Prophylactic Effects of Garlic Oil and Onion Oil Fractions as Compared to Vitamin E on Rats Orally Fed with Lead Acetate Solution

G. R. Sajitha¹ · K. T. Augusti¹ · Regi Jose²

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Abstract Heavy metal pollution is a global public health challenge due to its stable and persistent environmental contamination. Of these lead is considered to be one of the most common ubiquitous and industrial pollutants and at low concentration it exerts extensive damages to the tissues. Daily feeding of lead acetate solution (Dose: 10 mg/kg/day) to normal rats for a month adversely altered the parameters of blood, serum and tissues, viz; RBC, WBC, Hb, d- ALAD (Delta amino levulinic acid dehydratase), Pb content, lipids, oxidized lipids (TBARS), vitamins C and E and GSH levels and activities of AST, ALT and antioxidant enzymes viz; catalase, GR, Gpx and SOD. In order to study whether antioxidants have any effect to counteract the toxicity of lead we have selected comparatively better active allium fractions for the study viz: polar fraction of garlic (PFG) and polar fraction of onion (PFO). On feeding of these active fractions of garlic and onion oils i.e. their polar fractions and vitamin E (Dose 100 mg/kg/day) separately for a month along with or without lead acetate to rats each nutraceutical and vitamin E counteracted the adverse effects of Pb significantly ($p \leq 0.05$). Their effects are in the order of PFG > PFO > Vitamin E. All these results point out that garlic and onion oils contain natural disulfoxide compounds which act as antioxidant and anti toxic to lead compounds. Their comparative differences in action may be due to the presence and position of double bonds and disulfide oxide bonds in their molecules. i.e., in

PFG the allyl disulfide oxide group is present and in PFO saturated methyl and propyl groups and unsaturated propenyl group are present in place of allyl groups. The former group confers a better antioxidant activity on PFG, while the latter groups confer a lesser activity on PFO.

Keywords Polar fraction of garlic oil (PFG) · Polar fraction of onion oil (PFO) · Lead acetate · Antioxidant enzymes · Vitamin E

Introduction

There are over 50 elements that can be classified as metals, of which 17 are considered to be very toxic [1]. The important heavy metals included are Cadmium, nickel, copper, zinc, chromium, arsenic, mercury, lead etc. Toxicity level depends on the type of metal, its biological role and the type of organism that is exposed to it. Heavy metals enter into human or animal body and accumulate mainly in kidneys, liver, lungs, hair, adrenal gland, skin and brain. The human body cannot break down heavy metals and they readily form stable covalent complexes with tissue proteins, interfere with normal cell and tissue functions through multiple pathways including interactions with macromolecules such as proteins, enzymes, hormones etc. [2]. Heavy metal pollution is a global public health challenge due to its stable and persistent environmental contaminants causing various alterations in target tissues of exposed animals [3]. Of these lead is considered to be one of the most common ubiquitous and industrial pollutants and to be toxic even at low concentration and exerts extensive damages to the tissues. Recent studies suggest that oxidative stress is a potential contributor to lead toxicity, disrupt directly or indirectly the delicate pro oxidant/antioxidant balance in the

✉ K. T. Augusti
ktaugusti@yahoo.co.uk

¹ School of Health Science, Kannur University, Palayad, Thalassery, Kannur, India

² Sree Gokulam Medical College, Venjaramoodu, Thiruvananthapuram, India

cells [4]. Thus there has been increased interest in the treatment of lead toxicity by the plant products or medicinal plants with antioxidant properties in reducing reactive oxygen species induced tissue injury. Natural compounds involve a wide array of biological processes including activation of antioxidant defenses and many other processes [5]. Allium plants such as garlic and onion are the two sources of nutraceuticals such as nutritional medicines having sulfur containing compounds with multifunctional antioxidant activity. Swati et al. [6] reported that allium plants such as garlic has some beneficial effects in preventing the heavy metals such as nickel and chromium induced alteration in serum lipid profile. The preventive role of garlic oil and Vitamin E against the adverse effects of lead acetate and ethanol separately as well as in combination in the drinking water of rats were previously reported by our team [7]. So for very little work was carried out for the prevention of lead induced oxidative stress by using nutritional medicines (neutraceuticals). Therefore our study is designed to conduct prophylactic effects of active principles of allium oils (i.e. polar fractions of garlic and onion oils) against lead acetate toxicity for 1 month in rats as they were comparable in activity with vitamin E for preventing oxidative stress in lead acetate fed rats.

