Oxidative Stress Status and Lipid Level of Rats Co-Administered with Lead Acetate and Aqueous Extract of Sweet Bell Pepper (*Capsicum annuum L*.) Fruits

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Abstract: Lead is an environmental pollutant that causes damage to biological systems. The results of in vitro analysis of methanol and aqueous extract of Capsicum annuum L. (CA) fruits showed that the aqueous extract has higher antioxidant capacity than the methanol extract. The effect of this extract on lead induced oxidative stress, organ damage and derangement of lipid metabolism was investigated in thirty five wistar rats which were divided into seven groups with five rats in each group. The lead control group was given only 740mg/kg bw lead acetate while the normal control and extract control groups were given only feed and 500mg/kg bw extract, respectively. The other groups were co-administered with Lead and varying doses of the aqueous extract of CA fruits for a period of four weeks. It was observed that there was significant (*p < 0.05) decrease in glutathione reductase, reduced glutathione, catalase and superoxide dismutase with corresponding increase in malondialdehyde concentration, alanine amino transaminase, aspartate transaminase and triglycerides in Lead control group compared to other groups. The deleterious effect of Lead was prevented either completely or to a significant degree by the aqueous extract of CA fruits.

Keywords: Lead Acetate, Capsicum annuum L., Lipid Peroxidation, Oxidative Stress, Hyperlipidemia.

1. Introduction

In developing countries of the world, introduction of lead has been associated primarily with growth of industry and the expanding use of motor vehicles in transport. Lead is a heavy metal that generates free radicals and has the potential to disrupt many biological systems, particularly proteins because it forms complexes with important functional chemical groups including carboxy (-COOH), amine (-NH₂) and thiol (-SH). Thus, many enzymes are potential targets. Lead inhibit activities of antioxidant enzyme, such as superoxide dismutase and catalase and also decreases the level of glutathione, increasing lipid peroxidation which harms proteins, cell membranes and DNA, among others^{1, 2}. The lead-thiol interaction is the most stable, and an important example is the interaction between lead and dithiol bridges (S-S) to form S-Pb-S. One result of this is disruption of the cellular antioxidant activity of glutathione so that lead intoxication exacerbates oxidative stress. This in turn renders vital protein thiol groups more susceptible to lead toxicity since their source of protective glutathione is depleted. Lead ability to substitute for bivalent cations, particularly Zn2+ and Ca2+, is an important mechanism underlying many of its toxic effect³. The enzymes involved in the pathways of haem, myoglobin, cytochromes, are inhibited by lead. In the blood very low concentrations of lead inhibit the synthesis of haem and reduce the life span of erythrocytes. In the nervous system, lead disrupts mitochondrial function. In the kidney, lead produces lesions of the proximal tubules and the loop of Henle^{4,5,6,7}. The oral toxic dose of lead (Pb) for rats is 740 mg/kg body weight daily⁸. Antioxidants are molecules that can neutralize free radicals by accepting or donating an electron to eliminate the unpaired condition⁹. Typically this means that the antioxidant molecule becomes a 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. Vitamin C (ascorbate, AscH), for example, can donate a hydrogen atom to a free radical molecule (R) thereby neutralizing the free radical while becoming an ascorbate radical itself which can also neutralize the radical form of other antioxidants, such as glutathione (GS) and Vitamin E (tocopherol) (Toc):

$$AscH^{-} + Toc \longrightarrow Toc + Asc^{-}$$

Sweet peppers (Capsicum annum L.) are plump, bell-shaped vegetables featuring either three or four lobes. The antioxidant properties of the Capsicum annuum L. could be attributed to the presence of antioxidants, especially ascorbic acid, tocopherol and phenols ^{10, 11}. Hot cultivars are rich in capsaicinoids alkaloids with pharmacological properties giving the specific taste to pepper fruits ⁹. Capsicum annuum L. are spices that have been used as flavoring and coloring agents and as preservatives for thousands of years. Capsicum annuum L. has also been recognized to possess medicinal properties and their use in traditional systems of medicine has been on record for a long time ¹². With the advancement in the technology of spices and on knowledge of the chemistry and pharmacology of their active principles, their health benefits were investigated more thoroughly in recent decades.

The objective of this study was to assess the *in vitro* antioxidant activities of methanol and aqueous extracts of *Capsicum annuum L*. fruits and further evaluate the preventive effects of the more active extract of *Capsicum*

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annuum L. fruits on Lead- induced oxidative stress and consequent damage to liver and kidney as well as derangement of lipid metabolism.

