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The Protective Nature of Garlic, Ginger and Vitamin E on CCl_4-Induced Hepatotoxicity in Rats

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Abstract: The protective nature of garlic, ginger and vitamin E on CCl_4-induced liver damage in rats were investigated using selected haematological indices: PCV, Hb, WBC, polymorphonuclear cells and lymphocytes. PCV was lowest in rats administered garlic-ginger (1:2) and CCl_4 (0.5 mL kg^{-1} bw) with a mean value of 28.7%. However, highest PCV values were obtained in rats that were given rat pellets alone without CCl_4.Hb of rats pre-treated with vitamin E recorded the lowest mean value while rats treated with garlic and ginger (2:1) only, recorded the highest mean value of 101.2 g dL^{-1}). Hb levels were increased in rats pre-treated with ginger, garlic and ginger (1:1), garlic and ginger (1:2). CCl_4 treatment caused an elevation of WBC in rats pre-treated with garlic, ginger, vitamin E and mixtures of garlic and ginger. This increase was observed to be highest in rats treated with a mixture of CCl_4, garlic and ginger (1:2) (10860 mm^{-3} L^{-1}). However, rats in the group treated with feed only without CCl_4 recorded the lowest WBC. Among the groups injected with CCl_4, a lower WBC value of 6200 mm^{-3} L^{-1} was recorded in the group fed vitamin E. These findings suggest that the pre-treatment of rats with mixtures of garlic and ginger prior to CCl_4-administration would result in an increase in the PCV and Hb values and a decrease in WBC values.

Key words: Carbon tetrachloride, haematological parameters, garlic, ginger, vitamin E

INTRODUCTION

The Scientific Community has long respected garlic as a plant possessing impressive therapeutic activity. Louis Pasteur first demonstrated the antibacterial properties in 1858 and later Albert Schweitzer used garlic to treat amoebic dysentery (Herbs n Health, 2004).

More recently, researchers have demonstrated that garlic helps protect against heart disease and cancer and possesses remarkable antibiotic effects. Garlic juice and its constituents can slow, or kill, more than sixty fungi and twenty types of bacteria including some of the most virulent ones known to man (Herbs n Health, 2004).

Garlic is used extensively as a food and as an ingredient in foods. It is listed in the USA as GRAS (Generally Recognized As Safe) (Leung, 1980). Garlic has long been used universally as a flavouring ingredient, functional food and traditional medicine. It is believed that the medical and beneficial properties may be attributed to specific constituents found in garlic and it's extracts, many studies suggest that organosulphur compounds are responsible for the biological activities (Block, 1992). Aged Garlic Extract (AGE) contains unique organosulphur compounds, among which are S-allylcysteine (SAC) and S-allylmercaptocysteine (SAMC). These constituents have been reported to show variety of biological activities, including antioxidant function (Imai *et al*., 1994; Ide and Lau, 1999), cancer prevention (Amargase and Milner, 1993) and antiatherogenic (Efendy *et al*., 1997). Ginger on the

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other hand is widely used around the world as a spice or food additive. Ginger is fried and eaten plain and used in curry paste and other source in India. A ginger extract with carbonated water makes the popular drink we call ginger ale. Ginger was also used in the middle age in Europe to flavour beer (Skiner, 2004). Ginger is listed by the council of Europe as a natural source of food flavouring (category N2). This category indicates that ginger can be added to foodstuffs in small quantities, with possible limitation of an active principle (as yet unspecified) in the final product (Council of Europe, 1981). It is used widely in food as a spice. In the USA, ginger is listed as GRAS (Generally Regarded As Safe.) (Leung, 1980). Ginger contains gingerol, a ginger oleoresin (combination of volatile oils and resins) that accounts for the characteristic aroma of ginger and explains its therapeutic properties, (Herbs n Health, 2004). Components of gingerol (Zingiberone, Bisabolene, Camphene Geranial, Linalool and Borneol) have recently been studied and found to possess beneficial properties for the treatment of poor digestion, heartburn, vomiting and preventing motion sickness.