Structure of Garlic and Onion Oil Polar Fractions

Allicin (Di allyl disulphide oxide)

Dimethyl disulfide oxide

Dipropyl disulfide oxide

Dipropenyl disulfide oxide

Materials and Methods

Chemicals used in this study were of analytical grade supplied by British Drug House,

E-Merck and Sigma chemicals. Polar fractions of garlic and onion oils were prepared from the two vegetables after slicing and drying them according to a standard method [8]. Vitamin E was purchased from Sigma chemicals. Prior permission was obtained for the experiment from the Institutional ethical committee (114/ac/07/CPCSEA). Male Sprague-Dawley albino rats selected from our colony of rats and weighing around 150–200 g were divided into 5 groups of six in each and they were maintained under standard laboratory condition. The rats were fed with rat pellets.

(Hindustan Lever limited) and water ad libitum. 12 h light/dark cycle was maintained. According to a pilot study we have conducted earlier [7] to find out an effective dose of garlic oil to counteract the toxic effect of lead acetate (10 mg/Kg/day) in rats was found to be 100 mg/Kg/body weight. This lead acetate concentration in drinking water of rats was calculated on the basis of a previous work conducted by Hari Sekhar [9]. A rat of 150 g weight usually drinks water ad libitum 8 ml/day as we have observed in our animal house. Harisekhar supplied drinking water containing 160 mg of lead acetate/litre to his experimental rats. That means a rat of 150 g drinking 8 ml of water consumes 1.28 mg of lead acetate/day. In our experiment we have given 1.5 mg of lead acetate to a rat with 150 g weight/day so as to get a definite toxic effect on the animals. The analyses of onion oil and garlic oil polar fractions were not carried out because we have no facilities for gas chromatographic analysis at Trivandrum. More over the senior author analyzed the polar and non polar fractions of onion oil while he was in England during 1975 [10] and it was found that the polar fraction contained mainly methyl propenyl disulfoxide and traces of dimethyl sulfoxide, while the non polar fraction contained a large number of mono and di sulfides of dimethyl, methyl propenyl, propyl propenyl disulfides and methyl propyl and methyl propenyl trisulfides. Various workers [11–13] reported that most of these compounds are yielded due to decomposition of the original allium oils and later recombination to give various alkyl mono, di and tri sulfides. The above workers also remarked that the same happened with garlic oil fractions with allyl groups as found in ajoene with or without oxygen in polar and non polar fractions respectively. Moreover their sulfoxides are trapped in the columns of the analyzer. Therefore we felt that gas chromatographic analysis of the oils gives only the artifacts of the original oil. Our main aim of the work is to find which of the fractions of the oils i.e; polar or non polar is more effective between themselves.

The rats were divided into 5 groups as follows.

- Group I is kept as normal control
- Group II Lead acetate solution fed control (Dose of lead acetate 10 mg/Kg/day for 1 month)
- Group III Lead acetate (LA) as above + polar fraction of garlic oil (PFGO) in glycerol (Dose: 100 mg/Kg/body weight) was fed for 1 month
- Group IV Lead acetate as above + polar fraction of onion oil (PFOO) as above (Dose: 100 mg/Kg/body weight) was fed for 1 month
- Group V Lead acetate as above + Vitamin E (Dose: 100 mg/Kg/body weight) as above was fed for 1 month

Samples of lead acetate, active fraction of oils and Vitamin E solutions were orally fed to the rats daily by means of a stomach tube.

