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Full Length Research Paper

A study of two weeks administration of copper sulphate on markers of renal function and feeding pattern of Wistar rats

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This study aimed at determining the changes in food consumption, water intake, plasma and urine concentrations of some organic constituents which are often used in the assessment of renal function following two weeks' administration of two doses of copper sulphate to Wistar rats. Fifteen adult male Wistar rats were randomly divided into three groups of five rats each. Group I (control group) received distilled water; groups II and III were given 100 and 200 mg/kg/day *p. o* of copper sulphate for 14 days, respectively. Significant reductions in food consumption and water intake were observed in group II when compared with the control and group III rats, but their body weight increased insignificantly throughout the study. The plasma urea concentrations of the treated rats were not significantly different from the control rats. The plasma creatinine levels of the experimental rats rose slightly, but not significantly different from the control rats. The creatinine and urea concentrations in the urine fell significantly in group II when compared with the control group. This was accompanied by decrease in creatinine clearance. Photomicrographs of the kidneys of both the control and experimental rats revealed no alteration in the histology of their renal tissue. It is concluded that acute copper sulphate administration to rats induced anorexia and suppression of renal function, thereby indicating the potential toxicity of the salt if ingested for a longer period.

Key words: Copper sulphate, kidney, creatinine, urea, rats.

INTRODUCTION

Copper (Cu) is an essential trace element and one of the most important heavy metals capable of producing toxic effects in man and animals when ingested acutely or chronically in excess. Copper compounds are widely used

in electrical industry, metallurgy, photography, painting, leather manufacture and water purification. Burning of copper sulphate in houses and shops (as a good luck charm and for religious activities) is a common practice among Buddhists and Hindus. Among the medicinal applications of copper is its utilization in certain types of dental amalgam and intrauterine contraceptive devices (IUCD). It appears in several enzymes, facilitates the absorption of iron, and helps to transmit electrical signals in the body. In high doses, however, the metal can be extremely toxic (Saravu et al., 2007). The circulation and proper utilization of copper in the body requires good functioning of the liver, gall bladder and adrenal glands. If any of these organs are impaired, the body cannot properly excrete and utilize copper. Initially, the copper will build up in the liver, further impairing its ability to excrete copper. As copper retention increases, it will build up in the brain, the joints and the lungs, adversely affecting the structure and function of the tissues. Copper is a powerful oxidant causing inflammation and free radical damage to the tissues. To avoid these toxic effects, it must be bound to the binding proteins, ceruloplasmin and metallothionein. These proteins can become deficient due to impaired adrenal and liver function which allows free copper to build up (Sinkovic et al., 2008).

Copper sulfate, one of the most available salts of copper, is a blue and odorless salt that is employed in various products such as fungicides, herbicides and insecticides (Blundell et al., 2003; Oldenquist and Salem, 1999). Copper sulfate is also found in chemistry laboratories as wettable powders and fluid concentrates. It can be absorbed through the gastrointestinal tract, lungs and skin causing both systemic and local toxicity including stupor, coma, convulsion, hypotension, shock, respiratory failure, pallor and jaundice (Oldenquist and Salem, 1999; Agarwal et al., 1993). Ingestion of significant quantities of copper sulphate carries a risk of multi organ failure.

Recently, the adverse effect of copper sulphate poisoning on sperm quality and testicular histopathology has been reported (Sakhaee et al., 2011). Studies carried out by Babaei et al. (2012) showed that short term administration of copper sulphate (14 days) at a dose of 100 and 200 mg/kg had deleterious effects on intracellular organelles of rat ovarian cells. Literature is scanty on the influence of short term administration of copper sulphate on the feeding pattern and renal function of rats hence, we decided to investigate the effects of acute ingestion of copper sulphate on the feeding pattern and some markers for the assessment of kidney function in Wistar rats at the same doses that have been reported to be toxic to their reproductive organs.

MATERIALS AND METHODS

Animal care and management

Fifteen (15) adult male Wistar rats weighing 120 - 150 g were used for this study. The rats were obtained from the Animal House of the College of Health Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria. Each rat was housed in a separate metabolic cage (Ohaus R Model; Ohaus, Pine Brook, NJ, USA) during the experiment to obtain a 24 h urine sample. The rats were kept under normal environmental conditions with a natural light/dark cycle and free access to standard rodent pellet diet (Caps Feed PLC, Osogbo, Nigeria) and water *ad libitum*. They were allowed to acclimatize in the laboratory for one week before the commencement of the study. The experimental procedures adopted in this study were in strict compliance with the guidelines on Experimental Animal Care and Use of Laboratory Animals in Biomedical Research, College of Health Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria.

Experimental design

The rats were randomly divided into three groups of five rats each. Group I (control group) received distilled water; groups II and III were given 100 and 200 mg/kg/day *p.o* of copper sulphate for 14 days, respectively.

Twenty-four hours after the last dose of treatment, the rats in each group were sacrificed by cervical dislocation and blood was obtained by cardiac puncture into separate heparinized bottles for hematological analyses. The blood was centrifuged for 20 min at 4000 rpm using a cold centrifuge (Centrium Scientific, Model 8881). The plasma was separated and analyzed for organic constituents that are routinely used in the assessment of kidney function. Thereafter, the kidney of each rat was carefully excised and fixed inside 10% formo-saline for histopathological studies.

Measurement of body weight

The body weight of the animals were measured once in a week using a weighing balance (Camry; Zhongshan Guangdong, China) during the experiment to access the weight gain or loss in each group.

Measurement of food consumption and water intake

The food consumption and water intake of each rat were determined daily. The volume of water and weight of food given to each rat was measured with a measuring cylinder and a weighing balance respectively. The difference between the previous day volume of water and weight of food, and the left-over was taken as the daily food consumption and water intake of the rats.

Haematological indices

The haematocrit (HCT), hemoglobin (Hb) concentration, red blood cell (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), white blood cell (WBC), granuloctye, monocytes, lymphocytes and platelet counts were measured using an auto-analyzer machine (SFRI Blood Cell Counter, H18 Light, France).

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Table 1. Effect of copper sulphate on food consumption (gram) of rats.

Week	I Control (Water)	II (100 mg/kg)	III (200 mg/kg)
Pre-treatment	20.51 ± 1.39	20.80 ± 1.66	21.71 ± 0.63
1	19.94 ± 1.11	13.71 ± 1.40$^{*\#}$	19.57 ± 1.02§
2	19.09 ± 1.60	15.86 ± 1.50	17.89 ± 0.98$^{\#}$

Values are given as mean ± SEM (n =5). * = Significantly different from control. § = Significantly different from group II. $^{\#}$ $^{=}$ Significantly different from pre-treatment ($p < 0.05$).

Table 2. Effect of copper sulphate on the body weight of rats (gram).

Week	I Control (Water)	II (100mg/kg)	III (200mg/kg)
Pre-treatment	167.0 ± 8.46	176.0 ± 8.72	178.0 ± 3.74
1	191.2 ± 9.02	174.0 ± 10.77	201.0 ± 4.00$^{\#}$
2	212.0 ± 11.68$^{\#}$	191.0 ± 12.39	212.0 ± 4.64$^{\#}$

Values are given as mean ± SEM (n=5). $^{\#}$ $^{=}$ Significantly different from pre-treatment ($p < 0.05$).

Table 3. Effect of copper sulphate on water intake (ml) of rats.

Week	I Control (Water)	II (100mg/kg)	III (200mg/kg)
Pre-treatment	45.63 ± 1.54	44.09 ± 4.84	49.66 ± 2.98
1	43.60 ± 1.50	31.40 ± 1.87*	39.69 ± 2.45§
2	38.89 ± 2.27	29.60 ± 2.85$^{*\#}$	38.21 ± 0.24$^{\#\S}$

Values are given as mean ± SEM (n=5). * = Significantly different from control. § = Significantly different from group II. $^{\#}$ = Significantly different from pre-treatment ($p < 0.05$).