Carbon tetrachloride (CCl_4) is a manufactured compound that does not occur naturally. It is a clear liquid with a smell that can be detected at low levels. It is most often found as a colourless gas. It is not flammable and does not dissolve in water very easily. These effects results from eating, drinking, or breathing it and possibly from exposure to skin. The liver is especially sensitive to carbon tetrachloride because it swells and cells are damaged or destroyed. The metabolic effects of CCl_4 inside mitochondria have been described (Brattin *et al*., 1984) and it has been found that damage to the calcium pump in the mitochondria (Albano *et al*., 1985) is dependent upon haloalkylation. It is well established that CCl_4 is metabolized in the liver to the highly reactive trichloromethyl radical and this free radical leads to auto-oxidation of the fatty acids present in the cytoplasmic membrane phospholipids and causes functional and morphological changes in the cell membrane (Recknagel, 1967). Lipid peroxidation, a free radical-induced mechanism, is implicated in the pathogenesis of several acute and chronic disorders. This is estimated using Malondialdehyde (MDA) concentration (Trible *et al*., 1987).

Ginger, garlic and a combination of ginger and garlic have been found to be useful in the prevention of severe hepatic injuries caused by a single dose of CCl_4 in rats (Patrick-Iwuanyanwu *et al*., 2007).

The aim of this study was to investigate improvements in haematological parameters such as PCV, Hb and WBC (Total and differential) by garlic, ginger, Vit. E and different mixtures of garlic and ginger in CCl_4-induced hepatotoxicity.

The induction of hepatic injury in rats would be achieved by the administration of CCl_4. However, prevention of CCl_4-induced liver injury by the drugs would be determined by monitoring the levels of selected haematological parameters: PCV, Hb and WBC

MATERIALS AND METHODS

Plant Material

Ginger (*Zingiber officinale*) and garlic (*Allium sativum*) were purchased from Fruit Garden Market D/Line, Port Harcourt, Nigeria.

Preparation of Ginger and Garlic

The samples were sundried for 4 days, ground into powdery form using an electric blender (moulinex) and stored in air tight containers.

Animals

Forty five adult male Wister albino rats (150-170 g) used in this study were obtained from the Animal House, Department of Biochemistry, University of Port Harcourt, Port Harcourt, Nigeria.

Table 1: Treatments administered to different rat groups

Groups	Treatments	No. of rats
1	Normal feed + CCl_4	5
2	Normal feed + Ginger + CCl_4	5
3	Normal feed + Garlic + CCl_4	5
4	Normal feed + Vit. E + CCl_4	5
5	Normal feed +Garlic: Ginger (1:1) + CCl_4	5
6	Normal feed + Garlic: Ginger (2:1) + CCl_4	5
7	Normal feed + Garlic: Ginger (1:2) + CCl_4	5
8	Normal feed-CCl_4 (control 1)	5
9	Normal feed + Garlic:Ginger (1:1)-CCl_4 (control II)	5

They were housed in standard cages (Griffin and George Modular Cage System) and left to acclimatize for 7 days to laboratory conditions before the commencement of the experiment. During the acclimatization, the animals were fed with pelleted rat chow and water *ad libitum*.

This study was conducted at the Animal House of the Department of Biochemistry, University of Port Harcourt, Nigeria in November, 2005.

The forty five rats were arranged into nine groups of 5 animals each.

Induction of Hepatic Injury

Carbon tetrachloride (CCl_4)-induced liver damage was achieved by injecting 0.5 mL kg^{-1} intraperitoneally after 28 days of feeding animals of groups 1 to 7 with commercial feed, ginger, garlic and vitamin E as stated in Table 1.