2. Materials and Methods

2.1Animals

A total of forty eight (48) two months old wistar strain albino rats of both sexes weighing between 120-150mg/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 Care¹³. The rats were acclimatized for two weeks. Thirteen (13) of the rats were used for the acute toxicity study of the aqueous extract of *Capsicum annuum L*.¹⁴. The remaining Thirty five (35) rats were divided at random into seven groups with five rats in each of the groups for the *in vivo* antioxidant studies. The rats were fed with Grower's mash from Vital Feeds Company Plc. Jos and water was provided *ad libitum*. Ethical clearance was obtained from the Health Committee on Ethical Clearance of the Institution

2.2 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 were of analytical grade.

2.3 Drying and Extraction of Capsicum annuum L. Fruits

Two solvents: distilled water and methanol were used for the extraction by cold maceration ¹⁵. *Capsicum annuum L*. fruits were oven dried at 40^oC Eighty grams (80g) of the powdered *Capsicum annuum L*. fruits was divided into two equal portions. Using a separating funnel, 500ml Distilled Exactly 500ml methanol was added to the second portion and allowed to soak for twenty four hours after which the filtrate was collected differently each into separate crucible and evaporated to dryness using a water bath at 60° C. The percentage yield of each extract was calculated. The methanol and aqueous extracts were reconstituted with distilled water and the *in vivo* antioxidant capacities of the two extracts were evaluated.

2.4 In vitro Evaluation of Antioxidant Properties of the Aqueous and Methanol Extracts of Capsicum annuum L. Fruits.

DPPH (2, 2- diphenyl-2-picrylhydrazine) is a stable nitrogen centered free radical which produces violet colour in methanol solution. DPPH reacts with suitable reducing agents during which the electrons become paired off and the solution loses colour stoichiometrically depending on the number of electrons taken up. As antioxidants donate protons to this radical, the absorbtion decreases. (DPPH) scavenging activity. DPPH was determined according to the method described by Mensor *et al.*,¹⁶.Thiobarbituric Acid Reactive substances (TBARS) was determined according to the method described by Ottolenghi¹⁷. The formation of malondialdehyde is the bases for the well -known TBA method used for evaluating the extent of lipid peroxidation. At low pH and high temperature (100^oc), malondialdehyde binds TBA to form red complex that can be measured at 532nm. The increase in amount of the red pigment formed with oxidative rancidity of the lipid. correlates Determination of Total reducing power was as described by Mathew and Abraham¹⁸. The principle is based on the reduction of Fe (III) to Fe (II). The yellow colour of the test solution changes to various shades of green and blue depending upon the reducing power of each extract. The presence of reductants (antioxidants) in the Capsicum annum L. fruits extract causes the reduction of Fe^{3+} (ferricynide complex to ferrous form. Therefore, Fe^{2+} can be monitored by measuring the formation of perl's Prussian blue at 700nm. The determination of total antioxidant capacity was as described by Kumaran and Karunukaran¹⁵ Phosphomolybdenum method was used for determination of total antioxidant capacity of the extract which was based on the reduction of Mo (VI) to Mo (V). Thirteen wistar (13) rats were used for the acute toxicity studies of aqueous extract of *Capsicum annuum L*.fruits. as described by Lorke¹⁴.

2.5 In vivo Evaluation of Antioxidant Properties of Aqueous Extracts of Capsicum annum L. Fruits

Experimental Design

The rats were divided into seven groups with five rats in each group. All the rats were given water and pelleted feed ad libitum. The toxic dose of Lead acetate given to the rats was 740mg/kg b.w orally. The induction was daily for twenty eight days. The s seven groups consisted of the normal control group (NC) which was given only feed and water and referred to as the normal control group. Group LC -served as negative control group and treated with only lead acetate. Group EC- received 500mg/kg. b.w extract only. Group L500- received lead acetate + 500mg/kg b.w extract. Group L250- received lead acetate + 250mg/kg b.w extract. Group L125- received lead acetate + 125mg /kg b.w extract.Group LVITS- received lead acetate + 100mg/kg and 10mg/kg b.w Vitamin C and Vitamin E respectively. Weekly packed cell volume was determined by microhaematocrit methods 20. The animals were anaesthetized with chloroform and sacrificed after an overnight fast. Blood samples were collected via heart puncture and centrifuged to get the serum. The animals were dissected to get the Livers and kidneys immediately, 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 was centrifuged at 10,000 rpm for 20 min at 4°C twice to get the enzyme fraction. The supernatant was used for biochemical assays. The remaining portions were fixed in formalin for histological examination.

