European Journal of Molecular & Clinical Medicine (EJMCM)
ISSN: 2515-8260 Volume 07, Issue 09, 2020

Original research article

Ameliorative Effect of Emblica Officinalis and Zingiber Officinalis as Antioxidant on Arsenic-Lead Toxicity Induced Oxidative Changes on Brain and Testis

Mohd Fazal[1] V.Vishnupriya[2*] Mujahid Mohammed[3] J. Selvaraj[4] Bushra Tahreen[5]

[1]Research Scholar, Saveetha University, Chennai. Asst.Professor, Govt. Medical College Siddipet, Telangana, India.

[2*]Professor Department of Biochemistry, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai 600 077, India.

[3] Professor Department of Physiology, Mamatha Academy of Medical Sciences, Hyderabad, Telangana, India

[4] Associate professor Department of Biochemistry, Saveetha Dental College, Saveetha Institute of Technical and Medical Sciences, Saveetha University, Chennai, Tamilnadu India

[5] Department of Dentistry, International Dental Care, Banjara Hills, Hyderabad, Telangana, India.

**Corresponding Author: V. Vishnu Priya. Professor Department of Biochemistry Saveetha Dental College and Hospitals,
Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-600 077, India.
Email: drvishnupriyav@gmail.com**

Abstract

Aim: This study was aimed to assess the protecting impact of Emblica officinalis (E.officinalis) and monocot genus officinalae (Z.officinalae) leaf extract on reactive element species, inhibitor potential changes in arsenic and lead- evoked toxicity in male rats.
Experimental Design: Adult male Wistar rats were used and divided as Group I: Control; Group II: lead and sodium arsenite together were administered through gastric intubation during 14 consecutive days Group III to Group IX: arsenic and lead induced rats treated with ethanolic extract of Emblica officinalis, *Z.officinalae* orally for 45 days with different concentrations). At the end of the treatment, control and experimental animals were subjected to oxidative stress markers such as H2O2, *OH, and lipid peroxidation (LPO), antioxidant enzymes of Brain and Testis.
Results: arsenic and lead evoked rats showed a major increase within the levels of reactive element species (H2O2, OH* and LPO) with concomitant alterations within the Brain and gonad tissues. Conversely enzymic and non-enzymic inhibitor levels were found to be belittled. However, Associate in Nursing oral effective dose of E.officinalis and Z.officinalae (120 + one hundred twenty mg/kg body weight/ day multiplied the inhibitor enzymes and retrieved the altered levels of ROS and LPO that were evoked by asenic and lead.

Conclusion: it's over from these findings that E.officinalis and Z.officinalae leaf extract exhibits neuroprotective and recovers gonad harm through the restoration of reactive element species and inhibitor enzymes within the brain and gonad tissue of arsenic and lead-induced neurotoxicity and gonad toxicity in rats. Hence, E.officinalis and Z.officinalae leaf extract could also be one amongst the therapeutic choices for the treatment of metal toxicity- evoked brain and gonad diseases.
KEYWORDS: Antioxidant enzymes, Neurotoxicity, Testicular toxicity, Oxidative stress

