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Antioxidant activity of β – Sitosterol isolated from *Dodoneoa viscosa* leaf extract on lead induced oxidative stress in rats

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Abstract

Oxidative stress is considered a possible molecular mechanism involved in Lead (Pb) toxicity. Considering the vulnerability of the developing heart, liver and brain to Pb toxicity, this study was carried out to investigate the free radical scavenging activity of β – Sitosterol from methanol extract of D*odonoea viscosa linn* leaf on Pb exposure on the heart, liver and regions of the brain. Adult male albino rats Wister strain were exposed to 200ppm of Pb, as Pb acetate in their drinking. The activities of antioxidant enzymes such as superoxide dismutase (SOD), Glutathione peroxidase (GPx) and Catalase (CAT) were determined in the heart and liver of male adult rats. Superoxide dismutase (SOD), gluthione peroxidase (GPx) and Glutathione reductase (GRed) were also determined in the brain of rats. In addition levels of non-enzymatic antioxidants namely vitamin E, vitamin C from plasma along with total reduced glutathione from liver and heart were also determined. In the Pb-exposed rats, the diminished activity of super oxide dismutase, Glutathione reductase and glutathione peroxidase in the brain of plant extract treated rats was significantly enhanced. The diminished activity of SOD, CAT, GPx in the heart and liver was increased. The decreased activity of vitamin E and vitamin C was normalized in the plasma of plant treated. There was a significant effect of the plant extract on the enzyme activity and the three regions of the brain evaluated in pb exposed rated. Based on the present results, it seems that oxidative stress due to decreased antioxidant function is normalized in methanol extract of *Dodonoea viscosa linn* solution treated rats. Toxicity in the organs tested and neurotoxicity in the brain is due to Pb exposure. And Spectral analysis of the methanol extract of *Dodoneoa viscoasa lin* was carried out.

Keywords: Lead; Lead exposure; Antioxidant system; *Dodoneoa viscosa linn*; Antioxidant enzymes; β – Sitosterol.

1. Introduction

Lead (Pb) is one of the common non-essential toxic heavy metal widely distributed in the environment Chronic exposure to low levels of Pb has been a matter of public health concern in many countries. Pb poisoning exerts its most severe consequences in the developing cardiovascular system (Milton, R 1979), blood (Haeger-Aronen,B 1966), kidney (Cramer, K1974) , liver (Scoppa,P 1973) and brain(Burdette and Goldstein, 1986) due to the intense cellular proliferation, differentiation and synaptogenesis. Moreover, the developing organism presents a five fold greater absorption of Pb (Lockitch, 1993) and lacks a functional blood brain barrier (Goyer, 1990).

Absorption of inorganic lead can lead to certain biochemical and metabolic toxicities (Murthy, G.K1971). The effect of lead toxicity may be due to its impact on the release of free radicals like hydroxyl (Ding, Y.2000). Therefore, a need for a reliable antioxidant which must be capable of scavenging the free radicals when a system is exposed to lead arises. Free radical attack is indicated by change in the levels of some biomolecules in the body. The main products of lipid peroxidation are Malondialdehyde (MDA), conjugated dienes and Hydroperoxides (HYPDX). Moreover, lead is also reported to release free radicals (hydroxy) thereby stimulating the process of lipid peroxidation. Lipids, particularly polyunsaturated fatty acids (PUFA), are the major class of biomolecules susceptible to oxidative damage by free radicals (lipid peroxidation), (Ray, S. 1992 and Akocyznska,A.1993). Lipid peroxides have been shown to impair tissue membranes, which is a risk factor in many diseases. Lead is reported to have an inhibitory action on the membrane bound enzymes such as Na+-K+-ATPase and Mg2+-ATPase in various vital organs (Siegel, G.J. 1977).