Thiobarbituric acid Reactive substances (TBARS) was assayed according to the method described by Ohkawa *et al.*,²¹. Reduced glutathione (GSH) concentration measurement was done according to Ellman ²².Glutathione reductase was measured according to the procedure of Hsiao ²³. Superoxide Dismutase (SOD) activity was determined by

the method described by Fridovich ²⁴. Catalase activity was determined by the method described by Sinha ²⁵. Enzymatic kits (Randox Laboratory) were used for evaluation of biochemical indices of liver and kidney function.

Statistical Analysis

Data are expressed as mean \pm S.D. Statistical analysis were performed with one-way analysis of variance (ANOVA) and test for differences between two means was performed using the students' t test. The values of *p<0.05 was considered as significant.

3. Results

<u>In vitro Antioxidant Capacity of Capsicum annuum L.</u> <u>Fruits</u>

The results of the *in vitro* evaluation of the antioxidant capacity of *Capsicum annuum L*. fruits are shown in figures 1, 2, 3 and figure 4. The aqueous extract of sweet bell pepper exhibited higher DPPH scavenging activity (figure 1), percentage inhibition of lipid peroxidation (figure 2), total antioxidant capacity (figure 3) and total reducing capacity (figure 4) than the methanolic extract. In view of this result the aqueous extract was selected for *in vivo* studies and its acute toxicity was determined. The aqueous extract of *Capsicum annuum L*. fruits was relatively safe at 5000mg/kg.b.w. No signs of toxicity or death were observed in the experimental rats at 1st and 2nd phases of the acute toxicity test. Thus, the LD₅₀ is greater than 5000mg/kg.



Figure 1: DPPH Scavenging Activity of Methanol and Aqueous Extract of *Capsicum annuum L*. Fruits. Relative to 0.03% Ascorbic Acid



Figure 2: Inhihibition of Lipid Peroxidation of Methanol and Aqueous Extract of *Capsicum annuum L*. Fruits Relative to 0.03% Ascorbic Acid



Figure 3: Total Antioxidant Capacity of Methanol and Aqueous Extract of *Capsicum annuum L*. Fruits Relative to 0.03% Ascorbic Acid.



Figure 4: Reducing Capacity of Methanol and Aqueous Extract of *Capsicum annuum L*. Fruits Relative to 0.03% Ascorbic Acid.



Figure 5: Packed Cell Volume of Rats Co-administered with Lead Acetate and Various Doses of the Aqueous Extract of Capsicum annuum L. Fruits.

Packed Cell Volume and Body Weight of Rats Coadministered with Lead Acetate and Aqueous Extract of Capsicum annuum L. Fruits

Figure 5 depicts effects of lead co-administered with varying dosages of aqueous extract of Capsicum annuum L. fruits on packed cell volume (PCV) of wistar rats. There was significant decrease in the PCV of the lead-intoxicated rats (LC) compared to the (NC) normal control group (p<0.05), this effect was seen to be prevented in the groups given lead concomitantly with aqueous extract of Capsicum annuum L. fruits. The decrease in PCV caused by Lead intoxication was also seen to be prevented in the groups (LVITS) treated with lead concomitantly with 100mg/kg and 10mg/kg Vitamin C and E respectively, (Figure 5). There was no significant (*p<0.05) difference between the groups (L125) treated with Lead acetate concomitantly with 125mg/kg aqueous extract of Capsicum annuum L. fruits and the lead control group (*p < 0.05). Figure 6 and 7depict the effects of lead coadministered with varying dosages of aqueous extract of Capsicum annuum L. fruits on the body weight of rats . There was significant decrease (*p<0.05) in the body weight of lead control group compared to the normal control group. The groups that were co-administered with lead acetate and the aqueous extract of Capsicum annuum L. fruits were seen to be prevented from drastic weight reduction.