Introduction

Arsenic associated lead toxicity represents an uncommon, nevertheless clinically important, medical condition. The rise in environmental pollution ends up in serious metal toxicity. If overlooked or sadly molested, this metal toxicity may end up in important morbidity and mortality. Metals are found naturally within the atmosphere and their compositions vary among totally different localities, leading to spatial variations of encompassing concentrations. Distribution of those serious metals within the atmosphere is examined by the properties of the given metal and by varied environmental factors [1]. Serious metals are typically said as those metals that possess a selected density of over five g/cm3 and adversely have an effect on the surroundings and living organisms [2]. In terribly low concentration these metals are essential to take care of varied organic chemistry and physiological functions in living organisms, however they become vesicatory after they exceed bound threshold concentrations. Though it's accepted that these metals have several adverse health effects and last for a protracted amount of your time, exposure to those serious metals continues and is increasing in several components of the planet. Arsenic and lead enter the environment by natural means that and thru human activities. varied routes of arsenic and lead exposure embody wearing, natural weathering of the earth's crust, mining, industrial effluents, urban runoff, sewerage discharge, insect or sickness management agents applied to crops, and lots of others [3]. Earlier study states that aerobic deterioration of biological macromolecules is primarily thanks to binding of serious metals to the DNA and nuclear proteins [4]. Indian gooseberry (Phyllanthus emblica officinalis) extracts, has been employed in ancient drugs to treat symptoms starting from constipation to cancer treatment for hundreds of years within the Indian system of medication [5]. E.officinalis has been shown to be a potent radical scavenging agent, thereby preventing carcinogenesis and cause. A dose of a hundred mg/kg weight has shown to cut back the incidence of growth by just about hour [6,7] Ginger (Zingiber officinale Roscoe) is additionally another natural dietary part usually employed in complementary and medicine (CAM). Varied ginger and ginger leaf extracts are reported positive response in dominant cancer proliferation [8]. However, therapeutic effects of E.officinalis and Z.officinalae on metal-toxicity on multiple organ injury have not been explored. Hence, the current study was aimed toward assessing impact of E.officinalis and Z.officinalae in arsenite and lead-induced toxicity in male anomaly rats.

MATERIALS AND METHODS:

All chemicals and reagents used in the present study were molecular and analytical grade; and they were purchased from Sigma Chemical Company, St. Louis, MO, USA; Amersham Biosciences, Little Chalfont, Buckinghamshire, United Kingdom; and Sisco Research Laboratories, Mumbai, India; Arsenic and Lead was purchased from Sigma Chemicals Company, USA. Biochemical assay kits used in the present study were purchased from Spin react, Spain.

Animals

Animals were maintained as per the guidelines and protocols approved by the Institutional Animal's Ethics Committee and by the regulatory body of the government (IAEC No:

BRULAC/SDCH/SIMATS/IAEC/09-2018/009). Healthy male albino rats of Wistar strain (*Rattus norvegicus*) weighing 180–210 g (150–180 days old) were used in this study. Animals were obtained and maintained in clean polypropylene cages under specific humidity (65 ± 5%) and temperature (27 ± 2 °C) with constant 12 h light and 12 h dark schedule at Biomedical Research Unit and Lab Animal Center (BRULAC), Saveetha Dental College & Hospitals, Saveetha Institute of Medical & Technical Sciences, Chennai– 600 077. They were fed with standard rat pelleted diet (Lipton India, Mumbai, India), and clean drinking water was made available *ad libitum*.

Experimental design

Adult male Wistar rats were used and divided as Group I Control: Animals were given 1 ml distilled water daily by oral intubation for 14 days. Group II: Lead & Sodium arsenite-induced metal toxicity (animals were induced metal toxicity by the administration of arsenic (13.8 mg/kg body weight) and lead (116.4 mg/kg body weight) together. These doses were administered through gastric intubation during 14 consecutive days [The arsenic dose was selected based on the previous method (Brown, et al., 1976; Kerkvliet et al., 1980; Bekemeier et al., 1989; Goyer et al., 1991; Mejia et a., 1997) and dose of lead was selected based on the previous publication (Gelman and Michaelson, 1979; Jason et al., 1981;Mejia et al., 1997)]. Group III: Toxicity induced animals were treated with *E.officinalis* (60mg/kg body weight). Group IV: : Toxicity induced animals were treated with *E.officinalis* (120mg/kg body weight). Group V: Toxicity induced animals were treated with *Z.officinale* (60mg/kg body weight).Group VI: Toxicity induced animals were treated with *Z.officinale* (120mg/kg body weight).VII: Toxicity induced animals were treated with *E.officinalis (60mg/kg* body weight*)+Z.officinale* (60mg/kg body weight). VIII: Toxicity induced animals were treated with *E.officinalis (120mg/kg* body weight*)+Z.officinale* (120mg/kg body weight). Group IX: Normal Control animals were treated with *E.officinalis (120mg/kg* body weight*)+Z.officinale* (120mg/kg body weight). *E.officinalis* and *Z.offcinale* were administered orally for 45 days.
At the end of the treatment, control and experimental animals were subjected to oxidative stress markers, antioxidant enzymes and Brain and Testis function markers.