Biochemical analysis

Levels of creatinine and urea were assayed by the use of appropriate biochemical kits purchased from Randox Laboratories (Crumlin, Co. Antrim UK). The plasma creatinine was estimated by alkaline picrate method (Bonsnes and Taussky, 1945). Urea assay was carried out in the plasma according to the method of Berthelot (Fawcett and Scott, 1960). The urine concentrations of urea and creatinine were estimated in the last samples of urine collected from the rats, using the same methods that were used in the analysis of plasma. Creatinine clearance was calculated.

Histopathological evaluation

The fixed kidney samples were dehydrated in graded alcohol and embedded in paraffin wax. They were then cut into 7-8 µm thick sections and stained with haematoxylin-eosin for photomicroscopic assessment using a Leica DM 750 Camera Microscope at 100 and 1000x magnifications.

Statistical analysis

The results obtained were expressed as mean ± SEM. The data were analyzed using one way ANOVA followed by Tukey's multiple comparison test using GraphPad 5.03 (GraphPad Software Inc., CA, USA). The results were considered significant when $p < 0.05$.

RESULTS

Food consumption and body weight

In the first week of treatment, a significant reduction in food consumption was observed in group II when compared with the control and group III rats (Table 1). Similarly, the food consumption of group II dropped significantly during the 1st week when compared with the pre-treatment value. Group III showed a significant decrease in food consumption during the 2nd week when compared with the pre-treatment value.

Although, a significant decrease in food consumption was observed in group III during the 2nd week of treatment, the body weight of rats in this group was significantly higher during the 1st and 2nd week than that of the pre-treatment Table 2.

Water intake and urinary volume

During the 1st and 2nd week, water intake fell significantly in group II when compared with the control and group III rats (Table 3). This reduction was accompanied

Table 4. Effect of copper sulphate on urine output (ml) of rats.

Week	I Control (Water)	II (100mg/kg)	III(200mg/kg)
Pre-treatment	5.34 ± 0.93	6.04 ± 1.04	10.24 ± 1.27*§
1	5.83 ± 0.59	4.01 ± 0.61	7.02 ± 0.83§
2	5.96 ± 1.13	5.65 ± 0.99	7.53 ± 2.13

Values are given as mean ± SEM (n=5). * = Significantly different from control. § = Significantly different from group II ($p < 0.05$).

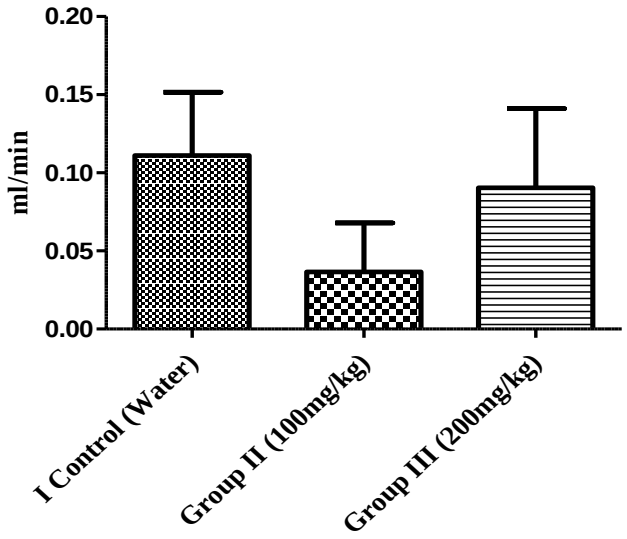

Figure 1. Effect of copper sulphate on creatinine clearance of rats. Values are given as mean ± SEM (n=5). No significant difference was observed between groups.

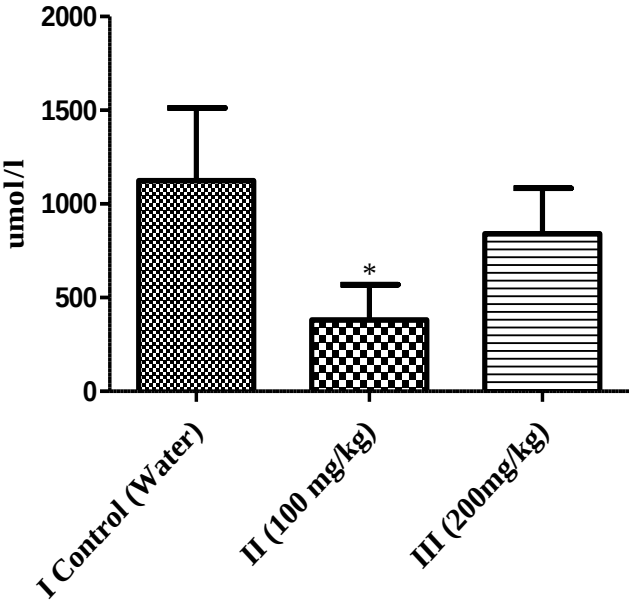

Figure 2. Effect of copper sulphate on urine creatinine concentration of rats. Values are given as mean ± SEM (n=5). * = Significantly different from control ($p < 0.05$).

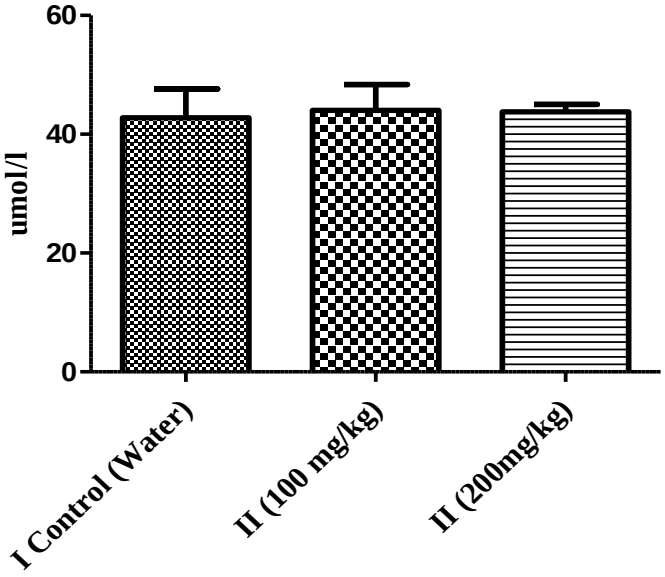

Figure 3. Effect of copper sulphate on plasma creatinine concentration of rats. Values are given as mean ± SEM (n=5). No significant difference was observed between groups.

by a fall in urine volume which was not significantly different from the pre-treatment (Table 4). A significant decrease in water intake was observed in groups II and III during the 2nd week when compared with the pre-treatment. The decrease in water intake of group III was accompanied by a significant fall in urine volume during the 1st week when compared with the pre-treatment.

Plasma creatinine, urine creatinine and creatinine clearance

A significant reduction in urine creatinine was seen in group II when compared with the control rats (Figure 2). There was also a fall in creatinine clearance in this group of rats (Figure 1). The plasma concentration of creatinine rose slightly but it was not significantly different from that of the control rats (Figure 3).