Oxidative Stress Status and Organ Damage of Rats Coadministerd with Lead and Aqueous Extract of Capsicum annuum L. Fruits

Table 1 and table 2 showed significant (*p< 0.05) decrease in glutathione reductase, reduced glutathione, catalase and superoxide dismutase in the liver and kidney of the lead control group compared to the groups treated with varying doses of the extract. There was significant (*p<0.05) increase in the Malondialdehyde concentration in the lead control group compared to the normal control group (Table 1 and Table 2).

annuum L. Fruits									
Groups	NC	LC	EC	L500	L250	L125	LVITS		
GSH RD(mmol/min /g tissue)	0.089 <u>+</u> 0.016 ^{ade}	0.048 ± 0.016^{b}	$0.145 \pm 0.025^{\circ}$	0.099 <u>+</u> 0.012 ^{dcg}	0.079 ± 0.012^{ef}	$0.069 \pm 0.01^{\rm f}$	0.11 ± 0.01^{g}		
Catalase(mols/min/g tissue)	0.093 ± 0.014^{acd}	0.03 ± 0.006^{b}	0.107 <u>+</u> 0.011 ^c	0.080 ± 0.009^{a}	0.08 ± 0.01^{ace}	0.034 ± 0.006^{b}	0.092 ± 0.003^{de}		
GSH (mg/g tissue)	99.9 <u>+</u> 11.0 ^{ac}	39.93 <u>+</u> 6.55 ^b	106.92 <u>+</u> 7.11 ^c	80.25 <u>+</u> 12.25 ^d	81.34 <u>+</u> 8.65 ^{ad}	54.29 ± 2.02^{e}	84.16 <u>+</u> 6.59 ^d		
MDA (µmols/g tissue)	3.66 ± 0.46^{a}	7.29 ± 0.81^{b}	$2.02 \pm 0.36^{\circ}$	3.64 ± 0.33^{a}	3.99 ± 0.72^{a}	7.85 ± 0.66^{b}	3.63 ± 0.31^{a}		

 $202.9 \pm 6.3^{\circ}$

 $241.3 + 28.5^{\circ}$

 $119.1 + 19.9^{b}$

Table 1: Oxidative Stress Markers in the Liver of Rats Co-administered with Lead and Aqueous Extract of Capsicum

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

 226.5 ± 30.0^{a}

Key: NC =normal control group, LC=lead control group, EC=extract control group, L500= lead treated concomitantly with 500mg/kg bw aqueous extract of Capsicum annuum L. fruits, L250= lead treated concomitantly with 250mg/kg bw

aqueous extract of Capsicum annuum L. fruits, L125= lead treated concomitantly with 125mg/kg bw aqueous extract of Capsicum annuum L. fruits and LVITS= lead treated concomitantly with 100mg/kg bw and 10mg/kg bw Vitamin C and Vitamin E. respectively. Superoxide dismutase Malondialdehyde (MDA), (SOD), Glutathione reductase(GSH Rd) and Reduced glutathione (GSH).

 $135.9+23.2^{t}$

197.3+35.3ª

Table 2: Effects of Co-administration of Lead Acetate and Aqueous Extract of Capsicum annuum L. Fruits on Oxidative NЛ ст

Stress Markers in the Kidney of Lead-Intoxicated Rats							
Groups	NC	LC	EC	L 500	L250	L 125	L VITS
GR(mmol/min /g tissue)	0.088 ± 0.018^{ac}	0.042 <u>+</u> 0.012 ^b	$0.111 \pm 0.021^{\circ}$	0.085 ± 0.014^{ac}	0.069 ± 0.019^{ad}	0.059 ± 0.009^{d}	$0.087 \pm 0.014^{\mathrm{ac}}$
Catalase (mols/min/g tissue)	0.087 ± 0.007^{ac}	0.029 ± 0.002^{b}	0.093 <u>+</u> 0.009 ^c	0.078 ± 0.016^{ac}	0.081 ± 0.005^{ac}	0.034 ± 0.009^{b}	0.082 ± 0.001^{a}
GSH(mg/g tissue)	43.37 <u>+</u> 8.09 ^{ac}	20.71 <u>+</u> 2.71 ^b	50.7 <u>+</u> 4.37 ^c	46.68 <u>+</u> 6.65 ^{ac}	45.21 <u>+</u> 5.23 ^{ac}	20.01 ± 6.95^{b}	47.19 <u>+</u> 2.71 ^{ac}
MDA(µmoles/g tissue)	6.46 ± 0.37^{a}	9.72 ± 0.88^{b}	5.08 <u>+</u> 0.37 ^c	$\begin{array}{c} 6.44 \underline{+} \\ 0.26^{a} \end{array}$	$\frac{6.87 \pm 0.72^{a}}{0.72^{a}}$	10.79 <u>+</u> 0.13 ^b	6.4 ± 0.94^{a}
SOD (u/g tissue)	758.2 ± 28.9^{a}	335.9 <u>+</u> 70.1 ^b	893.9 <u>+</u> 96.9 ^c	680.9 <u>+</u> 77.9 ^{ad}	623.9 <u>+</u> 84.1 ^{de}	544.6 <u>+</u> 95.4 ^e	728.6 <u>+</u> 109.3 ^a