Animal Scarification and tissue collection:

At the end of the 45 days, rats were anaesthetized with an intraperitoneal injection of sodium thiopentone (40 mg/kg body weight). Lateral incision about 5-6cm was made through the integument and abdominal wall just beneath the rib cage and a small incision was made in the diaphragm using the curved, blunt scissor. The thoracic cavity was opened through the center using retractor clamp to expose heart and the aorta was mounted on a stainless steel cannula. While holding the heart steady with forceps, appropriate needle was used to collect 2-3ml of blood sample from the heart preferably from the ventricle slowly to avoid collapsing of heart. After blood collection needle was inserted directly into the protrusion of left ventricle to extend straight up to 5mm. Released the valve of intravenous tube which is attached to the needle to allow slow and steady flow of around 20 ml/20 min of 0.9% saline solution. A cut was made in right atrium with sharp scissor to make sure that the solution flowed freely. The flow of saline without blood out of the atrium indicates that the perfusion is completed. The perfusion was stopped and the testis and brain tissues were dissected out, washed in ice-cold physiological saline for the assessment of various parameters. Sera was separated from the blood and used for the assay of various parameters.
Volume of sample taken: 100µl

Chemicals and reagents

All chemicals and reagents used in the present study were of molecular and analytical grade; and they were purchased from Sigma Chemical Company, St. Louis, MO, USA; Amersham Biosciences, Little Chalfont, Buckinghamshire, United Kingdom; and Sisco Research Laboratories, Mumbai, India. Biochemical assay kits used in the present study were purchased from Spinreact, Spain. The RT kit used was from Eurogentec (Seraing, Belgium) and Real-time PCR kit was obtained from Takara. MAPK, ERK, JNK, p38, ERK, TGF-β, KIM-1, IL-18 and TNF-α primers were Sigma Chemical Company, St. Louis, MO, USA;

Collection of plant material

Emblica officinalis (Fruit) and Zingiber offcinale (ginger) powder were collected from Chennai District, Tamil Nadu, India respectively and used for ethanolic extraction.

Preparation of plant extracts

One kilo grams (1kg) of dry powders were taken in individual aspirator bottle; 3 litres of ethanol was used and the mixture was shaken occasionally for 72 hours. Then the extract was filtered. This procedure was repeated three times and both extracts were decanted and pooled. The extracts were filtered before drying using Whattman filter paper no.2 on a Buchner funnel and the solvent was removed by vacuum distillation in a rotary evaporator at 40˚C; the extracts were placed in pre-weighed flasks before drying (Saxena and Yadav, 1983).

Assessment of oxidative stress markers

Estimation of lipid peroxidation (LPO)

In the present study, LPO levels were measured by Devasagayam and Tarachand (1987). Briefly, the reaction mixture consisted of 1.0ml 0.15 M Tris-HCl buffer (pH7.4), 0.3ml 10mM KH_2PO_4 and 0.2ml of tissue extract in a total volume of 2ml. The tubes were incubated at 37°C for 20min with constant shaking. The reaction was stopped by the addition of 1ml 1% TCA. The tubes were shaken well and 1.5ml TBA was added and were heated in a boiling water bath for 20 min. Standard tubes containing 10, 20, 30, 40 and 50nM/ml were also run simultaneously. The tubes were centrifuged and the color developed was measured at 532nm. The malondialdehyde content of the samples was calculated using the following formula:

$$\frac{\text{OD of unknown}}{\text{OD of standard}} \times \text{Standard concentration} \times \text{dilution factor} \times \frac{1}{\text{mg protein}}$$

The malondialdehyde concentration of the samples is expressed as nmoles of MDA formed/min/mg protein.