Urine urea and plasma urea

The concentration of urea in the urine of group II fell

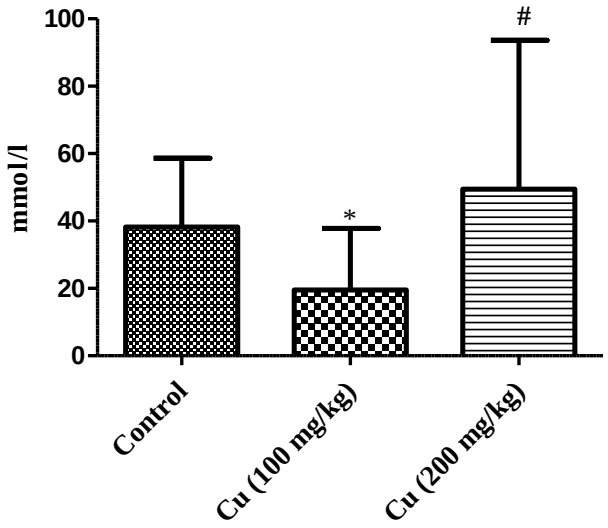

Figure 4. Effect of copper sulphate on urine urea concentration of rats. Values are given as mean ± SEM (n=5). * = Significantly different from Control. # = Significantly different from Group II ($p < 0.05$).

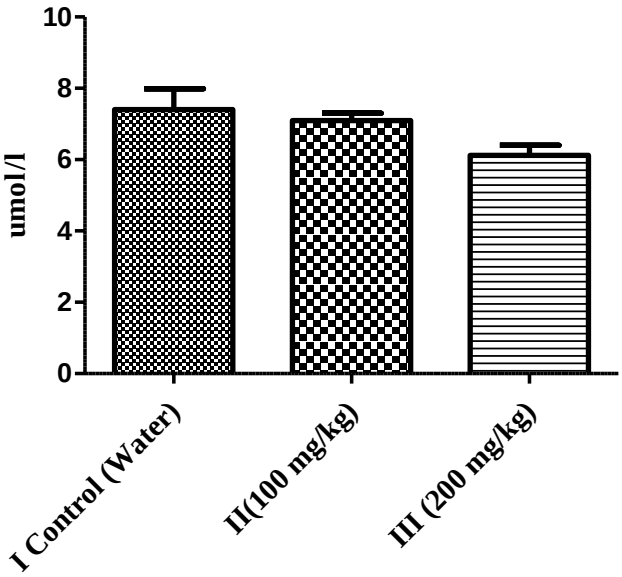

Figure 5. Effect of copper sulphate on plasma urea concentration of rats. Values are given as mean ± SEM (n=5). No significant difference was observed between groups.

significantly when compared with the control and group III rats (Figure 4). However, there was no significant difference in plasma urea concentration of group II when compared with the control and group III rats (Figure 5).

Haematological indices

A significant reduction in red blood cell count and hemoglobin concentration was seen in group III when compared with the control rats (Table 5). There was also a significant decrease in red blood cell count and haemoglobin concentration in this group when compared with group II.

Table 5. Effect of copper sulphate on haematological indices of Wistar rats.

	I (Control)	II (100 mg/kg)	III (200 mg/kg)
WBC count	4.08± 1.11	3.32± 0.64	4.04± 0.72
LYM (%)	78.46± 3.28	77.30± 3.00	76.80± 1.25
MON (%)	9.52± 0.61	10.28± 1.23	12.30± 0.39
GRAN (%)	12.02± 2.74	12.42± 1.98	10.90± 1.09
LYM count	3.26±0.99	2.52± 0.41	3.12± 0.57
MON count	0.38± 0.09	0.36± 0.08	0.52± 0.10
GRAN count	0.44± 0.09	0.44± 0.17	0.40± 0.07
RBC count	7.43± 0.18	7.81± 0.24	6.74± 0.30*§
HGBg count	14.60± 0.21	15.06± 0.58	13.22± 0.41*§
HCT (%)	44.18± 1.60	46.70± 1.33	41.42± 1.27
MCV (fl)	59.66 ±1.90	59.86± 0.68	61.70± 1.11
MCH (pg)	19.64± 0.31	19.22± 0.25	19.70± 0.27
MCHC (g/DI)	33.18± 1.18	32.18± 0.37	32.02± 0.37
PLT/UL	507.6 ± 36.01	635 ± 79.87	519.8 ± 37.54
MPV (fl)	6.56 ± 0.12	6.74 ± 0.09	6.94 ± 0.19
PCT (%)	0.32 ± 0.03	0.42±0.06	0.36±0.04

Values are given as mean ± SEM (n=5). *= significantly different from control. § = significantly different from group II ($p < 0.05$). WBC = White blood cells, LYM = lymphocyte, MON = monocyte, GRAN = granulocyte, HGB = haemoglobin, MCH = mean corpuscular haemoglobin, MCHC = mean corpuscular haemoglobin concentration, HCT = haematocrit, MCV = mean corpuscular volume, MPV = mean platelet volume, PLT = platelet count, PCT = platelet crit.

Photomicrographs of the kidneys

Photomicrographs of the kidneys of the experimental rats show normal glomerulus with distinct and intact glomerular spaces. Macula densa and epithelial cells appear normal when compared with the control rats (Figure 6).

DISCUSSION

This study demonstrated that oral administration of 100 and 200 mg/kg of copper sulphate for 2 weeks did not significantly alter the plasma concentration of some markers for the assessment of kidney function in the experimental rats. The most remarkable changes caused by copper sulphate were those related to food consumption and water intake. The changes were not dose dependent.