SOD (u/g tissue)

 $202 + 10.2^{a}$

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Data are mean \pm standard deviation for five replicates. Values with different superscripts across a row are significantly different (*p<0.05).

Key: NC :normal control group, LC=lead control group, EC=extract control group, L500= lead treated concomitantly with 500mg/kg bw aqueous extract of *Capsicum annuum L*. fruits, L250= lead treated concomitantly with 250mg/kg bw aqueous extract of *Capsicum annuum L*. fruits, L125= lead treated concomitantly with 125mg/kg bw aqueous extract of *Capsicum annuum L*. fruits and LVITS= lead treated concomitantly with 100mg/kg bw and 10mg/kg bw Vitamin C and Vitamin E. respectively. Superoxide dismutase (SOD), Malondialdehyde (MDA),Glutathione reductase(GR) and Reduced glutathione (GSH).

Table 3 shows significant (*p<0.05) decrease in the serum total protein and albumin of the lead control group compared to the normal control group. It is also shown in Table 3 that there was no significant (*p<0.05) difference between the normal control group and the groups co-administered with lead and *Capsicum annuum L*. fruits .Table 3 also shows significant (*p<0.05) increase in the serum urea and creatining of the lead control group compared to the normal control groups and the groups co-administered with lead and aqueous extract of *Capsicum annuum L*. fruits. There was significant increase(*p<0.05) in serum alanine transferase, alkaline phosphatase and aspartate transaminase in the lead control group compared to the normal control group and the groups co-administered with lead and aqueous extract of *Capsicum annuum L*. fruits (Table 3).

 Table 3: Effects of Co-administration of Lead Acetate and Aqueous Extract of Capsicum annuum L. fruits on Serum

 Biochemical Markers of Organ Damage

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Groups	NC	LC	EC	L500	L250	L125	LVITS	
TP (g/dl)	6.33+	4.36+	6.89+	6.07+	5.36+	4.31+	6.14+	
	0.51 ^{ac}	$0.97^{\overline{b}}$	0.41 ^c	$0.44^{\overline{ad}}$	1.32 ^{bcd}	0.85^{b}	0.34 ^{ad}	
Albumin(g/dl)	4.66 <u>+</u>	$2.38 \pm$	5.35+	4.39 <u>+</u>	3.75 <u>+</u>	3.35+	4.42 <u>+</u>	
	0.34^{a}	0.48^{b}	0.84^{c}	$0.45^{\overline{ac}}$	1.14 ^{ac}	0.47^{d}	$0.45^{\overline{ac}}$	
TB(µmol/l)	14.06+	25.42 <u>+</u>	14.85 <u>+</u>	16.45 <u>+</u>	19.15 <u>+</u>	23.49 <u>+</u>	15.73 <u>+</u>	
	2.78^{ac}	6.45^{bd}	2.47^{c}	2.17 ^{ace}	2.08 ^{de}	7.01 ^{deg}	3.71 ^{aceg}	
Urea(mmol/l)	60.12 <u>+</u>	116.57+	55.41 <u>+</u>	67.03 <u>+</u>	9422 <u>+</u>	99.72 <u>+</u>	70.38 <u>+</u>	
	15.31 ^{ac}	12.22 ^{bd}	9.44 ^c	13.69 ^{abc}	20.76 ^{de}	22.47 ^d	10.78 ^{ace}	
Creatinine(mg/dl)	1.49 <u>+</u>	5.32 <u>+</u>	$1.05 \pm$	1.97 <u>+</u>	3.14 <u>+</u>	401 <u>+</u>	1.53+	
	0.54 ^{ac}	0.33 ^b	0.28°	0.41 ^a	0.41 ^d	0.55 ^e	0.37 ^a	
AST(u/l)	11.30+	29.40 <u>+</u>	$10.54 \pm$	$12.25 \pm$	$15.50 \pm$	26.25 <u>+</u>	11.50+	
	1.91 ^{ac}	7.66 ^b	3.15 ^c	4.53 ^{acd}	2.23 ^d	2.56 ^b	1.87 ^{ac}	
ALT(u/l)	4.02 <u>+</u>	7.06 <u>+</u>	3.76 <u>+</u>	4.35 <u>+</u>	4.70 <u>+</u>	6.13 <u>+</u>	4.47 <u>+</u>	
	0.77^{a}	1.33 ^b	0.69 ^a	0.89 ^{ac}	1.66 ^{ac}	1.63 ^{bc}	0.52 ^{ac}	
ALP(u/l)	118.68+	230.92+	$64.40 \pm$	$109.02 \pm$	110.40+	133.17+	106.72 <u>+</u>	
	10.32^{a}	6.88 ^b	13.89 ^c	19.75 ^a	19.52 ^a	4.93 ^d	14.66 ^a	