Perinatal exposure to low levels of Pb has been involved in behavioural and neuro chemical alterations detected in both suckling and adult rats (Lasley and Lane, 1988; Moreira et al., 2001;Moresco et al., 1988; Rodrigues et al., 1996; Widzowski et al., 1994). One possible molecular mechanism involved in the Pb neurotoxicity is the disruption of the pro oxidant/antioxidant balance (Adonaylo and Oteiza, 1999a; Hermes-Lima etal., 1991; Sandhir et al., 1994), which can lead to heart, liver, kidney and brain injury via oxidative damage to critical biomolecules, such as lipids, proteins and DNA..

To date, the studies that have evaluated oxidative stress in heart, liver and brain following Pb exposure were carried out in adult animals (Sandhir et al., 1994; Adonaylo and Oteiza, 1999b; Rehman, 1984) or in newborns exposed post natally to high doses of Pb (Hsu, 1981; Valenzuela et al., 1989). Based on the above considerations, this study was carried out to investigate the effects of Pb exposure on the antioxidant system of heart, liver and brain. Albino rats were exposed to lead acetate through drinking water for 30 days to induce oxidative stress. Anti oxidant levels in heart, liver,blood and brain (Moreira, et.al.,2001) of rats exposed to Pb were evaluated. The non-enzymatic and enzymatic antioxidants evaluated were: Reduced Glutathione (SGH), Vitamin C, VitaminE, superoxide dismutase (SOD), glutathione peroxidase (GPx) and, Catalase (CAT). The levels of Iron, Phospho lipids and Malondialdehyde (MDA) were also analysed.

2. Materials and Methods

2.1. Plant material

The Leaves of *Dodonoea viscosa linn* were collected during the month of December – January in India. The collected plant material (leaf) was washed thoroughly with fresh water and dried under shade. The shade dried leaf material was powdered and sieved through muslin cloth.

2.2. Methanol Extract preparation

The root extract was prepared first by refluxing it with petroleum ether (50–60 ◦C for 72 h) and then with methanol (60–80 ◦C for 72 h) in a Soxhlet apparatus. The extract was then concentrated in vacuo, kept in a dessicator at room temperature and expressed in terms of dry weight. Before use, the extracted material was dissolved (10 mg/ml) in 0.9% saline and centrifuged at 2000 rpm×10 min at room temperature.

2.3. Isolation and purification of active plant constituent

2..3.1. Silica gel column chromatography

The methanolic leaf extract (100 g) of *Dodonoea viscosa* was dissolved in methanol, centrifuged (2000 rpm ×30 min). The suspension was added to silica gel and evaporated to dryness. The residue was placed on top of the silica gel (60–120 mesh) column (85 cm ×18 cm) and was eluted with petroleum ether:chloroform (9:1, 4:1, 2:1, 1:1, 0:1, 500 ml ×10 fractions each), chloroform:methanol (98:02, 95:05, 90:10, 500 ml ×10 fractions

each). The fraction elutes were evaporated to dryness and tested for (i) homogeneity through TLC and (ii) venom neutralization in experimental animals. For thin layer chromatography, a mixture of silica gel G in water was degassed and poured on TLC plates (20 cm ×10 cm ×1 cm). The plates were activated at 110 .C for 2 h. The eluted fractions were tested for the homogeneity of the compounds. Spots were developed in iodine chamber and *R* f calculated. Each fraction was then tested for venom neutralization.

The extract of *Dodoneoa viscosa* (100 g) fractionated over silica gel (60–120 mesh) column chromatography (Chatterjee et al.2006) gave an active fraction, which was eluted with chloroform: methanol (95:05, v/v). This active fraction when further rechromatographed on silica gel (100–200 mesh) column chromatography resolved into a pure compound eluted by chloroform: methanol (50:50, v/v). On TLC, this compound produced a single spot at *R* f 0.60 using benzene: ethyl acetate (70:30, v/v). Yield of the active compound was 0.2%. Chemical characterization by various spectroscopy viz. IR spectra, NMR spectra and mass spectrum indicated the active compound to be β – Sitosterol.