After treating each group as above for 1 month the rats were deprived of food overnight, sacrificed and their blood and tissues such as liver, kidney and heart were collected in clean dry test tubes and ice cold containers respectively. The following blood, serum and tissue parameters were determined and histopathology diagrams of liver and kidney tissues were prepared by standard methods; Hb level, RBC and WBC counts, were obtained from a data of complete blood count analysis using a fully automated Sysmex hematology instrument. Serum and tissue enzymes such as catalase [14], SOD [15], Glutathione peroxidase [16], Glutathione reductase [17], Lipid per oxidation products (TBARS) [18], reduced glutathione (GSH) [19], Lipid profile such as tri acyl glycerol (TAG) [20], total cholesterol [21], HDLcholesterol [22] and LDL cholesterol [23] and vitamins E [24] and C [25] levels and protein [26] were determined. Serum lead levels were determined on a flame atomic absorption spectrometer [27]. Histopathology pictures of tissues were obtained as per the procedures followed before [28]. The statistical analysis of the parameters were determined using a statistical program SPSSPC +, version 15 (SPSS, Inc., Chicago, IL, USA). A one-way ANOVA was employed for comparison among the groups. Duncan's post hoc multiple comparison tests of significant differences among groups were determined. $p \leq 0.05$ was considered to be significant.

Results

Daily feeding of lead acetate to rats adversely altered the blood, serum and tissue parameters significantly. Results on blood levels of Hb, RBC and WBC count and

d-aminolevulinic acid dehydratase (d-ALAD) and serum levels of lead are given in Table 1. Blood levels of Hb and RBC and WBC counts and d-ALAD activity were significantly reduced on feeding lead acetate solution for 1 month, while serum level of lead significantly increased. On treatment with polar fractions of garlic oil and onion oil along with the lead acetate feeding to rats significantly reversed the above altered parameters. d-ALAD activity doubly increased in comparison with the decrease in serum lead levels of treated groups (III–V). Feeding of active fractions of garlic and onion oil showed very highly significant ($p \leq 0.001$) protective effects when compared with the feeding of vitamin E.

Results of serum and tissue lipid parameters are given in Tables 2 and 3 and in Fig. 1. Results of lipid parameters such as serum total cholesterol, LDL cholesterol and TAG increased significantly by feeding lead acetate solution for 1 month. A similar pattern of lipid profile was observed in the liver, kidney and heart tissues of lead acetate fed groups. Treatment with active fractions of garlic oil and onion oil or vitamin E along with lead acetate in groups III–V, protects the deleterious effects of lead on the tissues and serum very high significantly ($p \leq 0.001$). Results on vitamin C and E are given in the Fig. 2. Both vitamin C and E levels very high significantly ($p \leq 0.001$) decreased in lead acetate solution fed group (II) for 1 month. On the other hand in lead acetate fed groups III–V feeding of active fractions of garlic and onion oils or vitamin E increased the levels of both vitamins C and E levels in serum. The levels of vitamin E and C increased in a very high significantly manner in vitamin E treated group when compared with the active fractions of garlic and onion oil treated groups.

Results on serum, heart, liver and kidney lipid peroxidation in terms of TBARS are given in Fig. 3. TBARS level increased very high significantly ($p \leq 0.001$) in lead

Table 1 Blood hemoglobin (Hb), RBC, WBC counts and serum Pb concentration AND d-ALAD[#]

No.	Groups	Hb (g %)	RBC (million/mm ³ blood)	WBC (10 ³ /mm ³ blood)	Lead (μg/ml)	Delta ALAD [#] (U/L)
I	Normal	17.7 ± 1.6	7.9 ± 0.72	10.3 ± 0.93	0.752 ± 0.09	3.57 ± 0.34
II	Lead acetate	11.2 ± 1.0 ^{a,*}	6.1 ± 0.56 ^{a,*}	5.3 ± 0.48 ^{a,*}	1.65 ± 0.06 ^{a,*}	2.23 ± 0.17 ^{a,*}
III	LA + PFG	17.0 ± 2.7 ^b	6.8 ± 0.64 ^b	8.6 ± 0.79 ^b	0.645 ± 0.05 ^{b,c}	4.88 ± 0.25 ^{b,c,***}
IV	LA + PFO	16.9 ± 1.6 ^b	7.2 ± 0.65 ^b	8.3 ± 0.76 ^b	0.622 ± 0.07 ^{b,c}	4.27 ± 0.18 ^{b,c,***}
V	LA + Vitamin E	16.7 ± 1.5 ^b	7.1 ± 0.65 ^b	8.2 ± 0.75 ^b	0.701 ± 0.02 ^b	3.96 ± 0.08 ^b

