.

Measurement of biochemical parameters

Assay for malondialdehyde was done through the method of Janeiro (1998) by measuring thiobarbituric acid reactive substances produced during lipid peroxidation. Catalase activity was estimated by the procedure of Sinha (1972). Determination of serum reduced ascorbic acid level was by the method of Urbach et al. (1951). Concentrations of cholesterol and phospholipids were determined using the methods described by Searcy and Bergquist (1960) and Connerty et al. (1961) respectively. Cholesterol/phospholipids ratio was calculated.

Statistical analysis

Results were presented as mean±S.E.M for five determinations. Experimental data were analysed using analysis of variance (ANOVA). Duncan's multiple range tests was used to determine significant differences between means. The statistical analysis systems (SAS) package was used for statistical analysis.

RESULTS

The results obtained for the effects of extracts of green tea and lemongrass on serum levels of primary products of lipid peroxidation (malondialdehyde) and activity of a primary antioxidant enzyme, catalase of rats intoxicated with paracetamol are presented in Table 1. Treatment of experimental animals with paracetamol produced a significant ($p<0.05$) increase in the level of malondialdehyde and catalase activity in the serum. Values obtained for the serum level of malondialdehyde for normal rats (10.24±2.15 nm/h) was significantly different from the value obtained for control rats (47.23±2.87 nm/h). A significant depletion of serum reduced ascorbic acid in rats treated with paracetamol (21.02±2.28 mg/100 ml) was obtained as against the value (89.23±3.25 mg/100 ml) obtained for normal rats.

Administration of 100 mg/kg body weight of extracts of green tea and lemongrass significantly inhibited elevation serum levels of malondialdehyde and catalase activity and caused pronounced increase in the level of serum reduced ascorbic acid (Table 1). Rats fed with extracts of green tea recorded 27.59±3.56 nm/h, 65.35±1.04 mg/100 ml and 4.23±0.36% activity while 15.23±2.10 nm/h, 75.81±2.78 mg/100 ml and 5.42±1.04% activity were recorded for rats treated with lemongrass for serum levels of malondialdehyde, reduced ascorbic acid and catalase activity respectively.

Moreover, treatment of rats with paracetamol produced an increase in the serum level of cholesterol, a decrease in the level of phospholipids and a subsequent increase in the cholesterol to phospholipids ratio (from 0.46±0.03 in normal rats to 0.95±0.04 in control rats) (Table 2). This result is an indication of membrane rigidity caused by paracetamol. However, administration of green tea and lemongrass extracts significantly prevented changes in

Table 2. Effects of extracts of green tea and lemongrass on serum cholesterol and phospholipids.

Groups	Cholesterol (mg/dl)	Phospholipids (mg/dl)	Cholesterol/Phospholipids ratio
Normal (No treatment)	38.65 ± 2.54[a]	84.87±1.45[a]	0.48±0.03[a]
Control (Paracetamol alone)	58.95±3.19[b]	62.25±5.15[b]	0.95±0.04[b]
Test 1 (Green Tea + Paracetamol)	46.15±1.54[c]	94.64±2.56[c]	0.48±0.03c
Test 2 (Lemongrass + Paracetamol)	55.29±3.24[c]	102.68±4.13[c]	0.54±0.01[c]

Values are means ± standard deviation for five rats per group. Means in the same column having different superscript are significantly different (P <0.05).

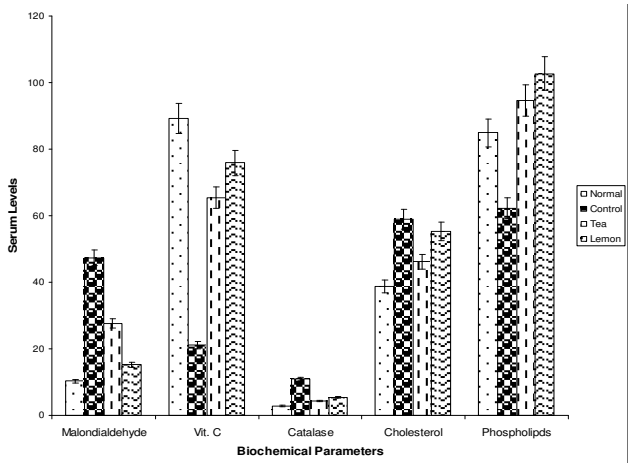

Figure 1. Inhibitive effects of extracts of green tea and lemongrass in rats treated with paracetamol.

