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Full Length Research Article

CURATIVE EFFECTS OF AQUEOUS EXTRACT OF SWEET BELL PEPPER (*CAPSICUM ANNUUM L.*) FRUITS AGAINST LEAD INDUCED OXIDATIVE STRESS

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ABSTRACT
Lead is a heavy metal which poses environmental and occupational hazard to the populace due to its extensive use in developing countries like Nigeria. This study was aimed at evaluating the curative effects of aqueous extract of *Capsicum annuum L*. fruits on Lead induced oxidative stress in wistar rats. Lead acetate was administered to rats for 4 weeks and subsequently treated with 125mg/kg, 250mg/kg and 500mg/kg of the aqueous extract for 4 weeks. The results showed significant ($p \geq 0.05$) difference in the malondialdehyde concentration in all the groups except the extract control group (E-c). Activities of glutathione reductase, reduced glutathione, superoxide dismutase and catalase were reduced in the lead control group (L-c) compared to the treated groups. The deleterious effect of lead was ameliorated in the groups treated with 500mg/kg aqueous extract and the vitamins treated group. It is thus concluded that the aqueous extract amiliotrated the oxidative damage caused by lead in rats.

Keywords: Lead acetate: *Capsicum annuum L.:* Recovery: Oxidative Stress.

INTRODUCTION

Lead compounds are found to be toxic and miners are prone to occupational hazards from misuse and over exposure. Lead sheathing for telephone and television cables continues to be a sizable outlet for lead. The unique ductility of lead makes it particularly suitable for this application because it can be extruded in a continuous sheath around the internal conductors. The use of lead in pigments has been a major outlet for lead but is decreasing in volume. White lead, $2PbCO_3 \cdot Pb(OH)_2$, is the most extensively used lead pigment. Other lead pigments of importance are basic lead sulfate and lead chromates. Lead also may have a role as a cofactor in carcinogenesis (Arif *et al*., 2015). Acute poisoning from a single exposure is rare but may result from the ingestion of solutions of soluble lead salts (lead acetate, lead carbonate). The primary symptoms are related to local irritation of the gastrointestinal tract and include: vomiting and abdominal colic. Pain in the legs, cramps and paresthesiae may follow, with shock, haemolytic anaemia and renal dysfunction. Depression, coma and death occur within 1 to 2 days.

Capsicum annuum L. contains free (fatty) oils, steam volatile oil, carotenoids, resin, protein, fibre and mineral elements. The carotenoids are responsible for the diverse and brilliant colours as well as their antioxidant effects (Bartley and Scolnik, 1995). Capsanthin accounts for 30-60% total carotenoids while the rest is Casorubin with small quantities of carotene, zeaxanthin and cryptoxanthin in a full ripe pepper (Matsufiji *et al.,* 1998). Lee *et al.* (1998) observed the presence of flavonoids (quercetin and luteolin) in pepper fruits.

However, the aqueous extract of *Capsicum annuum L*. fruits was found to be relatively safe at 5000 mg/kg body weight of the rats (Zubairu *et al*., 2015).

It has been reported that the adminitration of nutrients may have a positive effect on an individual's health. Nutrients supply not only energy, essential amino acids, fiber, Vitamins, and minerals but also some phytochemicals such as antioxidants (tocopherols, carotenoids, Vitamin C and phenolic compounds.) that may have various important functions in the body. Antioxidants are molecules that can neutralize free radicals by accepting or donating an electron to eliminate the unpaired condition (Daood *et al.*, 1996). Typically, this means that the antioxidant molecule becomes a free radical in the process of neutralizing a free radical molecule to a non-free-radical molecule. But the antioxidant molecule will usually be a much less reactive free radical than the free radical neutralized. The antioxidant molecule may be very large (allowing it to "dilute" the unpaired electron), it may be readily neutralized by another antioxidant and/or it may have another mechanism for terminating its free radical condition. Molecules with loosely-held hydrogen atoms can use those hydrogen atoms like electrons to neutralize free radicals. The hydrogen atoms are called reducing equivalents, and the molecules having such hydrogen atoms are said to be in a reduced state.