Each value is the mean (±SD) of six animals

F value Hb 13.3, RBC 6.53, WBC 34.28, Lead 112.8, d -ALAD 118.52

* ≤0.05, ** ≤0.01, *** ≤0.001

[#] d-ALAD Delta amino levulinic acid dehydratase

^a $p \leq 0.05$ between normal control and lead acetate fed group. (I and II)

^b $p \leq 0.05$ between LA fed group and treated groups. (II and III, IV, V)

^c $p \leq 0.05$ between vitamin E fed group and PFG or PFO group (V and III, IV)

Table 2 Concentration of total cholesterol

No.	Groups	Serum (mg/dl)	Liver (mg/100 g tissue)	Heart (mg/100 g tissue)	Kidney (mg/100 g tissue)
I	Normal	80.3 ± 7.3	239.9 ± 21.9	221.4 ± 20.2	331.1 ± 30.2
II	Lead acetate	109.5 ± 9.9 ^{a,*}	540.6 ± 49.3 ^{a,*}	443.7 ± 16.5 ^{a,*}	568.1 ± 51.8 ^{a,*}
III	LA + PFG	78.7 ± 7.2 ^{b,***}	164.0 ± 14.9 ^{b,***}	116.9 ± 4.4 ^{b,***,c}	269.5 ± 24.5 ^{b,***}
IV	LA + PFO	80.3 ± 7.3 ^{b,***}	177.3 ± 16.2 ^{b,***}	137.4 ± 5.1 ^{b,***,c}	308.6 ± 28.7 ^{b,***}
V	LA + Vitamin E	82.7 ± 7.5 ^{b,***}	180.9 ± 15.8 ^{b,***}	200.9 ± 7.5 ^{b,***}	298.4 ± 31.7 ^{b,***}

Each value is the mean (±SD) of six animals

F value Serum 16.18, Liver 208.5, Heart 191.62, Kidney 73.0

* ≤0.05, ** ≤0.01, *** ≤0.001

^a $p \leq 0.05$ between normal control and lead acetate fed group. (I and II)

^b $p \leq 0.05$ between LA fed group and treated groups. (II and III, IV, V)

^c $p \leq 0.05$ between vitamin E fed group and PFG or PFO group (V and III, IV)

Table 3 Concentration of tri acyl glycerol (TAG)

No.	Groups	Serum (mg/dl)	Liver (mg/100 g tissue)	Heart (mg/100 g tissue)	Kidney (mg/100 g tissue)
I	Normal	57.2 ± 5.2	266.5 ± 24.3	43.5 ± 3.9	54.3 ± 4.9
II	Lead acetate	80.2 ± 7.3 ^{a,*}	612.2 ± 55.8 ^{a,*}	95.7 ± 8.7 ^{a,*}	93.9 ± 8.5 ^{a,*}
III	LA + PFG	54.8 ± 5.0 ^{b,***}	196.8 ± 17.9 ^{b,***}	34.9 ± 3.2 ^{b,***}	45.1 ± 4.1 ^{b,***}
IV	LA + PFO	56.2 ± 5.1 ^{b,***}	213.1 ± 19.6 ^{b,***}	37.1 ± 3.4 ^{b,***}	47.2 ± 4.3 ^{b,***}
V	LA + Vitamin E	56.3 ± 5.1 ^{b,***}	226.5 ± 20.7 ^{b,***,v}	39.4 ± 3.6 ^{b,***}	48.2 ± 4.4 ^{b,***}

Each value is the mean (±SD) of six animals

F value Serum 22.1, Liver 189.1, Heart 155.9, Kidney 82.9

* ≤0.05, ** ≤0.01, *** ≤0.001

^a $p \leq 0.05$ between normal control and lead acetate fed group. (I and II)