membrane lipids and fluidity. Cholesterol/phospholipids ratio of 0.48±0.03 and 0.54±0.01 were recorded for green tea and lemongrass respectively.

DISCUSSION

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 cellular damage through metabolic activation of the chemical to highly reactive compounds such as free radicals, carbenes and nitrenes (Gupta et al., 2004). Moreover, the hepatotoxicity of paracetamol has been attributed to the formation of toxic metabolites when a part of paracetamol is activated by hepatic cytochrome p450 (Savides and Oehme, 1983), to a highly reactive metabolite N-acetyl-P-benzoquinoneimine (NAPQI) (Vermeulen et al., 1992). NAPQI is initially detoxified by conjugation with reduced glutathione (GSH) to form mercapturic acid (Moore et al., 1985). However, when the rate of NAPQI formation exceeds the rate of detoxification by GSH, it oxidizes tissue macromolecules such as lipid or -SH group of proteins and alters the homeostasis of calcium after depleting GSH.

However, cells have a number of mechanisms to protect themselves from the toxic effects of free radicals generated by paracetamol and other toxicants. Superoxide dismutase (SOD) removes superoxide (O_2^-) by converting it to H_2O_2, which can be rapidly converted to water by catalase and glutathione peroxide (GPx) (Halliwell et al., 1992). In addition, a large reserve of reduced glutathione is present in hepatocytes and red blood cells for detoxification of xenobiotics or free radicals. However, oxidative stress results in toxicity when the rate at which the free radicals are generated exceeds the cell's capacity for their removal. 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 and aging. Malondialdehyde (MDA) is one of the end products in the lipid peroxidation process (Kurata et al., 1993).

In this study, paracetamol-induced damage to erythrocytes was confirmed by the increases in lipid peroxidation products (MDA) and catalase activities, and decreases in membrane fluidity and serum level of reduced ascorbic acid. These results corroborate the findings of Ojo et al. (2005). Figure 1 showed inhibitive effects of green tea and lemongrass extracts on paracetamol-induced lipid peroxidation. Pre-treatment of rats with green tea and lemongrass extracts exhibited antioxidative properties by causing a decrease in the activity of catalase by 82.25% and 67.69% respectively. 53.10% and 86.51% decrease in the concentration of lipid peroxidation products (malondialdehyde) were obtained for rats treated with green tea and lemongrass respectively (Figure 1). The same trend was observed for the prevention of Vitamin C depletion from the serum. 65.01% and 80.33% inhibition of serum reduced ascorbic acid were obtained for green tea and lemongrass respectively (Figure 1).

Administration of paracetamol resulted in increases in erythrocyte membrane peroxidation, which may also lead to haemolytic changes. It has been shown that microviscosity of a membrane increases markedly with increases in cholesterol to phospholipids ratio thus leading to cellular rigidity (McConnell and Hubbell, 1971). Intoxication of rats with paracetamol may have altered membrane structure and function as suggested by the increases in

cholesterol and subsequent decreases in phospholipids concentrations, hence increased choleste-rol to phospholipids ratio. Cooper et al. (1977) reported that, alteration of bio-membrane lipid profile disturbs its fluidity, permeability, activity of associated enzymes and transport system. However, pre-treatment of rats with extracts of green tea and lemongrass inhibited the alteration of lipid membranes and hence prevented alterations in the levels of cholesterol and phospholipids *in vivo.* These results suggest that both green tea and lemongrass green tea and lemongrass play a role in peroxidation by inhibiting free radical attacks on bio-membranes.

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