It has been suggested that one of the most consistent

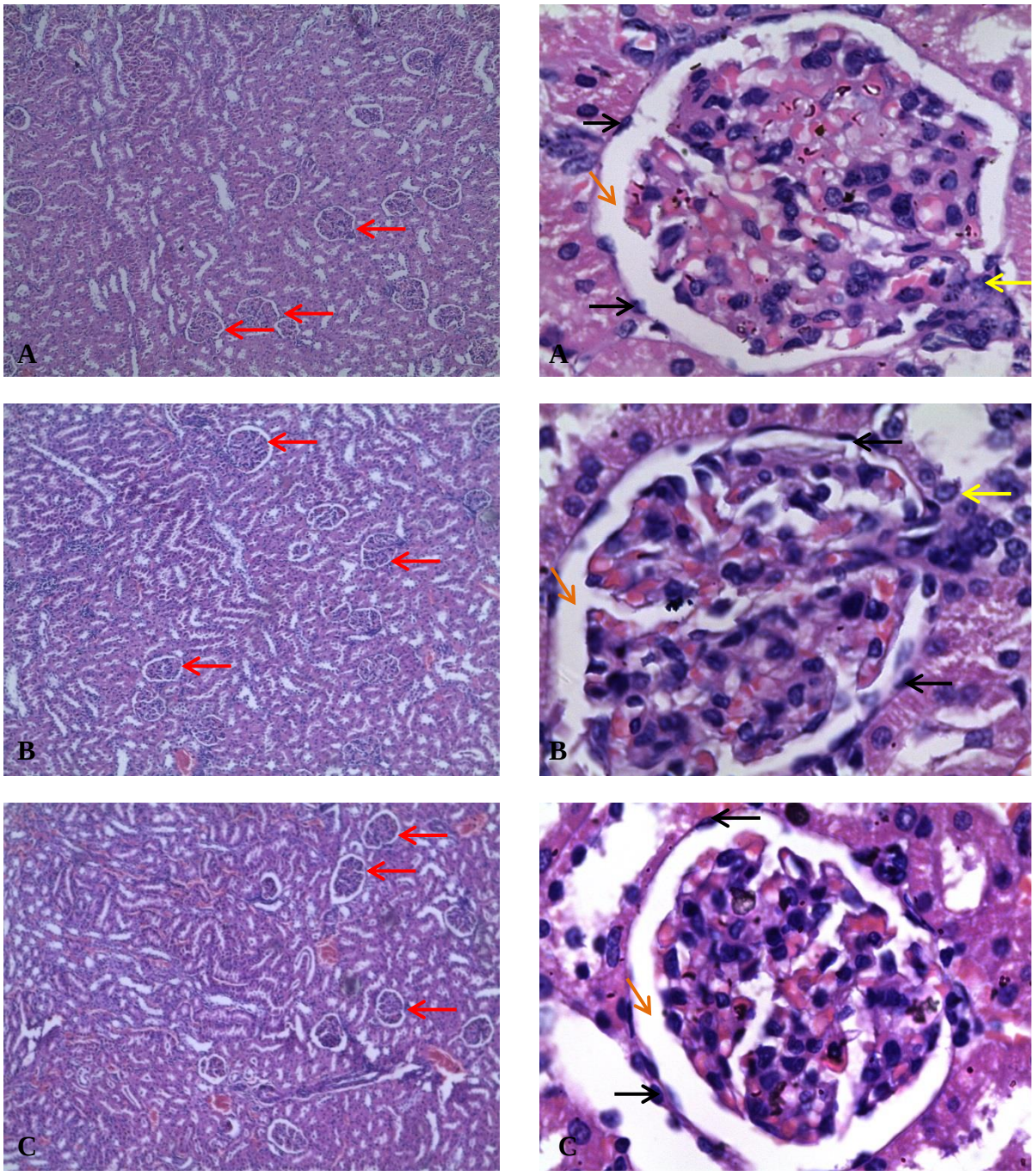

Figure 6. Photomicrographs of the renal cortex (A; Control, B; 100 mg/kg of $CuS0_4$, C; 200 mg/kg of $CuS0_4$) showing normal (red arrow) glomerulus. The glomerular spaces (brown arrow) are distinct and intact. Macula densa (yellow arrow) and epithelial cells (black arrow) appear normal across the groups. Magnification 100x (upper panels) and 1000x (lower panels).

clinical signs indicative of toxicity in animals administered with copper is a reduced growth rate (Haywood, 1979) which is accompanied by a fall in body weight. In the present study, a significant decrease in food consumption was observed in the experimental groups without a corresponding decrease in body weight. Water intake and urinary volume also fell in group II when compared with the control rats. The observed increase in body weight despite the significant reduction in food consumption and water intake appeared paradoxical. However, this could have been due to an increased ability of the rats to convert the reduced food they took into body mass (Thompson et

al., 1987) or water retention which was evident as reduced urine output. This needs to be further investigated.

There are reports indicating that copper exposure is associated with renal dysfunction (Galhardi et al., 2004; Sinkovic et al., 2008). Acute renal failure due to tubular necrosis is characterized by oliguria, anuria, increased blood urea nitrogen concentration, albuminuria and hematuria (Bauer, 1975). Tubular necrosis and cellular pleomorphy were reported in rats that received supplemented diet with a copper content of 3 g/kg for up to 5 weeks (Haywood et al., 1985). In this study, the plasma concentration of urea of the experimental rats was not significantly different from the control rats. The plasma creatinine level of the treated rats rose marginally, but was not significantly different from the control rats. The urea and creatinine concentrations in the urine was reduced significantly in rats that were administered 100 mg/kg of copper sulphate compared with the control rats (Figures 2 and 4). Similar observations have been reported in animal studies by Abou-Seif et al. (2003) who found that administration of copper (II) complexes in rats caused a significant increase in superoxide dismutase activity without alteration in blood urea and creatinine levels when compared with the control rats.

Plasma urea and creatinine are the most sensitive biochemical markers used in the assessment of renal tissue damage, because urea and creatinine are excreted through the kidneys. Therefore, in cellular damage, there is retention of urea and creatinine in the blood. The decrease in creatinine clearance is an indication of tissue damage, which was supposed to have been accompanied with a significant increase in plasma concentration of creatinine. The fact that the plasma levels of urea and creatinine did not rise significantly in the experimental rats could be due to the acute nature of this study. The fall in urine createnine is a further evidence of reduced ability of the renal tubules to extract and remove creatinine from the plasma of the experimental rats. The fall in urine excretion of urea may have resulted from diminished urea synthesis or a diminished intake of protein (Ganong et al., 2009).

The main function of red blood cells is the transporttation of oxygen into tissues of the body. Any pathological condition that affects the red blood cell alters its function and this may be detrimental to the body (Agbor et al., 2005). Substances that demonstrate significant effect on red blood cell and haemoglobin would have effects on bone marrow, kidney and haemoglobin metabolism (Young and Maciejewski, 1997). A significant decrease in red blood cell and haemoglobin was observed in group III when compared with the control rats. This may have resulted from the hemolysis of red blood cells or decreased ability of the kidney to secrete erythropoietin. Erythropoietin stimulates the bone marrow to produce red blood cells. This observed change is in accordance with the finding of Savaru et al. (2007) who reported that one of the major haematological manifestations of copper sulphate poisoning is intravascular haemolysis. Glucose-6-phosphate dehydrogenase, which has a major function in main taining the NADPH concentration in the red blood cell, is inhibited by copper (Joshi et al., 2002). NADPH is also necessary for maintaining the level of reduced glutathione, which in turn protects the red blood cell against the haemolytic effects of oxidizing substances. The inhibition of this enzyme by copper or impaired intestinal absorption of iron (Pamila et al., 1991) could explain the reduction in haemoglobin concentration that was seen in the experimental groups. Decrease in the haemoglobin levels may impair oxygen supply to various tissues resulting in slow metabolic rate and low energy production (Ahmad et al., 1995; Atamanalp and Yanik, 2003). Intravascular hemolysis and a direct action of copper on the kidneys often lead to tubular necrosis (Iyanda et al., 2011; Matovic et al., 2010). The hem pigment released due to hemolysis and direct toxic effect of copper released from lysed red cells contributes to tubular epithelial damage of the kidney. However, the photomicrographs of the kidneys of experimental rats revealed no significant alteration in the histology of their renal tissue. This suggests that copper sulphate induced tubular necrosis could require a longer period of exposure to develop in rats.

Conclusion

From the results of this study, it is concluded that acute copper sulphate administration to rats induced anorexia, and suppression of renal function, thereby indicating the potential toxicity of the salt if ingested for a longer period.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Full Length Research Paper

Radiation protection and anti-oxidative effects of garlic, onion and ginger extracts, x-ray exposed albino rats as model for biochemical studies

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The present study investigates and examines the comparative effects of plant extracts such as, garlic, ginger and onion on some organs (liver, kidney and heart) of x-ray exposed rats, using and assaying some biochemical enzymes. Twenty (20) albino rats with an average weight of (155.00 ± 2.01 g), divided into five groups were used for the study. The rats with exception of the control were exposed to x-ray with ionizing radiation at a dose of 525 kv/s. The results indicate some toxicity conferred on the rats were reversed when fed with diet containing garlic, ginger and onion, as evidently shown in some of the biochemical parameters examined that includes: body weight gain, plasma and femur alanine aminotransferase (ALP) activity; enzymatic changes in super oxide dismutase (SOD), catalase (CAT) level in the liver, kidney and heart. Feeding with ginger, garlic and onions extracts failed to restore the x-ray induced inhibition of aldenylate oxidase (AO) and sulphite oxidase (SO) activities in the liver and heart. Data of the study indicates that garlic and onions had more beneficial effects on radiation induced toxicity in rats, as increased body weight gain (P<0.05) of rats caused by radiation which was reduced by feeding with garlic and onion by -65.11 and -30.02%, respectively as against radiation exposed rats fed ginger (-3.17%) compared to rats treated with only x-ray. Together, the results obtained from this study suggest that garlic, ginger and onion may have significant anti-radiation properties, bearing the reversal and restoration observed after radiation exposure on some of the investigated biochemical parameters. Such properties properly harnessed will be helpful in combating cellular oxidative stress.

Key words: Radiation, x-ray, ionizing, radical scavengers, anti-oxidant, medicinal plants.