2.4. Spectroscopic analysis

Spectroscopic analysis was performed by Ultraviolet–visible (UV), Infrared (IR) and Nuclear Magnetic Resonance (NMR). UV absorption spectra were obtained in 1.0% H20 solution using a UV–vis U-2010 Spectrophotometer (Shimadzu). IR spectra were recorded in a Mattson Instruments FTIR spectrophotometer, using KBr disks and 4 cm−1 resolution. 1D and 2D (HSQC) NMR spectra were obtained in D20, taking dioxane or acetone as internal reference, using a Bruker DRX 400 AVANCE spectrometer. The NMR analyses were carried out at the “Sophisticated Analytical instrument facility, Department of chemistry, Indian Institute of Technology(IIT), Madras, India.

2.5. Animal and tissue preparation

Adult male albino rats Wistar strain weighing 100-120g and free from the pathogens were purchased from the Indian Institute of Sciences, Bangalore with the permission of Animal Ethics Committee. The rats were divided into five groups and housed in rat cages, which were exposed to sunlight. The drinking water given to them

was adulterated with 200ppm Pb, as Pb acetate. To prevent the formation of Pb precipitate, 0.5ml of glacial acetic acid was added while stirring to prepare 1000ml of Pb acetate solution. The treatment lasted throughout the period. The Pb exposure regimen in brain regions was chosen based on a previous study (Moreira et al., 2001).

The rats were randomly divided into 5 groups with each group containing 8 rats. All the animals were allowed to drink deionised water ad libitum throughout the study period (30days). (Group1) Normal animals received deionised water alone. (Group2) Control animals had free access to lead acetate in deionised water in a concentration of 200ppm for 30 days. (Group3) Animals were treated with the crude powder of *Dodonoea viscosa* linn leaf in physiological saline at a concentration of 50mg\100g body weight once daily for 30 days. They had free access to deionised water. (Group4) Drug Positive control; Animals were treated with crude powder of *Dodonoea viscosa linn* leaf in physiological saline at a concentration of 50mg\100g body weight once daily for 30 days and had free access to deionised water. (Group5) Lead exposed animals as per group2 were treated with the standard drug vitamin E in Sunflower oil at a concentration of 50 IU\kg.

At the end of 30 days the animals were anesthetized with pentobarbital sodium (60 mg/kg, intraperitoneally), killed under mild anesthesia and blood collected from the jugular vein in heparinised tubes. The body was cut open and tissues were dissected out and rinsed in ice-cold saline. After perfusion through the ascending aorta with 140 mM phosphate buffer saline (PBS) pH 7.4, the brain was removed and dissected using the method of Glowinski and Iversen (1966) into the three regions of interest: hypothalamus, hippocampus and striatum. Due to the small amount of tissue, tissue of three littermates was pooled. The tissue was weighed and homogenized (1 mg tissue/4 ml buffer) in ice-cold 140 mM PBS using 10 strokes in a Teflon/glass homogenizer. The tissue was maintained at 0-4°C throughout the dissection and homogenization procedures. The homogenate was centrifuged for 15 min at 1000*g* at 4 °C and the supernatant centrifuged again at 18 000*g* for 15 min at 4 °C. The organs such as liver, heart and brain were per fused with physiological saline and made into a 10% homogenate with tris sucrose buffer.