Dietary components, which are capable of acting as antioxidants, are likely to be beneficial by augmenting cellular defenses and protecting the cell against damage caused by free radicals, by acting as radical scavengers, reducing agents, potential complexes of pro-oxidant metals, and quenchers of singlet oxygen formation (Hochstein and Atallah, 1988; Gutteridge, 1993; Doblado *et al*., 2005: Oboh, 2005).

Recent studies have shown that anti-oxidants may prevent potentially diseases producing cell damage that can result from natural bodily processes and from exposure to certain hazardous chemicals (Klein *et al*., 2011).

MATERIALS AND METHODS

Animals

Thirty-five (35) two months old wistar strain albino rats weighing between 120-150 mg/kg b.w were used for this study. The rats were purchased from The Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. All rats received humane care in compliance with the guidelines of Animal Care by Kasenen and Inhila (2009). The rats were acclimatized for two weeks. The rats were divided at random into seven groups with five rats in each of the groups for the *in vivo* antioxidant studies.

Reagents

Lead acetate was purchased from Lois Co. England, DPPH, Reduced glutathione, oxidized glutathione, NADPH, adrenaline and DNTP were purchased from Sigma Laboratories Germany. Enzymatic kits were purchased from Randox Laboratories UK. All other reagent used of analytical grade.

Extraction and Percentage Yield of *Capsicum annuum L.* Fruits

Using a separating funnel, 500ml distilled water was added to the Forty grams (40g) of powdered *Capsicum annuum L.* fruits, the mixture was allowed to soak for 24 h after which the filtrate was collected into a crucible and evaporated to dryness using water bath at 60ºC (Zubairu *et al*., 2015). The percentage yield of the aqueous and methanol extracts of *Capsicum annuum L.* fruits were calculated according to the method described by Prashani *et al*. (2005) from the following formular:

Percentage yield: $\frac{W_2-W_1}{W_0} \times 100$

Where W_2 =Weight of the container + extract
W_1 =Weight of empty container W_0 =weight of initial dried sample
The aqueous extracts was reconstituted with distilled water and the *in vivo* antioxidant capacities of the extracts was evaluated.

Dosage and Treatment

The rats were divided into seven groups with five rats in each group and the experiment lasted for Eight weeks. The mode of administration of lead acetate (740 mg/kg) and varying dosages of the aqueous extract of *Capsicum annuum L.* fruits and Vitamins, respectively were orally by intubation. Feed and water were given *ad libitum.* The Composition of feed given to the rats were protein: 15%, calcium :1%, fat, : 7%, phosphorus : 0.35%, fibre : 10% and soluble carbohydrate : 66.35%.

Group 1: This group was given rat chow and water *ad libitum* and referred to as the Normal Control (N-c) group.

Group 2: This group was administered 740 mg/kg b.w Lead acetate for four weeks and subsequently, withdrawn from lead administration. This group is referred to as the Lead control (L-c) group.

Group3: This group was given only the aqueous extract of *Capsicum annuum L.* for four weeks. It is referred to as the extract control group (E-c).

Group 4: This group was given lead acetate (740 mg/kg b.w) for four weeks and then treated with 500 mg/kg b.w aqueous extract of *Capsicum annuum L.* fruits without further four weeks without administration of lead. This group is referred to as L-500.

Group 5: This group was given lead acetate (740 mg/kg b.w) for four weeks and then treated with 250 mg/kg b.w aqueous extract of *Capsicum annuum L.* fruits without further administration of lead. This group is referred to as L-250.

Group 6: This group was given lead acetate (740 mg/kg b.w) for four weeks and then treated with 125 mg/kg b.w aqueous extract of *Capsicum annuum L.* fruits without further administration of lead. This group is referred to as L-125.

Group 7: This group was given lead acetate (740 mg/kg b.w) for four weeks and then treated with 100 mg/kg b.w ascorbic acid and 10 mg/kg b.w. This group is referred to as L-Vits.

Weekly change in body weight was measured using a standard weighing balance (Blance model no.GF-2000) and packed cell volume was determined by microhaematocrit methods (Alexander and Griffins, 1993).