INTRODUCTION

The discovery of x-rays by Roentgen in the year 1895 and radioactivity by Becquerel in the year 1896 is considered a turning point in human health care as the x-rays allowed to peep inside the human body (Roentgen, 1895; Becquerel, 1896). Although harmful effects of ionizing radiations were reported within a few months of

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discovery of x-rays, the real magnitude was not known until, study of occupational workers like physicians and scientists handling radioactivity gave a clearer picture of the harmful effects of ionizing radiations, which was further strengthened after the study of Japanese atomic bomb survivors of 1945. It is now fairly well established that radiation produces deleterious effects on the organisms and widespread use of radiation in diagnosis and therapy, industry and, energy sector with inadvertent exposure during air and space travel, nuclear accidents and nuclear terror attacks requires concerted safeguards.

Ionizing radiations produce deleterious effects in the living organisms, the rapid technological advancement has increased human exposure to ionizing radiations enormously. Attempts of protection against the deleterious effects of ionizing radiation by pharmacological interventions were made as early as 1949 and there have been continuous efforts to search for radio-protectors that are of great help for human application (Nair et al., 2010).

X-ray, is an electromagnetic radiation. The wavelength of x-ray is between 0.01 and 10 nm corresponding to frequencies between 30 petahertz (PHz) to 30 exahertz (EHz) (3×10^{16} Hz) and energies in the range of 120 eV to 120 KeV (Novelline, 1997). They are shorter in wavelength than ultraviolet rays and longer than gamma rays. Exposure to x-ray as ionizing radiation can be a health hazard. Such exposure to radioactive agents has been shown to produce various pathological changes in living systems like lipid peroxidation (LPO) (Yagi, 1988) and damaging of cellular macromolecules. Further, studies had shown that fathers exposed to radiation are more likely to have infants who contract leukemia especially if such exposure is closer to conception or includes two or more x-rays of the lower gastrointestinal tract or lower abdomen (Focea et al., 2012). The risk of radiation is greater to unborn babies, so in pregnant patients, the benefits of x-ray should be balanced with the potential hazards, to the unborn foetus (Focea et al., 2012). Avoiding unnecessary x-rays (especially CT scans) will reduce radiation dose and any associated cancer risk (Foeca et al., 2012).

The use of plants, natural products are thoughts to be beneficial in protecting against radiation-induced damage, they are less toxic compared to synthetic compounds used at their optimum protective dose levels (Bhatia et al., 2006; Sharma and Sisodia, 2000). Thence, the interests has always existed in development of potential drug of plant origin, been a good sources of potent but non-toxic radioprotectors (Blokhina et al., 2003).

Antioxidants of plant origin include vitamin E, C, selenium, phenolic compounds, carotenoids and flavonoids (Chandha, 1996). Earlier studies in the laboratory indicated that oral administration of carotene (Sharma and Sisodia, 2000) and plant extract of spinach (Bhatia et al., 2006), amaranths (Yadav et al., 2004) and linseed (Bhatia et al., 2006), to Swiss albino mice protects various tissues against oxidative stress induced by radiation. It has been postulated (Souza et al., 2006) that mechanisms of action of these plants includes the activation of metabolizing enzymes which detoxify carcinogens, the suppression of DNA adduct formation, the inhibition of the production of reactive oxygen species, the regulation of cell-cycle arrest and the induction of apoptosis (Campana, 2004, Souza et al., 2006).

Radio-protective, anti-oxidative efficacy of garlic extract has been reported (Block, 1995; Singh et al., 2005). Onions contains quercetin that is believed to have anticancer, anticholesterol and antioxidant properties. Administration of the dried bulb *Allium cepa* at a concentration of 20 mg/kg was active against x-irradiation (Block, 1995).

This study was aimed to look at the effects of the plants (garlic, onion and ginger) extracts with specific biochemical enzymes such as, AO, SOD, CAT, SO and ALT, with comparative study on the effects of the extracts; we deduced that garlic and onions were more potent than ginger albeit, the results suggests each extract confer some degree of radio-protective combined with anti-oxidative properties.

MATERIALS AND METHODS

Experimental animals

Twenty (20) white female albino rats (wistar strain) bred in the Animal Unit of the College of Health Sciences, Delta State University, Abraka were used in the study. The animals were housed in standard rat cages and left to acclimatize to laboratory condition for two weeks. The laboratory animals were kept at room temperature with access to water in accordance with the international guide for the care and use of laboratory animals (Committee for update of the guide for the care and use of laboratory animals, 2011).

Plant materials

Fresh ginger (*Zingiber officinale*), garlic (*Allium sativum*) and onions (*Allium cepa*) were sourced locally in Warri, Delta State, Nigeria.

Preparation of extracts

Fresh bulbs of onions, garlic and ginger were carefully dressed and frozen at +4°C. About 100 ml of chilled distilled water were added to 100 g of each of onions, garlic and ginger and crushed in a homogenizer. The resultant slurry was squeezed and filtered through a fine cloth and the filtrates of garlic, onion and ginger extracts were quickly frozen at -20°C until used.

Treatment of animals

The animals were divided into five groups (garlic, ginger, onions, test and control) with four rats in each of them. Rats in group of garlic, ginger and onions received twice weekly 5 ml/kg of extracts of garlic, ginger and onions, respectively orally by intubation. This

treatment was maintained for five weeks during which the rats were given growers mash and water. During this treatment, rats in garlic, ginger, onions and test groups were exposed to x-ray. The control group received nothing except food and water and they were not exposed to x-ray. The initial and final weights of the rats in each group were also recorded.

Radiation dosage

The experimental albino rats (wistar strain) except those in the control group were exposed to the effect of ionizing radiation from x-ray at the Delta State University Health Center, Radiology Department, Abraka at a dose of 525 kv/s for 2 s.

Collection of samples

At the end of the treatment period each rat was anaesthetized in chloroform (May and Baker, England) saturated chamber, the rat, carefully dissected. The liver, heart, kidney, femur bone and blood of each rat were collected and stored at -20°C until required. The blood was collected directly from the heart using sterilized needle and syringe into well labeled heparinized containers.

Preparation of tissue homogenate

Ten percent homogenate of each organ was prepared in pre-chilled pestle and mortar using 4 ml, 1-x ice-cold phosphate buffersaline (PBS) solution(137 mMNaCl, 10 mM phosphate, 2.7 mMKCl pH 7.4). The homogenate was centrifuge at 5000 g for 10 min and the supernatant obtained were used for biochemical analysis. Also blood in the heparinized container was centrifuged at 3000 g for 10 min after which it was separated into plasma and red cells. The plasma at the top was pipetted carefully without the red portion into well labeled clean containers for estimation of the creatinine level present and enzyme analysis.

Enzymes assays

Different enzymes including AO, SOD, CAT, SO, and ALT, using Omarov et al. (1998), Misra and Fredorich (1972), Cohen et al. (1970), and Macleod et al. (1961) methods, the enzymes activities were assayed.

Statistical data analysis

The data are presented as ± SEM, and are analysed statistically by one-way analysis of variance (ANOVA), this is followed by Duncan's multiple range test using SPSS 10.0 computer software package (SPSS Inc., Chicago, U.S.A). The correlation analysis was performed, quoting the Pearson correlation coefficients and test of significance, with significance accepted at $P < 0.05$.

RESULTS

The present study explored the effectiveness of ginger, garlic and onions on the survival of albino rats after exposure to x-ray. Bearing, information related to the radio-protective and anti-oxidative effects of ginger, garlic and onion are yet been compared, ascertaining degree and potency of the different plants, we designed our experiments to get this established. Here we provide information on the radio-protective properties of the different plants, combined with the comparative indices of the plants in radiation protection.