Estimation of hydrogen peroxide generation (H_2O_2)

In the present study, H_2O_2 was measured by Devasagayam and Tarachand (1987). Briefly, the reaction mixture contained 1.64ml phosphate buffer, 54µl HRP, 30µl of 28nM phenol red, 165µl of 5.5 nM dextrose and 600µl of tissue extract. Blank was prepared simultaneously by adding all the reagents except tissue lysate. The reaction mixture was incubated at 35˚C for 30 min and the reaction was terminated by adding 60µl of 10N NaOH solution. Absorbance was read at 610nm against reagent blank on spectrophotometer. For the preparation of standard curve, known amount of hydrogen peroxide and the entire above reagent except enzyme source were incubated for 30min at 35˚C and then 60µl of 19N NaOH was added and optical density

was read at 610nm. The hydrogen peroxide concentration of the sample is expressed as µmol/min/mg protein.

Analysis of hydroxyl radical generation

According to the method of Puntarulo and Cederbaum (1988), hydroxyl radical generation was estimated. To 1ml of tissue extract, 0.2ml 1M phosphate buffer, 0.1ml each magnesium cholride, sodium azide, DMSO and NADPH were added and incubated for 10 min at 37˚C. The reaction was arrested by adding 0.5ml chromotropic acid and boiled for 30min and read at 570nm, against the reagent blank. The hydroxyl radical concentration of the samples is expressed as µmol/min/mg protein.

Estimation of antioxidant enzyme activities in brain and testis

Assay of superoxide dismutase (SOD)

In the present study superoxide dismutase was assayed by the method of Marklund and Marklund (1974). Briefly, to 1 ml of tissue homogenates 0.25 ml of ethanol and 1.25 ml of chloroform were added, kept in a mechanical shaker for 15 min and centrifuged at 20000xg for 15min. To 0.5 ml of the supernatant, 2.0 ml of 0.1 M Tris-HCl buffer pH 8.2; 1.5 ml of distilled water and 0.5 ml of pyrogallol were added. Change in optical density at 0, 1 and 3 min was read at 420 nm in a spectrophotometer. Control tubes containing 0.5 ml of distilled water were also treated in a similar manner against a buffer blank. The enzyme activity is expressed as Units/mg protein. One enzyme unit corresponds to the amount of enzyme required to bring about 50% inhibition of pyrogallol auto-oxidation.

Assessment of Catalase (CAT)

Catalase activity was assayed by the method of Sinha (1972).Briefly, to 0.1 ml of tissue homogenates 1.0 ml of buffer and 0.5 ml of hydrogen peroxide were added and the time was noted, and then 2.0ml of Dichromate acetic acid was added. The green colour developed was read at 570 nm using a spectrophotometer. Catalase activity is expressed as µmoles of H_2O_2 consumed/min/mg protein.

Assay of Glutathione peroxidase (GPx)

Glutathione peroxidase was assayed by the method of Rotruck et al. (1973). In brief, 0.2 ml each of EDTA, sodium azide, GSH, H_2O_2, buffer and tissue homogenates were mixed and incubated at 37°C for 10 min. The reaction was arrested by the addition of 0.5 ml of TCA and the tubes were centrifuged. To 0.5 ml of supernatant, 3.0 ml of phosphate solution and 1.0 ml of DTNB were added and the colour developed was read at 420 nm immediately against blank containing only phosphate solution and DTNB reagent. Graded amount of standards were also treated similarly. GPx activity is expressed as µg of glutathione utilized/min/mg protein.

Glutathione-S-Transferase (GST)

The enzyme activity in the brain and testis was assayed by the method of Habig et al. (1974). In brief, to 1.0 ml of buffer, 0.1 ml of homogenate, 1.7 ml of distilled water and 0.1 ml of CDNB were added and incubated at 37˚ C for 10 min. After incubation, 0.1 ml of reduced glutathione was added. The increase in optical density was measured against that of the blank at 360 nm. Enzyme activity is expressed as µmoles of CDNB utilized/minute/mg protein at 37°C.