^b $p \leq 0.05$ between LA fed group and treated groups. (II and III, IV, V)

^c $p \leq 0.05$ between vitamin E fed group and PFG or PFO group (V and III, IV)

Fig. 1 Serum (HDL& LDL)

Units (mg/dl). *≤0.05, ***≤0.01, ****≤0.001 Each value is the mean (±SD) of six animals. ^a $p < 0.05$ between normal control and lead acetate fed group. (I and II) ^b $p < 0.05$ between LA fed group and treated groups. (II and III, IV, V) ^c $p < 0.05$ between vitamin E fed group and PFG or PFO group (V and III, IV) F value: HDL53.5, LDL91.05

acetate fed group (II) but on treatment with active fractions of garlic and onion or vitamin E the TBARS levels significantly decreased. When compared between the treated groups the garlic oil active fraction treated group showed an effect of very high significance ($p \leq 0.001$) more than

vitamin E treated group. Results on serum, heart, liver and kidney GSH (reduced glutathione) levels are given in Table 4. Reduced glutathione level decreased highly significantly in lead acetate fed group but on treatment with the active fractions of garlic and onion oil and vitamin E

Fig. 2 Serum (vitamin E & C) Units-mg/100 ml. $*\leq 0.05$, $**\leq 0.01$, $***\leq 0.001$ Each value is the mean ($\pm$ SD) of six animals. *a* $p < 0.05$ between normal control and lead acetate fed group. (I and II) *b*

$p < 0.05$ between LA fed group and treated groups. (II and III, IV, V) *c* $p < 0.05$ between vitamin E fed group and PFG or PFO group (V and III, IV) F value: Vitamin E 98.75, Vitamin C 14.67

Fig. 3 TBARS Units[#]-mMol/100 ml for serum mMol/100 g tissue *TBARS Thiobarbituric acid reacting substances # Unit of TBARS is expressed as millimol/100 ml for serum and as millimol/100 g for tissue. 0.05, $**\leq 0.01$, $***\leq 0.001$ Each value is the mean ($\pm$ SD) of six animals. *a* $p < 0.05$ between normal control and lead

acetate fed group. (I and II) *b* $p < 0.05$ between LA fed group and treated groups. (II and III, IV, V) *c* $p < 0.05$ between vitamin E fed group and PFG or PFO group (V and III, IV) F-value serum 76.82, liver 204.47, heart 381.16, kidney 28

Table 4 Concentration of glutathione[#] (Expressed as mMol/100 g for tissue and mMol/100 ml for Serum)

No.	Groups	Serum	Liver	Heart	Kidney
I	Normal	13.33 $\pm$ 1.22	228.6 $\pm$ 20.9	257.3 $\pm$ 23.5	174.3 $\pm$ 15.9
II	Lead acetate	8.24 $\pm$ 0.75 ^{a,**}	182.9 $\pm$ 16.38 ^{a,**}	145.3 $\pm$ 13.1 ^{a,**}	95.9 $\pm$ 8.79 ^{a,**}
III	LA + PFG	15.38 $\pm$ 1.40 ^{b*,c}	282.9 $\pm$ 25.82 ^{b*,c}	303.4 $\pm$ 27.7 ^{b*,c}	170.2 $\pm$ 15.52 ^{b*,c}
IV	LA + PFO	14.35 $\pm$ 1.31 ^{b,*}	265.5 $\pm$ 24.2 ^{b,*}	244.5 $\pm$ 13.5 ^{b,*}	164 $\pm$ 14.97 ^{b*,c}
V	LA + Vitamin E	13.33 $\pm$ 1.21 ^{b,*}	251.2 $\pm$ 22.9 ^{b,*}	249.1 $\pm$ 22.7 ^{b,*}	147.6 $\pm$ 13.47 ^{b,*}

Each value is the mean ($\pm$ SD) of six animals

F-value serum 31.526, Liver 18.068, Heart 45.823, kidney 31.661

$*\leq 0.05$, $**\leq 0.01$, $***\leq 0.001$

[#] Unit of glutathione is expressed as millimol/100 ml for serum and as millimol/100 g for tissue

^a $p < 0.05$ between normal control and lead acetate fed group. (I and II)

^b $p < 0.05$ between LA fed group and treated groups. (II and III, IV, V)

^c $p < 0.05$ between vitamin E fed group and PFG or PFO group (V and III, IV)

groups III–V increased reduced their glutathione level significantly.