We investigated effects of garlic, ginger and onion on body weight gain and organ/body weight ratio of x-ray exposed rats (Figure 1A, B and C). We observed exposure to x-rays significantly increased ($P < 0.05$) the body weight gain of rats (Figure 1A, B and C). Whilst, feeding ginger, onions and especially garlic to these rats indicates a reversal of weight gain of the rats to a level comparable to the control (Figure 1A), and onion with relative effect (Figure 1C). Conversely, there seems to be no significant difference ($P>0.05$), observed in the liver/body weight ratio of rats in all experimental groups, except those feed with garlic (A). The parameter value remained significantly ($P>0.05$) unchanged, after feeding x-ray exposed rats with ginger (Figure 1B) and onion (C) however, similar feeding with garlic significantly increased (26.3%) heart/body weight ratio relative to the control (A). Thus, the kidney/body weight ratio of x-ray exposed rats was significantly ($P < 0.05$) decreased as compared to the control (B, C). Prior treatment of x-ray exposed rats with ginger and onion had no significant effect on the kidney/body weight ratio, but similar treatment with garlic restored the value to a level comparable to the control (A).

Moreover, the effects of same extracts (garlic, ginger and onion), on the enzymatic activity of ALT in the plasma, liver, kidney and heart of x-ray exposed rats were analyzed (Tables 1 and 4). The results indicate exposure to x-rays significantly ($P < 0.05$) increased plasma ALT activity relative to control, whilst feeding of garlic to x-ray exposed rats reversed the effect of x-ray however, ginger and onion had no significant ($P>0.05$) effect on plasma ALT activity relative to the test (Tables 1 and 4). Also, no significant change ($P>0.05$) was observed in the activity of ALT in the liver, kidney and heart of rats in all the experimental groups (Table 1)

Similarly, Table 2 presents its effects on the activity of aldehyde and sulphite oxidases in the organs of rats exposed to x-rays. The liver AO and SO activities were significantly ($P<0.05$) increased in the x-rays exposed rats (Table 4). The feeding with garlic, ginger and onion (AO excluded) had no effect on radiation-induced increase in liver AO and SO activities. Conversely, feeding of onion reversed the effect of x-ray on the liver AO activity, as the value obtained was comparable to control (Tables 2 and 4). Like in the liver, the heart AO and SO activities of x-ray treated rats were significantly ($P<0.05$) increased relative to control. The heart AO and SO activities remained significantly ($P<0.05$) increased in x-ray exposed rats fed with garlic, ginger and onion. On the other hand, feeding of ginger restored the level of heart SO activity to a level comparable to control (Tables 2 and 4). And no significant ($P>0.05$) changes were observed in the kidney AO and SO activities of rats in all

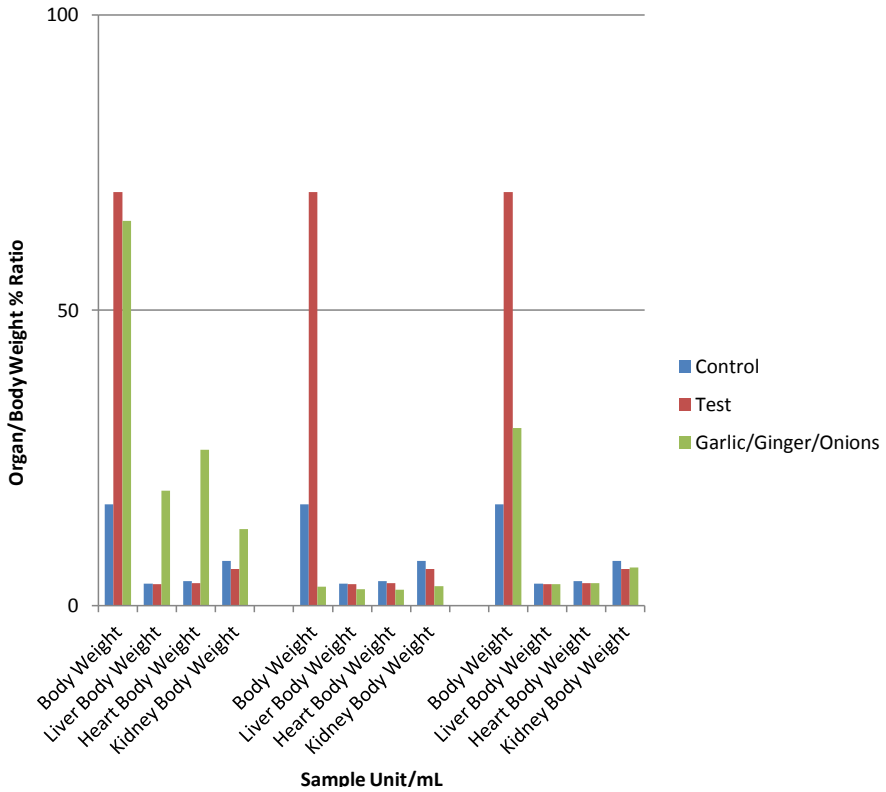

Figure 1. Comparative effects of the extracts (A) garlic, (B) ginger and (C) onion.

Table 1. Effect of garlic, ginger and onions on the activity of alanine aminotransferase (ALT) in the plasma, liver, kidney and heart of x-ray exposed rats.

Parameter	Control	Test	Garlic	Ginger	Onions
Plasma	272.5±47.4^{a}	626.8±119.9^{b}	337.5±58.1^{a} (-46.16%)	132.5±33.4^{b} (-78.86%)	107.5±20.4^{b} (-82.85%)
Liver	1729±317.6^{a}	1120±175.0^{a}	1736±369.3^{a} (+55.00%)	1582±234.4^{a} (+41.25%)	1337±341.8^{a} (+19.38%)
Kidney	476.8±108.4^{a}	385±25.1^{a}	527.3±29.9^{a} (+36.96%)	450.0±59.2^{a} (+16.88%)	836.0±116.6^{a} (+117.14%)
Heart	505.75±131.49^{a}	295.2±143^{a}	507.50±138.28^{a} (+71.92%)	649.25±215.31^{a} (+119.94%)	315.00±31.44^{a} (+6.71%)

Results are expressed as mean ± SEM. Means of the same row with different letters as superscript are significantly different (P < 0.05). %= Percentage efficiency in restoring the level of enzyme. Activity of ALT is in units/ml.

Table 2. Effect of garlic, ginger and onions on the activity of aldehyde oxidase and sulphite oxidase in the organs of rats exposed to x-ray.

Parameter	Control	Test	Garlic	Ginger	Onions
Liver					
AO	66.86±0.03^{a}	73.05±0.03^{b}	73.40±0.16^{b} (+0.48%)	73.10±0.10^{a} (+0.07%)	66.86±0.24^{a} (-8.47%)
SO	11.12±0.06^{a}	15.49±0.32^{b}	14.71±0.24^{b} (-5.04%)	13.88±0.24^{b} (-10.39%)	14.88±0.30^{b} (-3.94%)
Heart					
AO	48.32±1.08^{a}	67.35±0.15^{b}	68.78±0.18^{b} (+2.12%)	68.86±0.65^{b} (+2.24%)	63.54±0.59^{c} (-5.66%)
SO	9.17±0.44^{a}	13.40±0.42^{b}	11.9±0.48^{b} (-11.19%)	10.73±0.43^{a} (-19.93%)	13.57±0.70^{b} (+1.27%)
Kidney					
AO	72.74±0.02^{a}	72.30±0.07^{a}	73.03±0.02^{a} (+1.01%)	71.37±0.10^{a} (-1.29%)	72.24±0.18^{a} (+0.08%)
SO	10.14±0.64^{a}	11.89±0.32^{a}	10.29±0.53^{a} (-13.46%)	10.92±0.57^{a} (-8.16%)	11.26±0.14^{a} (-5.30%)

Results are expressed as mean ± SEM. Means of the same row with different letters as superscript are significantly different (P < 0.05). Activity of AO is in units/g tissue. Activity of SO is in Units/g tissue.