Statistical Analysis

The data were subjected to statistical analysis using one-way analysis of variance and Duncan's multiple range test to assess the significance of individual variations between the control and

treatment groups using a computer-based software (GraphPad Prism version 5). In Duncan's test, the significance was considered at the level of $P < 0.05$

RESULTS

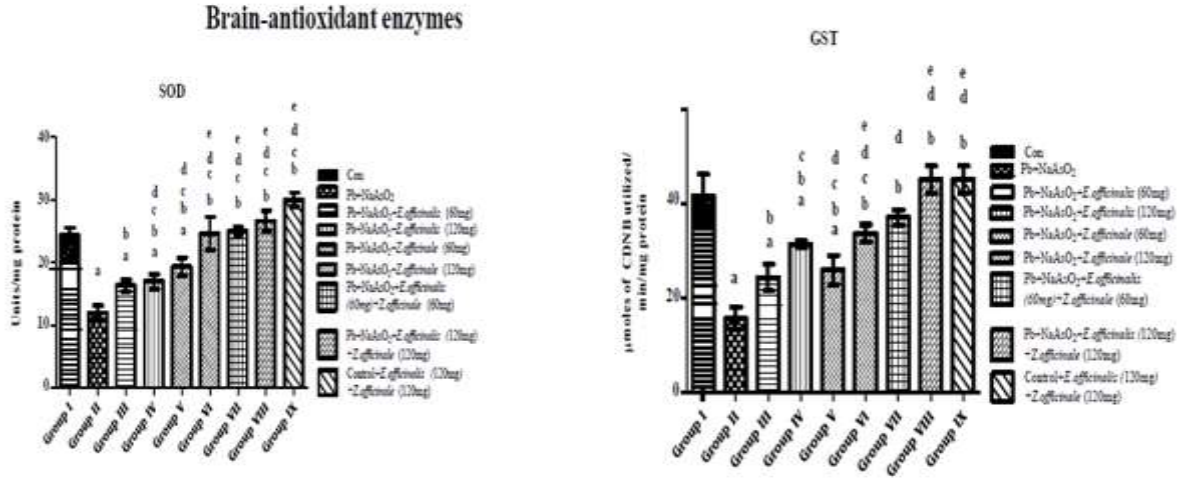
Emblica officinalis and Zingiber officinalis Extract Upraises the Antioxidant Enzymes in Brain of Arsenic-Lead induced Toxicity in Rats

There was a marked decrease observed in antioxidants enzymes (SOD, CAT, GPx, GST, GR, GSH) in the brain of Arsenic-Lead induced Toxicity group compared to control. Treatment with extract of *E.offcinalis* and *Z officinalis* at the doses of 60mg/kg body weight could not increase the antioxidant enzyme levels to that of the control [Figure 1a- d]. However, combined treatment with extract of *E.offcinalis* and *Z.officinalis*120 mg/kg b.wt dose significantly restored the brain antioxidant enzymes level to control level. Control rats treated with *E.officinalis* and *Z.officinalis* extract did not showed any significant change showing the effective dose of leaf extract does not have any toxicity. *E.officinalis* and *Z.officinalis* efficiently increased the level of antioxidant enzymes compared to the induced group due to the presence of antioxidants in the extract [Figure 1 (a-d)].

Emblica officinalis and Zingiber officinalis Extract Upraises the Antioxidant Enzymes in Testis of Arsenic- Lead induced Toxicity in Rat

There was a marked decrease observed in antioxidants enzymes (SOD, CAT, GPx, GST,) in the testis of arsenic- lead induced toxicity group compared to control. Treatment with extract of *E.offcinalis* and *Z.officinalis* at the doses of 60mg/kg body weight could not increase the antioxidant enzyme levels to that of the control [Figure 2a-d]. However, Combined treatment with extract of *E.offcinalis* and *Z.officinalis*120 mg/kg body weight dose significantly restored the testis antioxidant enzyme level to control level. Control rats treated with *E.officinalis* and *Z.officinalis* extract did not showed any significant change showing the effective dose of leaf extract does not have any toxicity.

E.officinalis and *Z.officinalis* efficiently increased the level of antioxidant enzymes compared to the induced group due to the presence of antioxidants in the extract [Figure 2 (a-d)].