2.6. Enzyme assays

The final supernatant from brain tissue homogenate was used for the evaluation of the activity of SOD (McCord and Fridovich, 1969), GPx (Sies et al., 1979) and GRed (Sies et al., 1979). Enzymatic antioxidants such as Superoxide dismutase (SOD), Glutathione Peroxidase (GPx), Catalase (CAT) present were determined from the liver and heart tissue homogenates. Non enzymatic antioxidants namely vitamin E and vitamin C from plasma along with total reduced Glutathione from liver and heart were estimated. The excised liver and heart tissues of albino rats were rinsed in ice-cold physiological saline and then homogenized in Tris_HCL buffer (pH7.4). The tissue homogenates and plasma were used for the following estimations. (1) Thiobarbituric acid reactive substances (TBARS) (lipidperoxide) were estimated according to the method of Nichans and Samuelson (1968). (2) Estimation of Serum Iron by the method of Ramsay et al., ((1958). (3) Phospholipids were estimated by the method of Zilversmith and Davis methods (1950). (4) Alpha-Tocopherol was determined according to the method of Baker and Frank (1980). (5) Ascorbic acid was estimated by the method of Roe and Kuether (1943). (6) Reduced glutathione was estimated by the method of Ellman (1959). (7) The activity of superoxide dismutase (SOD) in heart and liver was assayed by the method of Beauchamp and Fridovich, (1971). (8) Catalase (CAT) activity was assayed by the method of Sinha (1972). (9) The activity of glutathione peroxidase (GPx) was assayed according to the method Rotruck et al., (1973) and SOD, GPX and GRed in the brain were assayed by the method of Morreia et. al.,(2001).

Protein content was determined by the biuret method (Kit Proti A/G, Celm, Brazil). SOD result is expressed as one unit U/mg of protein and GPx and GRed results as one thousand units mU/ mg of protein. All chemicals were obtained from Sigma (St. Louis, MO).

2.7. Blood Pb determination

Pb concentration was determined in blood of albino rats by atomic absorption spectrophotometry with a Zeeman-corrected graphite furnace (Model Spectra AA-220Z, Varian, Australia), as described by (Moreira et al., 2001).

2.8. Statistics

Data are expressed as mean ± SD. The Mann Whitney non-parametric tests and paramertric student's t-test (SAS.1985) were used for tests of significance of differences between groups. A probability of less than 0.05 was accepted as significant. Statistical calculations were performed using SPSS (version 9) software.

3. Results

3.1. Identification of different compounds of *Dodoneoa viscosa leaf*

The fraction E on TLC plate showed 5 bands and designated as E1, E2, E3, E4 and E5 with R_F values of 0.66, 0.60, 0.55, 0.48 and 0.44 cm, respectively. After elution, the Fraction E2 with anti snake venom activity showed single band in TLC plate with the R_F value 0.61 cm.

3.2. Spectral analysis of the compound from *Dodoneoa viscosa*

The UV–vis spectrum of methanol extract in 1.0% aqueous solution shows bands at 206.0 nm (1% 0.60) Uv λ_{max} (MeOH): 206 nm . The UV–vis spectrum of Methanol extract showed bands characteristic of phenol group (Okuda, 1999 The IR spectrum of methanol extract shows absorption bands (KBr disks) at:IRυcm[1]3423,2956.2937.2866,1647,1461,1311,1261,1056,1022,960,789 (Fig.3.6) The 1 H and 13 C NMR of methanol extract was obtained in D2O. Table 2 shows the 1 H NMR and 13 C NMR chemical shifts in ppm(δ): ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm) values: H- 2(1.48,2H,m); H-3(1.99,2H,m); H-(1.82,2H,m); H-5,(3.53,1H,m); H-6(2.27,1H,m); H-7(1.82,2H,m); H-8(1.99,1H,m); H-9(5.36,1H,m); H-10(1.48,1H,m); H-11(1.48,1H,m); H-12(1.11,2H,m); H-13(1.48,1H,m); H-14(1.148,1H,m); H-15(1.48,2H,m); H-16(1.98,1H,m); H-17(1.23,2H,m); H-18(1.11,2H,m); H-19(1.82,1H,m); H-20(1.48,1H,m); H-21(0.82,3H,s); H-221'(0.82,3H,s); H-22(1.08,2H,m); H-23(0.93,3H,s); H-24(0.93,3H,s); H-25(1.00,3H,s); H-26(0.67,3H,s) and ^{13}C NMR ($CDCl_3$, 300MHz, δ ppm) values: C-1,36.46;2,37.19; C-2',140.70, C-3,42.25; C-4,31.60; C-5,71.77; C-6,31.85; C-8,50.05; C-9,121.71; C-10,56.70; C-11,39.70; C-11',42.25; C-12,21.03; C-13,55.97; C-14,28.22; C-15,24.27; C-16,36.11; C-

17,33.65; C-18,25.93; C-19,45.75; C-20,33.87; C-21,18.98; C-21',18.98; C-22,22.99; C-23,12.37; C-24,19.38; C-25,19.81; C-26,12. Finally, the NMR data showed that the aliphatic resonances predominate, indicating the higher proportion of the carbohydrate moieties in methanol extract constituents. Furthermore, the NMR data confirm the tentative structure for this compound as given β – Sitosterol (Fig.1.).