Table 3. Effects of garlic, ginger and onions and the levels of superoxide dismutase (SOD), catalase and lipid peroxidation in the organs of rats exposed to x-ray

Parameter	Control	Test	Garlic	Ginger	Onions
Liver					
SOD	46.67±5.45[a]	33.33±5.45[b]	33.33±10.90[b]	48.67±6.62[a]	53.33±5.45[b]
CAT	1.03±0.092[a]	0.919±0.023[b]	0.390±0.087[c]	0.735±0.080[b]	0.631±0.040[b]
LP	0.53±0.05[a]	0.34±0.03[b]	0.89±0.12[c]	0.41±0.17[a]	0.62±0.03[a]
Heart					
SOD	26.64±10.61[a]	33.33±5.45[a]	40.00±9.44[b]	37.33±4.35[a]	40.00±0.00[b]
CAT	0.207±0.005[a]	0.231±0.004[b]	0.234±0.002[b]	0.232±0.001[b]	0.257±0.001[b]
Kidney					
SOD	33.33±5.45[a]	26.67±10.61[b]	40.00±9.44[a]	37.33±4.35[a]	40.00±0.00[a]
CAT	0.189±0.002[a]	0.197±0.002[b]	0.195±0..002[b]	0.195±0.004[b]	0.202±0.010[b]
LP	0.43±0.03[a]	0.77±0.01[b]	0.32±0.04[a]	0.79±0.13[a]	0.25±0.05[a]

Results are expressed as mean ± SEM. Means of the same row with different letters as superscript are significantly different (P < 0.05). Activity of SOD is in Units/g tissue. Activity of CAT is in Units/g tissue. LPO is expressed in Units/g tissue.

Table 4. comparative effects of garlic, ginger and onions on the activity of different enzymes including; alanine aminotransferase (ALT), aspartate aminotransferase (AST), plasma creatinine,

Parameter	Control	Test	Garlic	Ginger	Onions
Plasma					
ALT	272.5±47.4[a]	626.8±119.9[b]	337.5±58.1[a]	132.5±33.4[b]	107.5±20.4[b]
AST	196.0±11.8[a]	549.9±46.6[b]	213.3±10.9[a]	180.0±8.2[a]	236.7±2.70[a]
Creatinine	16.1±2.6[a]	12.9±5.5[a]	21.5±0.2[a]	15.2±6.8[a]	14.8±4.6[a]
Liver					
ALT	1729±317.6[a]	1120±175.0[a]	1736±369.3[a]	1582±234.4[a]	1337±341.8[a]
AST	868±217.4[a]	592.0±76.0[a]	345.3±16.6[a]	751.3±137.1[a]	1820±233.6[c]
AO	66.86±0.03[a]	73.05±0.03[b]	73.40±0.16[b]	73.10±0.10[a]	66.86±0.24[a]
SO	11.12±0.06[a]	15.49±0.32[b]	14.71±0.24[b]	13.88±0.24[b]	14.88±0.30[b]
Kidney					
ALT	476.8±108.4[a]	385±25.1[a]	527.3±29.9[a]	450.0±59.2[a]	836.0±116.6[a]
AST	180.7±16.9[a]	176.0±46.6[a]	434.2±61.9[b]	845.7±140.2[c]	491.8±10.92[b]
AO	72.74±0.02[a]	10.14±0.64[a]	73.03±0.02[a]	71.37±0.10[a]	72.24±0.18[a]
SO	10.14±0.64[a]	11.89±0.32[a]	10.29±0.53[a]	10.92±0.57[a]	11.26±0.14[a]
Heart					
ALT	505.75±131.49[a]	295.2±143[a]	507.50±138.28[a]	649.25±215.31[a]	315.00±31.44[a]
AST	3290.0±540.71[a]	1963.5±596.18[b]	3430.0±418.05[a]	770.0±239.09[b]	3930.5±376367[a]
AO	48.32±1.08[a]	67.35±0.15[b]	68.78±0.18[b]	68.86±0.65[b]	63.54±0.59[c]
SO	9.17±0.44[a]	13.40±0.42[b]	11.9±0.48[b]	10.73±0.43[a]	13.57±0.70[b]

Aldehyde oxidase (AO) and sulphite oxidase (SO) in Liver, Kidney and Heart of x-ray Exposed Rats. Results are expressed as mean ± SEM. Means of the same row with different letters as superscript are significantly different (P < 0.05), % = Percentage efficiency in restoring the level of enzyme. Activity of ALT is in Units/ml. Activity of AST is in Umits/ml. Activity of ALP is in Units/ml. Creatinine concentration is expressed in µmol/L. Activity of AO is in Units/g tissue.

the experimental groups (Tables 2 and 4). Exposure to radiation significantly (P<0.05) decreased liver LPO relative to control (Table 3). However, the liver LPO was increased in x-ray rats by feeding ginger (+20.6%) and

onion (82.4%) to levels comparable to control. Feeding with garlic of x-ray exposed rats significantly increased (+161.7%) the level of LPO as compared to rats treated to only x-ray (Table 3). The heart SOD activity of radiation-exposed rats was not significantly different (P>0.05) from control (Table 3). The heart SOD activity of x-ray exposed rats also remained at a level not significantly (P>0.05) different from control. However, upon feeding with ginger, garlic and onion extracts, there was noticeable difference (Table 3), with significant increment on feeding with garlic (+20.0%) and onion (+20.0%) as compared to the test (Table 3). There was no, significant change recorded on the CAT activity of x-ray exposed rats relative to control. It remained significantly the same after feeding with garlic, ginger and onion (Table 3).

DISCUSSION

A major interest in radiation biology and chemistry is identification of chemical agents that are able to protect humans from ionizing radiation. Hence, the study and use of plants and natural products that may be beneficial in protection against these radiation induced damage are of significant; they are less toxic or in most cases, practically nontoxic compared to synthetic compounds. Here, we look at the effects of the plant extracts on aldehyde oxidase, super oxide dismutase, catalase, sulphite oxidase and alanine aminotransferase, the results suggests these extract to confer anti-oxidative properties. Using same extracts, we have further study its effects on other biochemical enzymes including, aspartate amino-transferase, alkaline phosphatase, plasma creatinine and lipid peroxidation (manuscript in preparation), and the results indicates the extracts possesses radioprotective efficacy against the damaging effects of ionizing radiation from x-ray.

Changes in the body weight and organ/body weight ratio have often been used as indices of toxicity (Bhatia et al., 2001). The significant alteration (Figure 1) observed in these parameters in the x-ray exposed rat is an indication of x-ray toxicity and this is in agreement with earlier reports (Bhatia et al., 2001).

Changes in SOD, CAT and LPO have often been used as an index of oxidative stress. These parameters were studied in view of the free radical generating capacity of radiations. The results obtained indicate that the activity of SOD and CAT in the liver and kidney of rats treated with x-ray was significantly (P<0.05) decreased relative to control (Tables 3 and 4). This decrease may be due to the effect of x-ray exposure. Such decrease in antioxidative enzymatic activities in response to X -ray had also been reported previously (Focea et al., 2012).

A previous study (Ernst and Pittler, 2000), indicated that plant extracts eliciting radioprotective efficacy contain immunostimulants, cell proliferators, anti-inflammatory and antimicrobial agents, some of which may act in isolation as well as in combination with other constituents from the same plant. And may also augment the efficacy of compounds present in other plant species to provide protection against radiation induced damage. A number of plants studies including, *Allium sativum*, *Aloe vera*, *Centellaasiatica*, *Osimum sanctum*, *Zingiberofficinale* etc. (Chen et al., 1999), have bio-active constituents including flavonoids, exhibit anti-inflammatory properties, and the radioprotective response in several cases is mediated by this effect (Ernest and Pittler, 2000). Such plants have invariably also, showed anti-oxidative properties (Uma Devi and Gansoundari, 1995). Hence, the use of plants and their bio-active constituents with antioxidative properties and activities is highly relevant in mitigation of radiation-induced oxidative stress and damages (Souza et al., 2006).