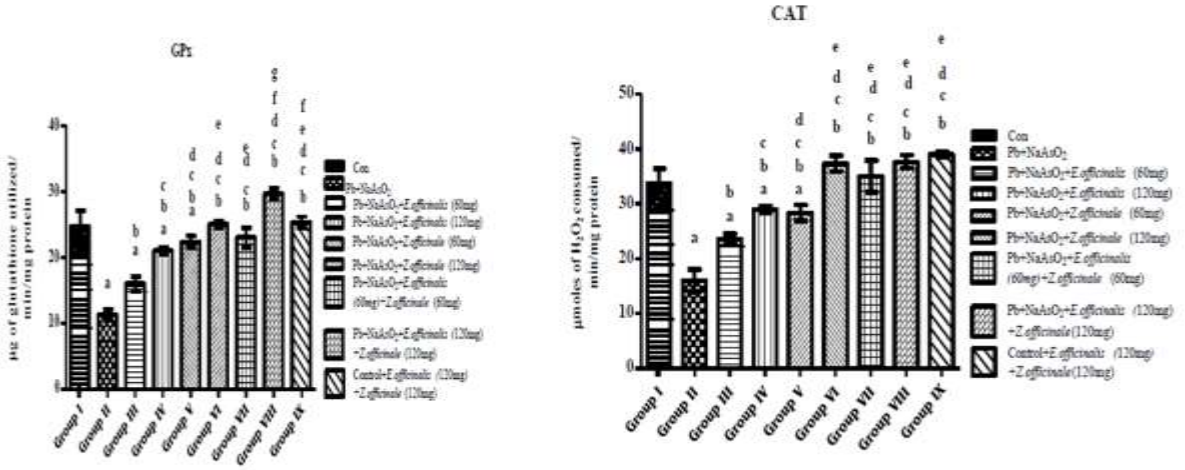


Figure 1 (a-d). Effect of *E.officinalis* and *Z.officinale* on antioxidant enzymes in the brain tissue of lead-arsenic-induced toxicity in adult male rat. Each bar represents mean ± SEM of 6 animals. Significance at p< 0.05, a-compared with control, b-compared with Lead-Arsenic toxicity, c-compared with Lead-Arsenic toxicity rats treated with 60mg *E.officinalis*; d- compared with Lead-Arsenic toxicity rats treated with 120mg *E.officinalis*; e-compared with Lead- Arsenic toxicity rats treated with 60mg *Z.officinale*; f-compared with Lead-Arsenic toxicity rats treated with 60 E.officinalis+60mg *G.officinale*.