The serum and tissues antioxidant enzymes such as catalase, SOD, glutathione reductase and glutathione peroxidase are given in the Tables 5, 6 and 7 and Fig. 4 respectively. In lead acetate fed group (II) all the above antioxidant enzymes decreased significantly. On feeding

active fractions of garlic and onion oils to the rats along with lead acetate solution they showed a reversing effect on the above parameters towards normal. Similarly vitamin E feeding to the lead acetate fed group also reversed the altered tissue and serum parameters significantly and the results obtained with feeding of polar fractions of garlic and onion oils are better than those with vitamin E.

Table 5 Activities of catalase (units/mg protein)

No.	Groups	Serum	Liver	Heart	Kidney
I	Normal	18.14 ± 1.66	51.66 ± 4.71	14.97 ± 1.37	25.93 ± 2.37
II	Lead acetate	9.15 ± 0.56 ^{a,*}	27.42 ± 2.51 ^{a,*}	8.69 ± 0.79 ^{a,*}	13.85 ± 1.26 ^{a,*}
III	LA + PFG	26.55 ± 2.42 ^{b,**,c}	65.29 ± 5.95 ^{b,**}	20.8 ± 1.89 ^{**b,c}	29.32 ± 2.68 ^{b,**}
IV	LA + PFO	23.47 ± 2.13 ^{b,*c}	61.81 ± 5.63 ^{b,*}	18.45 ± 1.68 ^{b,*c}	27.78 ± 2.53 ^{b,*}
V	LA + Vitamin E	19.46 ± 6.25 ^{b,*}	60.68 ± 5.54 ^{b,*}	16.71 ± 1.52 ^{b,*}	27.68 ± 2.53 ^{b,*}

Each value is the mean (± SD) of six animals

F value Serum 77.886, Liver 55.982, Heart 56.01, Kidney 43.864

units*Velocity constant/sec

* ≤0.05, ** ≤0.01, *** ≤0.001

^a $p < 0.05$ between normal control and lead acetate fed group. (I and II)

^b $p < 0.05$ between LA fed group and treated groups. (II and III, IV, V)

^c $p < 0.05$ between vitamin E fed group and PFG or PFO group (V and III, IV)

Table 6 Activities of SOD (Expressed as units*/mg protein)

No.	Groups	Serum	Liver	Heart	Kidney
I	Normal	1.38 ± 0.13	4.02 ± 0.37	5.29 ± 0.48	2.48 ± 0.23
II	Lead acetate	0.473 ± 0.43 ^{a,*}	2.14 ± 0.19 ^{a,*}	2.39 ± 0.22 ^{a,*}	0.94 ± 0.086 ^{a,*}
III	LA + PFG	2.31 ± 0.21 ^{b,**,c}	4.63 ± 0.42 ^{b,**,c}	7.78 ± 0.71 ^{b,**,c}	3.84 ± 0.3 ^{**b,c}
IV	LA + PFO	1.78 ± 0.16 ^{b,*}	4.44 ± 0.41 ^{b,*}	6.71 ± 0.61 ^{b,*}	3.09 ± 0.28 ^{b,*}
V	LA + Vitamin E	1.54 ± 0.14 ^{b,*}	4.12 ± 0.37 ^{b,*}	6.29 ± 0.57 ^{b,*}	3.03 ± 0.28 ^{b,*}

Each value is the mean (±SD) of six animals

F-value serum 125.065, liver 45.561, heart 84.715, kidney 105.086

Units*- enzyme concentration required to inhibit the chromogen production by 50 % in 1 min

* ≤0.05, ** ≤0.01, *** ≤0.001

^a $p < 0.05$ between normal control and lead acetate fed group. (I and II)