3.3. Antioxidant enzymes activity in Brain

3.3.1. Hypothalamus

The effect of Pb exposure during on the antioxidant enzymes activity of hypothalamus is shown in Table 1. The students t-test showed that SOD, GPx and GRed were decreased ($P<0.05$) in the Pb-exposed group. Plant treated animals showed statistically significant enhancement of the activity of SOD,GPx ($P<0.05$) and GRed ($P<0.05$) .

3.3.2. Hippocampus

The effect of Pb exposure during feeding on the antioxidant enzymes activity of hippocampus is shown in Table2. The students t-test showed that SOD, GPx and GRed were decreased ($P<0.05$) in the Pb-exposed group. Plant treated animals showed statistically significant enhancement in the activity of SOD ($P<0.05$), GPx ($P<0.05$) and GRed ($P<0.05$).

3.3.3. Striatum

The effect of Pb exposure during feeding on the antioxidant enzymes activity of striatum is shown in Table 3. The students t-test indicated a significant increase in the activities of SOD, GPx and GRed ($P<0.05$) when compared to Pb - exposed rats.

3.4. Enzyme assays

Significant differences in enzyme activities were found between the Pb exposed and plant treated liver, heart, plasma and brain of albino rats. As lipid per oxidation increased there was a significant increase in the level of MDA in plasma, liver, and heart of rats exposed to lead (Table 4). This MDA level was normalized in rats treated with *Dodonoea viscosa linn* leaf. The level of vitamin E, Vitamin C (Table 5) and Iron (Table

4) was significantly reduced ($P<0.05$) in all Pb exposed rat plasma samples when compared with normal and plant treated rat plasma samples.

The level of total reduced glutathione was significantly reduced ($P<0.05$) in all-Pb exposed rat tissues when compared with normal and plant treated rat tissues (Table 5). The activity of superoxide dismutase was significantly ($P<0.05$) reduced in all the Pb exposed tissues of rats, while it was highest in heart and liver of normal and plant treated rats. (Table 6), Catalase activity was higher in the heart and liver of normal rats. In Pb exposed rats there was a significant decrease ($P<0.05$) in catalase activity in heart and liver (Table 6). Glutathione peroxidase activity was highest in the heart and liver of normal rats while it was significantly lower ($P<0.05$) in all Pb exposed tissues of heart, liver and brain (Table 7). Overall, activity of antioxidants was significantly lower in all the tissues of Pb exposed rats when compared to equivalent tissues of normal and plant treated rats.

3.5. Blood Pb Levels in rats

The blood Pb levels in the rats were 57.6±4.7μg/dl for the Pb-exposed group. It was not detectable (i.e. 0.1μ g/dl) for the normal groups and plant treated goups.

4. Discussion

Oxidative stress is a term denoting an imbalance between the production of oxidants and the respective defense system of an organism. Oxidants encompass reactive oxygen species, sulfur centered radicals and various others. Oxidative damage may be involved in aging and pathogenesis of major diseases such as cancer, otherosclerosis and certain neurological disorders.

In the present study, a significant increase in the level of MDA in plasma, liver and heart of rats exposed to Lead indicates enhanced lipid per-oxidations. This is due to non- essential toxic heavy metal lead that releases free radicals (hydroxy). The elevated level of MDA in plasma clearly shows lipid per-oxidation in the polymeric fatty acid present within membrane phospholipids of myocardial and hepatic cells. Myocardial and hepatic MDA levels were found to be normalized in rats treated with *Dodonoea viscosa linn* leaves.