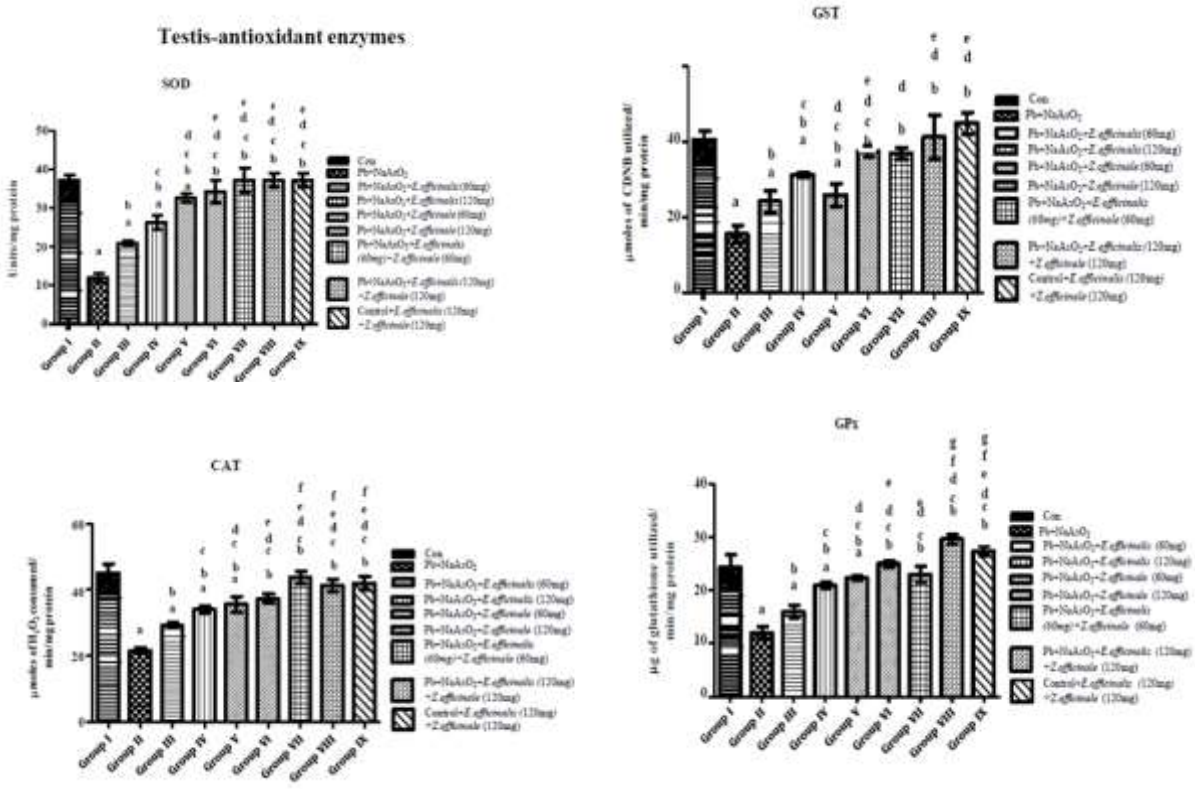


Figure 2 (a-d): Effect of *E.officinalis* and *Z.officinale* on antioxidant enzymes in the testis of lead-arsenic-induced toxicity in adult male rat. Each bar represents mean ± SEM of 6 animals. Significance at p< 0.05, a-compared with control, b-compared with Lead-Arsenic toxicity, c-compared with Lead-Arsenic toxicity rats treated with 60mg *E.officinalis*; d- compared with Lead-Arsenic toxicity rats treated with 120mg *E.officinalis*; e- compared with Lead- Arsenic toxicity rats treated with 60mg *Z.officinale*; f-compared with Lead-Arsenic toxicity rats treated with 60 E.officinalis+60mg *Z.officinale*.

Emblica officinalis and Zingiber officinalis Extract Treatment Modulates the Oxidative Stress in Brain of Arsenic-Lead induced Rats

Compared to control, the H_2O_2, OH*, and LPO in brain were significantly raised in Arsenic-Lead induced rats. Treatment with *E.officinalis* and *Z.officinalis* leaf extract notably brought down the rise in hydrogen peroxide, OH*, and LPO [Table-1].

Emblica officinalis and Zingiber officinalis Extract Treatment Modulates the Oxidative Stress in Testis of Arsenic-Lead induced Rats

Compared to control, the H_2O_2, OH*, and LPO in testis were significantly raised in Arsenic-Lead induced rats. Treatment with *E.officinalis* and *Z.officinalis* leaf extract notably brought down the rise in hydrogen peroxide, OH*, and LPO [Table-2].

OXIDATIVE STRESS MARKERS-BRAIN

Parameters	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII	Group VIII	Group IX
H2O2	0.7±0.05	2.61±0.06^{a}	2.0±0.05^{ab}	1.36±0.06^{abc}	1.2±0.05 abc	0.96±0.08 bcde	0.72±0.03 bcdef	0.9±0.05 bcdef	0.96±0.03 bcdef
LPO	7±0.57	15±1.45^{a}	12±0.88^{ab}	10±0.57^{abc}	10.8±0.44^{abc}	9±0.57^{bcd}	8.7±0.39^{bcd}	8±0.53 bcd	8±0.40 bcd
OH*	7±0.57	16±1.4 a	14±1.15^{ab}	9.3±0.89 abc	9.4±0.71 abc	8±0.57^{bcde}	6.7±0.39^{bcdef}	7±0.52 bcdef	8±0.57 bcde

Table 1. Effect of E.officinalis and *Z.officinale* on oxidative stress (H2O2 & OH*) and lipid peroxidation (LPO) in the brain tissue of lead-arsenic-induced toxicity in adult male rat. Each bar represents mean ± SEM of 6 animals. Significance at $p< 0.05$, a-compared with control, b-compared with lead-arsenic toxicity, c-compared with lead-arsenic toxicity rats treated with 60mg *E.officinalis*; d- compared with lead-arsenic toxicity rats treated with 120mg *E.officinalis*; e- compared with lead-arsenic toxicity rats treated with 60mg *Z.officinale*; f-compared with lead-arsenic toxicity rats treated with 60 E.officinalis+60mg *Z.officinale*.