^b $p < 0.05$ between LA fed group and treated groups. (II and III, IV, V)

^c $p < 0.05$ between vitamin E fed group and PFG or PFO group (V and III, IV)

Table 7 Activities of glutathione reductase (10^{-2} U*/mg protein)

No.	Groups	Serum	Liver	Heart	Kidney
I	Normal	15.79 ± 1.44	6.68 ± 0.61	8.26 ± 0.76	4.31 ± 0.39
II	Lead acetate	10.09 ± 0.92 ^{a,*}	4.77 ± 0.47 ^{a,*}	3.27 ± 0.29 ^{a,*}	2.72 ± 0.25 ^{a,*}
III	LA + PFG	17.12 ± 1.56 ^{b,**}	8.39 ± 0.77 ^{b,**,c}	10.57 ± 0.97 ^{b,**,c}	6.05 ± 0.55 ^{b,**,c}
IV	LA + PFO	16.56 ± 1.51 ^{b,*}	7.86 ± 0.72 ^{b,*}	9.53 ± 0.87 ^{b,*}	5.33 ± 0.49 ^{b,*}
V	LA + Vitamin E	16.82 ± 1.51 ^{b,*}	7.29 ± 0.67 ^{b,*}	8.81 ± 0.80 ^{b,*}	4.92 ± 0.45 ^{b,*}

Each value is the mean (±SD) of six animals

F value Serum 25.88, Liver 27.512, Heart 80.201, Kidney 49.569

U* 1 μm of NADPH oxidized/min

* ≤0.05, ** ≤0.01, *** ≤0.001

^a $p < 0.05$ between normal control and lead acetate fed group. (I and II)

^b $p < 0.05$ between LA fed group and treated groups. (II and III, IV, V)

^c $p < 0.05$ between vitamin E fed group and PFG or PFO group (V and III, IV)

Further the effects are slightly higher for polar fraction of garlic oil.

Histo pathological changes of the liver and kidney can be observed in Fig. 5. Treatment with active fractions of garlic oil/onion oil fractions or vitamin E regenerated the

damaged liver and kidney tissue to a great extent in lead acetate induced toxicity in rats. Their effects are in the order of PFG > PFO > Vitamin E. Total onion oil and vitamin E showed such corrective effects of nicotine toxicity in rats by some workers [29]. The extraction of garlic

Fig. 4 Activities of glutathione peroxidase (10^{-2} U*/mg protein). Each value is the mean ($\pm$ SD) of six animals. *a* $p < 0.05$ between normal control and lead acetate fed group. (I and II) *b* $p < 0.05$ between LA fed group and treated groups. (II and III, IV, V) *c* $p < 0.05$ between vitamin E fed group and PFG or PFO group (V and III, IV) F-Value serum 64.962, liver 89.575, heart 96.735, kidney 58.655

LIVER

Fig. 5 Histopathology of liver tissues **a** Normal: No pathological changes were observed in liver; **b** Lead acetate fed group: Normal structural organization of the hepatic lobules was impaired and congestion of central vein, vacuolization and necrosis of cells. Central

vein and sinusoids between hepatocytes were dilated; **c** PFG oil; **d** PFO oil; **e** Vitamin E treated groups showing very mild grade of degeneration and mild grade of focal necrosis

yield 20 % of polar fraction and 80 % of non polar fraction however on the extraction of onion yield 25 % of polar fraction and 75 % of non polar fraction Fig. 6.

Discussion

Lead is one of the cumulative tissue poisons; the haemopoetic system is one of the target organs in lead toxicity. Lead could affect RBC membrane flexibility, increase RBC fragility and decrease their mobility [30] leading to hemolysis [31]. In addition lead may induce oxidative stress in RBCs [32]. The current data showed that Hb content and WBC count and d-ALAD activity were

decreased in parallel with the decrease in RBC count in experimental rat. The decrease in hemoglobin and d-ALAD activity may coincide with higher serum lead level. Lead may inhibit the body's ability to make Hb by interfering with several enzymatic steps in the heme pathway. Specifically lead decreases heme biosynthesis by inhibition of d-aminolevulinic acid dehydratase and ferrochelatase activity [33, 34]