We set out to monitor the effects of the extracts on known biochemical enzymes involved in causing or managing oxidative stress. For instance, aldehyde oxidase (AO) is a known redox enzyme that catalyses both oxidation and reduction reactions. It helps in the oxidation of carbohydrates and other aldehyde including acetaldehyde produced from ethyl alcohol. It is involved in the intermediate metabolism of several agents in the metabolism of nicotine. The active enzyme is involved in the bio-activation of some known xenobiotics including the antiviral pro-drug, Faciclovir to the active metabolite, Peniclovir (Rashidi et al., 1997). Co-administration of famiclovir and a potent aldehyde oxidase inhibitor could reduce or abolish its antiviral efficacy.

The active super oxide dismutase enzyme catalysis the dismutation of superoxide into oxygen and hydrogen peroxide. It is an important antioxidant defense in virtually all cells exposed to oxygen species. SOD catalyzed reaction of dismutation of superoxide follows a simple path, with reactions:

$$\text{Cu, Mn, Fe} - \text{SOD} + O_2^- \rightarrow \text{Cu, Mn, Fe} - \text{SOD} + O_2$$

$$\text{Cu, Mn, Fe} - \text{SOD} + O_2^- + 2H^+ \rightarrow \text{Cu, Mn, Fe}^{(n+1)+} - \text{SOD} + H_2O_2$$

The oxidation state of the metal cation oscillates between n and n+1. The importance of SOD in biological systems are further exemplified by its potent ability to form a reactions with itself (dismutation), or with another biological radical such as nitric oxide (NO) to check mate release of oxidizing radicals. Reaction of the superoxide anion radical (O_2^-) is known to spontaneously dismutes to O_2 and hydrogen peroxide (H_2O_2) in a quite rapid timing of ~10^5 M^{-1} s^{-1} at a neutral pH 7. Moreover, the reaction rate of super oxide {E + S}, is thought to be diffusion limited because of its fast turnover number of ~10^9 M^{-1} s^{-1}, in comparison to other known enzyme with limiting factor, frequency of collision between itself and superoxide. The enzymatic mechanism of SOD is well

studied the active site of the cytosolic enzyme in eukaryotes contains a Cu^{2+} and Zn^{2+} that is coordinated to the side chain of histidine residue (Campana, 2004).

Thus a negatively charged superoxide is electrostatically binds to a very positively charged catalytic site at the bottom of a channel, where O_2 binds to Cu^{2+} and the guanido group of an arginine residue. Here electron is transferred from superoxide to cupric ion to form Cu^{+} and O_2, which are released, followed by a second superoxide in the active site, which binds to Cu^{+}, arginine and H_3O^{+}. The bound O_2 acquires an electron from CU^{+} and two protons from its binding partner to form H_2O_2 and regenerate the Cu^{+} state of the enzyme (Campana, 2004).

Catalase is a common enzyme found in nearly all-living organisms. Its functions include catalyzing the decomposition of hydrogen peroxide to water and oxygen (Ho et al., 2004). Catalase has one of the highest turnover rates of all enzymes; one molecule of catalase can convert millions of molecules of hydrogen peroxide to water and oxygen per second (Eisner and Aneshansley, 1999). The reaction of catalase in the decomposition of hydrogen peroxide is: $2\ H_2O_2 \rightarrow 2\ H_2O + O_2$. Although, the complete mechanism of catalase reaction is not fully known, it is however, believed to occur in two stages:

$$H_2O_2 + Fe(III)\text{-}E \rightarrow H_2O + O{=}Fe(IV)\text{-}E(.+)$$

$$H_2O_2 + O{=}Fe(IV)\text{-}E(.+) \rightarrow H_2O + Fe(III)\text{-}E + O_2$$

Herem Fe-E represents the iron centre of the heme group attached to the enzyme. Further, catalase is known to oxidize different toxins, such as formaldehyde, formic acid and alcohol. In doing so, it uses hydrogen peroxide according to the following reaction:

$$H_2O_2 + H_2R \rightarrow 2H_2O + R$$

Again, the exact mechanism of this reaction is not known. At the cellular level, it is a fact that H_2O_2 is a dangerous and harmful by-product of many normal metabolic processes, preventing cellular damage this is quickly converted into other, less dangerous substances. Hence, catalase, are frequently used by cells to rapidly catalyze the decomposition of hydrogen peroxide into less reactive oxygen species and water molecules (Blokhina et al., 2003).

SOD has been established to work in tandem with CAT to remove O_2^{-} and H_2O_2, respectively (Blokhina et al., 2003). Thus they are endogenous catalytic oxygen scavengers, and play key roles in cellular defense against reactive oxygen species under physiological conditions (Coudray et al., 1995). Moreover, SOD is inducible and the level of this enzyme will always increase with the toxic oxidations (Coudray et al, 1995). It follows therefore that the decrease in these antioxidant enzymes observed in the rats treated with x-ray may lead to lipid peroxidation occasioned by oxidative stress (manuscript in preparation). Thus the significant ($P<0.05$) decrease in SOD and CAT may account for the corresponding increase in level of LPO observed in the kidney of the x-ray exposed rats (manuscript in preparation). However, it is noteworthy that despite the significantly decreased activity of SOD and CAT in the liver of x-ray treated rats there was a significantly decreased LPO (manuscript in preparation). This seems to suggest that other antioxidative enzymes or molecules may be responsible for maintaining LPO at a level below that of the control. However, this is not surprising as the liver is better equipped at combating free radicals than other organs.

Sulphite oxidase is an enzyme present in mitochondria of eurkaryotic cells. It oxidizes sulphite to sulphate using cytochrome C, transfers the electrons produce to the electron transport chain, allowing generation of ATP in oxidative phosphorylation (Cohen et al., 1972; Tan et al., 2005; D'Errico et al., 2006). Sulphite oxidase is a metalloenzyme that utilizes a molybdopterine cofactor and a heme group. It is one of the cytochrome b_3 enzymes and belonging to the superfamily of oxo-transferase that include DMSO reductase, xanthine oxidase and nitrite reductase. In mammals, the expression levels of suphite oxidase, is high in the liver, kidney and heart and very low in spleen, brain, skeletal muscle and blood. The lack of functional sulphite oxidase has a disease phenotype known as sulphite oxidase deficiency. This rare but fatal disease causes neurological disorders, mental retardation, physical deformities, the degradation of the brain and death. Reasons for the lack of functional sulphite oxidase include a genetic defect that leads to the absence of molybdopterin cofactor and point mutation in the enzyme (Karakas and Kisker, 2005).

Though there is scarcity of information on the effect of x-ray on both AO and SO, the decreased activity of these enzymes in the liver and heart of x-ray exposed rats (Tables 2 and 4) is indicative that exposure to x-ray may impair biotransformation of xenobiotics. Although, the mechanism of x-ray induced inhibition of these oxidative enzymes cannot be offered with certainty.

Conclusions

The objective of this study was to examine, the comparative effects of garlic, ginger and onions on some biochemical parameters in organs of x-ray exposed rats. Changes in the body weight and organ/body weight ratio as observed in the x-ray exposed rats are indicative of x-ray toxicity. The study further showed that garlic, ginger and onion contain bioactive substances, which are radio-protective further consolidating garlic and onion with more radio-protective and anti-oxidative properties than ginger. Our results indicate these plants could exert these functions through modulation in activity of several meta-

bolizing enzymes that activate and detoxify (SOD, ALT, AO and SO), carcinogens and inhibit DNA adduct formation. Most of these enzymes have antioxidative and free radicals scavenging properties thus, involved in regulation of cell proliferation, apoptosis and immune responses.

Conflicts of Interests

We declare that there are no conflicts of interests.

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