Collection of Blood Samples and Tissues

After administration of the last dose, the animals were anaesthetized with chloroform and sacrificed after an overnight fast. Blood samples were collected through heart puncture in plain tubes, allowed to clot and centrifuged at 3000 g for 10 minutes using Denley BS400 centrifuge (England) to obtain the serum for biochemical assays. The livers and kidneys were dissected out, weighed and rinsed in ice-cold saline. Portions of liver and kidney were minced and homogenized (10 % w/v) in ice-cold 0.1 M sodium phosphate buffer (pH 7.4). The homogenate were centrifuged at 3500 g for 20 min at 4 ^{o}C twice to obtain the supernatant. The supernatant was used for biochemical assays. The remaining portions of the tissues were fixed in formalin for histopathological examination.

Biochemical Assays

Thiobarbituric acid Reactive substances (TBARS) were assayed according to the method descibed by Ohkawa *et al*., 1979. Reduced glutathione (GSH) concentration measurement was done according to Ellman (1959).Glutathione reductase was measured according to the procedure of Hsiao *et al.,* (2001).Superoxide Dismutase (SOD) activity was determined by the method described by Fridovich (1989). Catalase activity was determined by the method described by Sinha (1972). Enzymatic Randox kits were used for kidney, liver function tests and other relevant biochemical parameter.

Statistical Analysis

Data are expressed as mean ± S.D. Statistical analyses were performed with one-way analysis of variance (ANOVA software: SPSS 17.2) and test for differences between two means was performed using the student's t test. The values of $p \leq 0.05$ was considered as significant.

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RESULTS

Oxidative Stress Status in the Liver of Rats Exposed to Lead and Treated with Aqueous Extract of *Capsicum annuum L*. Fruits.

Table 1 showed the effects of the aqueous extract of *Capsicum annuum L*. in enhancing the reversal of oxidative stress in the liver of Lead-intoxicated rats. There was no significant (P>0.05) difference in the malondialdehyde concentration in all the groups except the extract control group (E-c) which had the lowest concentration of malondialdehyde significantly (p< 0.05) different from others. The activities of glutathione reductase (GR), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase were found to be reduced in the lead control group (L-c) and the group treated with the lowest dose of aqueous extract of *Capsicum annuum L*. fruits (L-125) compared to the normal control group. The recovery from oxidative stress is seen to be enhanced in the groups treated with the 500mg/kg, 250mg/kg and Vitamins, respectively

Table 1: Oxidative Stress Status in the Liver of Rats Exposed to Lead and Treated with the Aqueous Extract of *Capsicum annuum L*. Fruits.

Groups	N-c	L-c	E-c	L500	L-250	L-125	L-vits
GR(mmol/min/g tissue)	0.116± 0.012^{ad}	0.091± 0.019^{be}	0.145± 0.025^{c}	0.104± 0.016	0.100± 0.01^{ef}	0.096± 0.0085^{f}	0.090± 0.028^{abdf}
Catalase (mol/min/g tissue)	0.099± 0.015^{ac}	0.085± 0.003^{bd}	0.107± 0.011^{c}	0.094± 0.009^{ad}	0.058± 0.034^{de}	0.058± 0.016^{e}	0.086± 0.017^{abd}
GSH(mg/g tissue)	84.39± 9.52^{a}	68.10± 6.41^{b}	106.92± 7.11^{dc}	103.05± 11^{c}	99.39± 7.61^{c}	66.26± 10.31^{db}	107.09± 6.57^{cd}
MDA (µmoles/g tissue)	3.49± 0.58^{ac}	3.66± 0.90^{a}	2.02 ±0.36^{b}	3.69± 0.24^{c}	3.10± 0.18^{a}	3.74± 0.91^{ac}	3.04± 0.41^{a}
SOD(U/g tissue)	296.7± 14.8^{a}	214.3± 15.4^{b}	241.3± 28.5^{a}	233.9± 9.1^{b}	275.7± 33.4^{a}	198.7± 33.4^{b}	280.8± 23.4^{ab}

Data are mean ± standard deviation for five replicates. Values with different superscripts across a row are significantly different ($p \leq 0.05$).

Oxidative Stress Status in the Kidney of Rats Exposed to Lead and Subsequently Treated with the Aqueous Extract of *Capsicum annuum L*. Fruits.