By pollution or feeding lead induced toxicity in the body affects metabolism through derangement of antioxidant enzymes which may upset the metabolism of lipids such as cholesterol, lipoproteins and triglycerides. Here inhibition of anti oxidant bio molecules such as GSH and anti oxidant enzymes adversely affected the repair of tissues due to lead

Fig. 6 Histopathology kidney **a** normal: No pathological changes were observed in the kidney; **b** Lead acetate fed group: disruption of the normal architecture, vacuolar spaces and disrupted proximal and

distal convoluted tubules; **c** PFG oil; **d** PFO oil; **e** Vitamin E treated groups showing very mild grade of degeneration and restored these pathological changes

toxicity. Treatment with natural herbal compounds may be preferable to other methods in reducing Lead toxicity in the exposed population as they have little or probably no side effects [35]. It was pointed out that garlic oil and onion oil contain natural Sulfur compounds which act as anti lead active substances [36–38]. Therefore free radical scavenging activities co-related with the total phenolics of the extracts [39]. Garlic extracts exerts antioxidant action by scavenging ROS, enhancing the cellular antioxidant enzymes, such as SOD, CAT, and GPX, increasing glutathione in the cells [40].

In our study lead administration lead to increased serum lipid profile TAG, total cholesterol, LDL cholesterol except, HDL cholesterol. Metal toxicity induced rise in serum LDL, VLDL and TAG and fall in serum HDL may be due to gene expression of some hepatic enzymes like HMG CoA reductase which in turn depresses LDL receptor gene expression [41]. The rise in serum lipid profile may also be attributed to increased lipolysis. All these events may lead to increased production of ROS including oxidative stress resulting in metabolic dysfunction. But treatment with garlic and onion oil fractions decreased the above parameters towards normal. It has been suggested that some constituents' garlic and related alliums like organic sulfides may have acted as inhibitors for some enzymes such as Hydroxyl methyl glutaryl COA reductase, which participates in cholesterol synthesis [42, 43]. The results on histopathology of liver and kidney tissues strongly suggest that the toxicological aberrations produced by lead

acetate feeding were counteracted and corrected by the antioxidant effects of PFG and PFO. Their effects are comparatively better than that produced by vitamin E. Here we may also note what Petkov did in such a situation. He used a garlic extract as a remedy in curing the occupational lead poisoning among the factory workers. He called it 'satlal'. He made a preparation of garlic called 'satlal' to be eaten by the works to get ride of their ailment. The sulfides may bind with lead and possibly help people to excrete the toxic metals [44].

A recent Nigerian study showed that auto-mechanics forms a quite vulnerable group to lead accumulation in their blood during a period of 1–5 years. Now a day's everybody is exposed to too much lead and other heavy metals due to polluted air in roads packed with vehicles and filled with heavy cigarette smokers [28, 45]. Therefore daily consumption of garlic, onion and vitamin E in the form of capsules or as food supplements may prevent the excessive production of free radicals that may oxidize LDL cholesterol so as to protect our body from the hazards of oxidative stress and other consequences due to the above mentioned environmental pollutants. The mechanism of action of organic disulfides was suggested by some previous workers [28, 46] against nicotine toxicity particularly based on their antioxidant action as follows.

Disulfide and thiols can also scavenge free radicals.

The thyl radical $\text{RS}^{\cdot}$ helps in the repair of free radicals and it does not react with critical molecules, hence they serve as very good scavengers of free radicals and as antioxidants. On the other hand, vitamin E is an effective antioxidant that delays LPO by reacting with chain – propagating peroxy radicals [47].

Therefore the above finding suggests that PFG, PFO and vitamin E are useful nutritional antioxidants and their supplementation in our diet may nullify the tissue damage or oxidative stress caused by lead pollution in water and food chain.

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Compliance with Ethical Standards

Conflict of Interest G. R. Sajitha, K. T. Augusti, Regi Jose declare that they have no conflict of interest.

Ethical approval “All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.”

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