Data are mean \pm standard deviation for five replicates. Values with different superscripts across a row are significantly different (*p < 0.05).

Key: NC =normal control group, LC=lead control group, EC=extract control group, L500= lead treated concomitantly with 500mg/kg bw aqueous extract of *Capsicum annuum L*. fruits, L250= lead treated concomitantly with 250mg/kg bw aqueous extract of *Capsicum annuum L*. fruits, L125= lead treated concomitantly with 125mg/kg bw aqueous extract of *Capsicum annuum L*. fruits, L125= lead treated concomitantly with 125mg/kg bw aqueous extract of *Capsicum annuum L*. fruits, L125= lead treated concomitantly with 125mg/kg bw aqueous extract of *Capsicum annuum L*. fruits and LVITS= lead treated concomitantly with 100mg/kg bw and 10mg/kg bw Vitamin C and Vitamin E. respectively. AST (aspartate transferase), ALT (alanine transferase) , ALP (alkaline phosphatase), total protein (TP), and TB (total bilirubin).

Relative Weight of Liver and Kidney of Rats Coadministered with Lead Acetate and *Capsicum annuum L*. Fruits

Figure 6 showed the relative weight of liver and kidney of rats co-administered with lead and *Capsicum annuum L*. fruits. There was no significant (*p > 0.05) difference in the relative weight of the liver of the lead control group compared to the normal control group and the groups co-administered with 500mg/kg b.w of the extract. There was significant (*p < 0.05) increase in the relative weight of the kidney and liver of the lead control group compared to the

respective normal control groups. There was no significant (*p>0.05) difference in the relative weight of kidney of the LC group compared to the L500 group.



Figure 6: Effects of the Co-administration of Lead Acetate and Varying Doses of the Aqueous Extract of *Capsicum annuum L*. Fruits on the Relative Weight of the Liver and Kidney of Rats

<u>KEY:</u> NC =normal control group, LC=lead control group, EC=extract control group, L500= lead treated concomitantly with 500mg/kg bw aqueous extract of *Capsicum annuum L*. fruits, L250= lead treated concomitantly with 250mg/kg bw aqueous extract of *Capsicum annuum L*. fruits, L125= lead treated concomitantly with 125mg/kg bw aqueous extract of *Capsicum annuum L.* fruits and LVITS= lead treated concomitantly with 100mg/kg bw and 10mg/kg bw Vitamin C and Vitamin E respectively.

Lipid Levels of Rats Co-administered with Lead Acetate and Aqueous Extract of *Capsicum annuum L*. Fruits

Figure 7 showed the hypolypidemic effects of the aqueous extract of Capsicum annuum L. fruits in rats administered with lead acetate. There was significant (*p<0.05) increase in the serum total cholesterol, respectively compared to the normal control group. The increase in the levels of lipids caused by the administration of lead was shown to be reduced in the groups co-administered with lead acetate and varying doses of the aqueous extract and Vitamins, respectively. There was significant (*p<0.05) decrease in the level of HDL-CHOL of the lead control group compared to the normal control group, this effect was observed to be protected in the groups co-administered with lead and varying doses of the extractThere was significant increase (*p<0.05) in the serum total cholesterol, LDL CHOL and triglycerides in the lead control group compared to the normal control group and the groups co-administered with lead and aqueous extract of Capsicum annuum L. fruits (figure 7).



Figure 7: Effects of Co-administration of Lead and Aqueous Extract of *Capsicum annuum L*. Fruits on Serum Lipid Profile of Lead intoxicated Rats.