The antioxidant enzymes SOD, CAT and GPx play an important role in scavenging toxic intermediates of reactive oxygen species. In the control group, there was a significant reduction in SOD, CAT, GPx in liver and heart. The antioxidant enzymes SOD, GPx and GRed play an important role in scavenging toxic intermediates of reactive oxygen species. In the control group, there was a significant reduction in SOD, GPx and GRed activity. In plant treated groups there was an increase in their antioxidant levels.

We have examined the antioxidant system status and the effect of *Dodoneoa viscosa linn* treatment on three regions of the brain (Hypothalamus,hippocampus and striatum) exposed to Pb. We have chosen to evaluate brain regions instead of whole brain because different regions may respond differently to oxidative stress (Sandhir et al., 1994; Shukla et al., 1988).

In male albino rats the only statistically significant effect that Pb exposure induced was decrease in the activity of SOD ,GPx and GRed in the hypothalamus. The diminished activity of SOD,GPX and Gred were normalized in *Dodoneoa viscosa linn* treated rats.

In adult rats, no effects of treatment were observed in any of the regions evaluated. One could imply that the lack of effects on adults is to be expected since Pb would no more be detected in the blood of these rats at the end thirty days. However, after (Moreira et al.,2001) have reported that Pb is still present in the brain tissue which could have a long term effect of Pb on the antioxidant enzymes.

Oxidative stress has been suggested as one possible mechanism for Pb neurotoxicity. Pb-induced oxidative stress damage could result from: (1) the inhibition of 5-aminolevulinic acid (ALA) dehydratase by Pb leading to the accumulation of ALA, a potential endogenous source of free radicals; (2) direct interaction of Pb with biological membranes, inducing lipid peroxidation; (3) increase of intracellular levels of calcium, impairing mitochondrial functions; and (4) Pb-induced decrease on free radical scavenging enzymes and glutathione (Hermes-Lima et al., 1991; Sandhir et al., 1994). The latter is mainly attributed to the high affinity of Pb for sulfhydryl groups or metal cofactor in these enzymes and molecules (Sandhir et al., 1994).

Based on the present results, it has been confirmed that oxidative stress due to decreased antioxidant function in pb exposed group has been normalized by treating with

Dodoneoa viscosa linn leaf extract The active compound of the extract was identified as β - Sitosterol which was confirmed by NMR Data fig.1. Moreira et al., 2001) have observed neurochemical alterations in different brain regions in adult rats exposed to a Pb exposure regimen similar to that employed in this study (Moreira et al., 2001). We have observed a behavioral alteration in our study. Oxidative stress is the major mechanism involved in the toxicity and neurotoxicity induced by Pb exposure in rats. The result of the present study suggests that *Dodonoea viscosa* leaf offers protection to myocardial cells, hepatic cells and brain tissues either by quenching free radicals or chelating iron enzymes.

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Table.1. Levels of SOD,GPx and GRed in hypothalamus

Groups	**SOD (U/mg protein)**	**GPx (mU/mg protein)**
Normal	32.0±1.3	38.6±1.4
Control	24.3±1.8*	26.0±3.1*
Leaf+Pb	27.0±1.4 *	35.0±1.2
Leaf	33.0±1.4 *	39.0±1*
Standard Vit.E	33.0±1.4 *	36.0±1.2

Data are presented as mean±SE (Eight rats in each groups). Statistical significance student's t-test ∗ (P <0.05).

Table.2. Levels of SOD,GPx and GRed in Hippocampus

Groups	**SOD (U/mg protein)**	**GPx (mU/mg protein)**	
Normal	20.7±1.4	35.6±1.4	
Control	17.3±1.8*	30.0±1.5*	
Leaf+Pb	19.0±1.4	35.0±1.2*	
Leaf	21.0±1.4*	34.0±1.2*	
Standard Vit.E	21.0±1.4	36.0±1.2	

Data are presented as mean±SE (Eight rats in each groups). Statistical significance student's t-test ∗ (P <0.05).