Table 2 showed the curative effects of the aqueous extract of *Capsicum annuum L*. fruits in enhancing the reversal of oxidative stress in the kidney of Lead-intoxicated rats. There was no significant (p> 0.05) difference in the malondialdehyde concentration in all the groups except the extract control group (E-c) which had the lowest concentration of malondialdehyde significantly different from others. The activities of glutathione reductase (GR), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase were found to be amiliorated in the lead control group (L-c) and the group treated with the lowest dose of aqueous extract of *Capsicum annuum L*.fruits (L-125) compared to the normal control group. The recovery from oxidative stress is seen to be enhanced in the groups treated with the 500mg/kg, 250mg/kg and Vitamins, respectively.

Table 2: Oxidative Stress Status in the Kidney of Rats Exposed to Lead and Treated with the Aqueous Extract of *Capsicum annuum L*. Fruits.

Groups	N-c	L-c	E-c	L-500	L-250	L-125	L-vits
GR(mmol/min/g tissue)	0.095± 0.025^{ac}	0.051± 0.014^{b}	0.111± 0.021^{c}	0.099± 0.03^{ac}	0.050± 0.016^{a}	0.087± 0.014^{b}	0.080± 0.022^{ac}
Catalase (mols/min/g tissue)	0.091± 0.016^{ab}	0.075± 0.011^{bc}	0.093± 0.009^{a}	0.090± 0.011^{ac}	0.093± 0.002^{a}	0.073± 0.01^{b}	0.093± 0.005^{a}
GSH (mg/g tissue)	37.54± 1.56^{ac}	29.54± 1.04^{b}	50.7+ 4.37^{c}	39.42± 2.18^{a}	39.29± 4.19^{a}	28.16± 3.39^{b}	40.11± 3.28^{a}
MDA (µmoles/g tissue)	7.74± 0.93^{ab}	8.68± 0.92^{b}	5.07± 0.37^{c}	6.99± 0.65^{a}	6.9± 0.51^{a}	8.54± 0.46^{b}	6.87± 0.51^{a}
SOD(U/g tissue)	858±49.9^{a}	770±53.7^{b}	893±96.9^{a}	824.0± 63.3^{ab}	809.5± 111.8^{ab}	784.9± 42.2^{ab}	868±93.9^{ab}

Data are mean ± standard deviation for five replicates. Values with different superscripts across a row are significantly different ($p \leq 0.05$).

DISCUSSION

The *in vitro* antioxidant capacity of fruit, vegetables, herbs and spices have received increasing attention recently for their potential role in prevention of human diseases as well as in food quality improvement (Kamatha *et al*., 2004 , Tangkanakul *et al*., 2009). Spices and Herbs are one of the most important targets to search for natural antioxidants from the point of view of safety.

The present study revealed that The antioxidant enzymes are found to be significantly increased in groups treated with the higher dose of the aqueous extract of *Capsicum annuum L*. fruits, similar results have been reported by Chu *et al.* (2002) where aqueous extracts of both peppers (ripe and unripe) significantly inhibited lipid peroxidation in in rat's brain in a dose-dependent manner. However, Chu *et al*. (2002) also reported that catalase had a higher inhibitory effect on lipid peroxidation in Rat's brain at a higher extract concentration. Glutathione reductase (GR), the enzyme responsible for recycling of GSH from the oxidized form (GSH disfulfide; GSSG) to the reduced form (reduced GSH) is also deprived by lead (Hunaiti *et al*., 1995). The decrease of GR enzyme activity during lead administration may be due to the inhibiting activity of some proteins essential for its synthesis by the lead acetate. The present study revealed that decreased levels of GR in lead treated groups may be due to increased reactive oxygen species (ROS) generation.

Conclusion

The aqueous extract of *Capsicum annuum L.* fruits significantly ameliorated the oxidative damage in rats administered with lead acetate.

Recommendations

Different phytochemical fractions of the *Capsicum annuum L.* fruits should be purified to determine the most active ingredient in terms of antioxidant capacity. The duration for the experiments on reversibility of oxidative damage of tissues should be extended to clearly establish the ability of self-recuperation of tissues

administered with lead acetate.

Declaration of Interests

The authors hereby declare that they have no known competing interests that could have appeared to influence the work reported in this paper.

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