4. 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 ^{26, 27}. Spices and Herbs are one of the most important targets to search for natural antioxidants from the point of view of safety. In this study the antioxidant capacity of methanol and aqueous extract of Capsicum annuum L. fruits were evaluated. The aqueous extract was found to have higher antioxidant capacity than the methanol extract. These results were in line with the results of Badami²⁸ who found that the water extract of coriander, ginger, sweet pepper were higher in antioxidant activity than the methanol extract. Therefore, the aqueous extract of Capsicum annuum L. fruits was selected for in vivo antioxidant capacity in lead intoxicated wistar rats. The results of this present study demonstrated significant decreases in packed cell volume (PCV), total protein, antioxidants(GR,GSH, SOD and catalase) and as well as albumin and a significant increase in malondialdehyde, creatine, urea, and serum enzymes (ALT, AST and ALP) in lead administered control group compared to the normal control group. These findings therefore, corroborates similar findings reported by Teijon et al., Mugahi et al., and Khan et al., 29, 30, 31. These results are indicative of increased oxidative stress and damage to cells and organs (liver and kidney) caused by lead poisoning. This present study revealed that lead caused significant increase in serum total CHOL., LDL - CHOL., triglycerides and decrease in serum HDL - CHOL. Several studies in humans have shown that occupational lead exposure induces alterations in serum lipid profiles ³². The deleterious effects caused by lead intoxication were prevented in animals given lead concomitantly with aqueous extract of Capsicum annuum L. or Vitamins, suggesting that the extract offered protection against lead-induced oxidative stress and organ damage in the animals. Anemia (reduction in PCV) result from reduction of the life span of circulating erythrocyte as well as by inhibition of the body's ability to make heamoglobin by interfering with several enzymatic steps in heam pathway ³³. In the present study increased oxidative stress caused by lead might have compromised the integrity of erythrocyte membrane, thereby reducing the life span of circulatory erythrocytes ³⁴. Co- administration of lead and aqueous extract of Capsicum annuum L. fruits and vitamins (E and C) to rats prevented decrease in packed cell volume and total protein. Similar results has been reported by Hassan³⁵ who suggested that administration of vitamin E to dams exposed to oxidative stress by cadmium chloride during lactation decreased the adverse effects produced by cadmium chloride on blood parameters. The extract used was shown to have antioxidant capacity and so might have exerted its effect in a manner similar to the antioxidant Vitamins used as controls. The antioxidant enzymes were significantly increased in groups treated with the higher dose of the aqueous extract of Capsicum annuum L. fruits and similar findings have been reported by Chu et al., ¹¹ where aqueous extracts of both peppers (ripe and unripe) significantly (*p < 0.05) inhibit lipid peroxidationin in Rat's brain in a dose-dependent manner. Glutathione reductase (GSH Rd), the enzyme responsible for recycling of GSH from the oxidized form (GSH disfulfide; GSSG) to the reduced form (reduced GSH) is also depleted by lead ³⁶. The decreased GSH Rd enzyme activity during lead administration may be due to the inhibiting activity of some proteins essential for its synthesis by the lead acetate ²⁹. However, Administration of aqueous extract of Capsicum annuum L. fruits and the Vitamins (E and C) improved the GSH Rd level and protected the organs from the oxidative damage of tissues by reacting with reactive oxygen species (ROS). There was prevention of Lead-induced increase in serum AST, ALT, ALP, urea and creatinine levels in rats treated with aqueous extract of Capsicum annuum L. The observed decrease in these serum enzymes shows that the aqueous extract of Capsicum annuum L. preserved the structural integrity of liver and kidney against lead induced damage. These were consistent with the observed increases in serum ALT, AST, ALP, creatinine and urea. These observations are in conformation with Sharma and Pandey ³⁷ . in the kidney architecture leads to impairment of the kidney function which is reflected in

the elevated levels of the serum creatinine and blood urea levels.

5. Conclusion

It is however, concluded that the aqueous and methanolic extracts of *Capsicum annuum L*. fruits have *in vitro* antioxidant activity with that of the aqueous extract being higher. The aqueous extract of *Capsicum annuum L*. fruits significantly prevented oxidative damage and hyperlipidemia in Lead-intoxicated rats. However, it is recommended that different phytochemical fractions of the *Capsicum annuum L*. fruits should be purified to determine the most active ingredient.

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