Preparation of Sample

Twenty four hour after the administration of CCl_4, the rats were anesthetized in Chloroform-saturated chamber. The animals were sacrificed by cervical dislocation and blood samples were collected using a 5 mL hypodermic syringe and needle through cardiac puncture. The blood samples for PCV, Hb and WBC were collected into EDTA containers without anticoagulant.

Analysis of the Haematological Parameters

The Red Blood Cells (RBC), White Blood Cells (WBC) and the differentials were estimated using the improved Neubauer counting chamber as described by Dacie and Lewis (1991). The Haemoglobin (Hb) concentration was determined by the Cyanmeth-haemoglobin method and the Packed Cell Volume (PCV) was determined by the micro method, also as described by Dacie and Lewis (1991).

Analysis of Data

The mean values of the control (s) and test samples were compared using the student's t-test (Zar, 1984). The significance level was set at $p \leq 0.05$.

RESULTS

Table 2 shows that the CCl_4-free groups (diet groups 8 and 9) positively improved the blood components. This observation is reflected by raised Packed Cell Volume (PCV), haemoglobin (Hb), decrease in White Blood Cell (WBC) and lymphocytes.The lowest level of PCV was observed in rats fed diets incorporated with garlic and ginger (1:2) (diet group 7), followed by rats fed with vitamin E (diet group 4). Rats that received mixtures of garlic and ginger (groups 5 and 6) had higher PCV values comparable to those that did not receive CCl_4 (groups 8 and 9) (Table 2). Also, higher Hb values were obtained in rats fed with mixtures of garlic and ginger (groups 5 and 6). Indeed, the Hb values of rats in group 6 were almost equal to those in group 9 (control II). Treatment with CCl_4 increased the WBC significantly ($p \leq 0.05$) in groups 1 and 7. However, low WBC values were obtained in rats in groups 4, 8, 9.

Table 2: The effects of garlic, ginger and vitamins on haematological parameters in CCl_4 hepatotoxicity

Group	Dietary formulae	PCV (%)	HB (g dL^{-1})	WBC (mm^{-3})	POLYS (%)	LYMPHS (%)
1	Feed + CCl_4	36.22±3.06^{a}	75.80±6.26^{a}	908 2.20±5268.97^{a}	49.60±10.11^{a}	50.40±10.11^{a}
2	Feed + Ginger + CCl_4	39.30±3.57^{a}	81.75±7.37^{b}	7750.00±700.00^{b}	69.00±9.02^{b}	31.00±9.02^{b}
3	Feed + Garlic + CCl_4	35.60±3.17^{a}	74.20±5.93^{a}	7000.00±2024.85^{c}	61.20±7.01^{c}	38.80±7.01^{c}
4	Feed + Vitamin E + CCl_4	31.81±2.48^{a,d}	67.00±5.10^{c}	6200.00±1584.30^{d}	74.80±7.46^{d}	25.20±7.46^{d}
5	Feed + (garlic + ginger) (1:1) +CCl_4	41.76±4.73^{b}	87.40±10.69^{d}	8040.00±1434.57^{b}	57.40±9.40^{c}	42.60±9.40^{c}
6	Feed + garlic + ginger(2:1) +CCl_4	48.16±1.82^{c}	100.40±3.85^{e}	7900.00±1102.27^{b}	53.60±4.04^{a}	46.40±4.04^{a}
7	Feed + garlic + ginger (1:2) +CCl_4	28.66±14.85^{d}	83.80±4.77^{b}	10860.00±907.19^{e}	67.00±9.22^{b}	33.00±9.22^{b}
8	Feed only (Control (1)	49.75±7.26^{c}	81.40±11.41^{b}	4960.00±1633.40^{f}	67.40±10.02^{b}	32.60±10.02^{b}
9	Feed garlic +ginger (1:1) (Control II)	48.72±3.45^{c}	101.20±7.30^{e}	5340.00±1388.52^{f}	66.20±7.40^{b}	33.80+7.40^{b}

*Values are means±SD for 5 replicates (n = 5), * Means with different superscripts are significantly different at the 0.05 level

DISCUSSION

Haematological parameters namely PCV, Hb, WBC and differentials were monitored during the chronic toxicity study in rats because of their role in providing reliable information concerning

haematological changes toxicants could cause. Haematological parameters have often been associated with health indices and are of diagnostic significance in routine clinical evaluation of the state of health.