Table.3 Levels of SOD,GPx and GRed in Striatum

Groups	SOD (U/mg protein)	GPx (mU/mg protein)	GRed (mU/mg protein)
Normal	24.2±0.8	35.6±1.0	44.0±1.5
Control	25.3±1.8*	33.0±1.1*	41.8±2.5*
Leaf+Pb	25.0±0.4*	34.0±1.2*	42.0±1.0*
Leaf	24.0±1.4*	33.0±1.2	45.0±1.7*
Standard Vit.E	26.0±1.4	38.0±1.2	43.6±1.4

Data are presented as mean±SE (Eight rats in each groups). Statistical significance student's t-test ∗ (P <0.05).

Table.4. Level of MDA and Iron

Groups	Malondialdehyde (nmoles/100g wt. Tissue) Liver	Malondialdehyde (nmoles/100g wt. Tissue) Heart	Malondialdehyde (nmoles/100g wt. Tissue) Plasma
Normal	176.7±20.03	776.58±103.79	127.9±22.58
Control	697.9±106 *	3427.03±266.3	1994.75±173.1*
Leaf+Pb	697.9±106 *	2138.4±102.2 *	505.9±22.73
Leaf	93.6±3.64	853.4±33.79 *	81.83±21.83 *
Standard Vit.E	443.97±30.94 *	1440.3±14.7 *	538.71±46.70 *

Data are presented as mean±SE (Eight rats in each groups). Statistical significance student's t-test ∗ (P <0.05).

Table 5. Level of Vit E, VitC and Reduced Glutathione

Groups	VitaminE (mg/dl) Plasma	VitaminC (mg/dl) Plasma	Reduced Glutathione (microgram/g wt.tissue) Liver
Normal	133.2±10.56	28.13±4.09	2.51±0.22
Control	58.18±12.5 *	6.03±4.09 *	0.52±0.51 *
Leaf+Pb	92.4±7.41 *	12.1±12.86 *	1.26±0.76 *
Leaf	126.8±3.7 *	33.7±2.2 *	1.77±0.75 *

Standard Vit.E	146.1±4.34 *	26.7±1.19 *	1.23±0.48 *

Data are presented as mean±SE (Eight rats in each groups). Statistical significance student's t-test ∗ (P <0.05).

Table.6. Level of SOD,CAT

Groups	Superoxide dismutase (Units/mg/protein) Liver	Superoxide dismutase (Units/mg/protein) Heart	Catalase Units/mg/protein) Liver	Catalase Units/mg/protei Heart
Normal	1.18+0.03	4.62+1.36	241.89+56.65	8.37+1.84
Control	0.84+0.27	3.39+1.84	413.33+93.52	237.19+57.54
Leaf+Pb	0.69+0.53	5.06+0.54	232.87+68.77	212.17+261.1
Leaf	0.71+0.35	6.10+0.59	186.95+26.49	276.23+36.59
Standard Vit.E	0.86+0.14	6.15+1.07	123.55+74.24	116.59+39.79

Data are presented as mean±SE (Eight rats in each groups). Statistical significance student's t-test ∗ (P <0.05).

Table.7. Level of Gluththione peroxidase and Phospholipids

Groups	Glutathione peroxidase (Units/mg/protein) Liver	Glutathione peroxidase (Units/mg/protein) Heart	Phospholipids (mg/dl) Liver
Normal	3.07+0.77	2.04+0.75	6.112±1.87
Control	0.62+0.30	0.84+0.17	30.36±2.44 *
Leaf+Pb	1.62+0.56	1.20+0.44	8.67±1.14 *
Leaf	1.26+0.49	1.12+0.09	5.75±0.47 *
Standard Vit.E	3.17+0.67	2.03+0.87	9.50±0.75 *

Data are presented as mean±SE (Eight rats in each groups). Statistical significance student's t-test * (P <0.05).