The high PCV value of the control groups 8 and 9 could be as a result of the absence of the toxicant (CCl_4-free) in the diet. This also implies that there were little or no damage to the internal organs.

Ginger, garlic and a combination of ginger and garlic (groups 2, 5, 6) improved PCV levels (Table 2), indicating that erythrocyte concentrations may not be adversely affected when ginger and garlic mixtures are administered to rats prior to CCl_4 administration.

Erythrocytopenia observed in this study in rats fed with vitamin E (group 4) and garlic-ginger mixture (1:2) (group 7) could be as a result of the suppressing effect of CCl_4 on erythropoiesis (Krishan and Veena, 1980). This is also in agreement with the findings of Moscrella *et al*. (1994) who established that high serum levels of MDA in patients with liver cirrhosis was correlated with lower serum levels of vitamin E. Haematological studies in pollution associated microbial diseases of marine animals have shown reduction of red blood cell count (Anderson *et al*., 1974).

Fairbarks (1967) showed that xenobiotics cause haemolytic anaemia when sulphydryl groups of the erythrocyte membrane are oxidized, which inflicts injury to the erythrocytes membrane. This is in agreement with PCV and Hb values in this study as rat exposed to CCl_4 alone (group 1) gave lower levels of PCV and Hb, respectively. Ginger appears to inhibit haemolytic anaemia induced by CCl_4 as the values approached those of rats in control 1 (group 8). Interestingly, Hb levels above those of rats in group 8 were obtained in rats fed with garlic and ginger (2:1) and garlic-ginger mixture (1:1) (groups 6 and 9, respectively). This finding suggests that the administration of mixtures of garlic and ginger to anaemic patients may increase their erythrocyte levels. It implies that mixtures of garlic and ginger may possess constituents that would trigger the erythropoietic system to produce red cells.

Results in Table 2 reveals that there was a slight increase in WBC of rats fed with garlic and ginger (1:2) (group 7) when compared with rats administered CCl_4 alone (group 1). This may be attributed to a combination of physiological and biochemical factors in the metabolic system of the animals. According to Cardwell and Smith (1971), total WBC count was found to increase in diseased fish. There was no significant increase ($p \leq 0.05$) in the WBC count of rats fed with whole ginger (group 2), garlic to ginger (1:1) (group 5), Garlic to ginger (2:1) (group 6) when compared with control groups 8 and 9. Also, increase in WBC observed in CCl_4-treated rats may be attributed to the defensive mechanism of the immune system Hoeney (1985). These findings corroborates those of Adisa *et al*. (1999) who reported increased WBC count and a corresponding decrease in PCV values in albino rats infected with *T. brucei*.

The result of differential count as shown in Table 2 reveals that there was a marked significant increase ($p \leq 0.05$) in the polymorphonuclear cells and lymphocytes of rats treated with CCl_4 only and those fed with garlic to ginger (2:1) (group 6) when compared with controls 8 and 9. However, rats fed with 100% ginger (group 2), garlic to ginger (1:2) (group 7) showed no significant increase in polymorphonuclear cells and lymphocytes when compared with control 8.

The results obtained from the present study confirms that the pre-treatment of rats with garlic, ginger and mixtures of garlic and ginger prior to CCl_4-administration would result in an increase in the PCV and Hb values and a decrease in WBC values. From the foregoing, it would be deduced that CCl_4 has potentials for inducing anaemia in rats. However, administration of the drugs studied for 28 days prior to the administration of CCl_4 would boost the defensive mechanism of the immune and erythropoietic systems.

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