Units: 1. H2O2- µmol/ming/mg protein

2. OH*- µmol/ming/mg protein

3. LPO-nmoles MDA formed/min/mg protein

OXIDATIVE STRESS MARKERS-TESTIS

Parameters	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII	Group VIII	Group IX
H2O2	2.5±0.18	5.5±0.29^{a}	4.2±0.17^{ab}	3.7±0.14^{abc}	3.6±0.20^{abc}	2.8±0.08 bcde	2.5±0.08 bcde	2.56±0.20 bcde	2.3±0.17 bcde
LPO	7.3±0.52	18±1.15^{a}	15±0.69^{ab}	12±0.57 abc	11±1.05 abc	8.3±0.8^{bcde}	9±0.57 bcde	9±0.5 bcde	8.6±0.8$_{a}$bcde

OH*	7.6±0.33	17±0.8 a	13±0.8 ab	11±0.3 a bc	12±0.57 abc	9±0.57 a bcde	8.7±0.57 bcde	9±0.57 bcde	9±0.57 a bcde

Table 2. Effect of *E.officinalis* and *Z.officinale* on oxidative stress (H2O2 & OH*) and lipid peroxidation (LPO) in the testis of lead-arsenic- induced toxicity in adult male rat. Each bar represents mean ± SEM of 6 animals. Significance at p< 0.05, a-compared with control, b-compared with lead-arsenic toxicity, c-compared with lead-arsenic toxicity rats treated with 60mg *E.officinalis*; d- compared with lead-arsenic toxicity rats treated with 120mg *E.officinalis*; e- compared with lead-arsenic toxicity rats treated with 60mg *Z.officinale*; f-compared with lead-arsenic toxicity rats treated with 60 E.officinalis+60mg *Z.officinale*.

Units: 1. H2O2- µmol/ming/mg protein

2. OH*- µmol/ming/mg protein

3. LPO-nmoles MDA formed/min/mg protein

DISCUSSION:

In the present study metal arsenite (NaAsO2) and lead resolution was accustomed induce toxicity. enhanced generation of ROS and increased lipid peroxidation area unit thought-about accountable for the toxicity of a large vary of compounds. The present study more confirms that metal arsenite (NaAsO2) and dyestuff increase radical production and/or decreases the anti-oxidative enzymes like SOD (SOD) and enzyme (CAT) and GPx that in all probability build the tissue additional liable to organic chemistry injury. GPx reduces lipid hydro peroxides into lipid alcohols within the presence of GSH. The decrease within the level of GSH and increase within the level of GSH/GSSG and lipid peroxidation (LPO) lead to a decrease GPx activity on arsenic and mercury exposure18. antioxidant plays a crucial role within the oxidoreduction sport of GSH to GSSG that's necessary for maintenance of the thiol content of the cell. GPX activity needs GSH as a chemical compound to exert its function; the lower GSH can limit the most activity of GPX. During this study once the experimental cluster was supplemented with E.officinalis and Z.officinalae the whole parameters were reversed within the direction of management cluster. The organic chemistry observation like SOD, GST, GPx and enzyme levels tends to maneuver within the direction of management cluster (Figure1&2). Treatment of E.officinalis and Z.officinalae enhanced aerophilous stability of sex gland and Brain tissues by increasing the endogenous antioxidants. It seems that the interference of E.officinalis and Z.officinalae in levelling the oxidoreduction state and dominant the activation of kinases and transcription factors which may have contributed to bring melioration within the level of affected inhibitor standing. The role of E.officinalis and Z.officinalae has been related to the management of lipid peroxidation, as E.officinalis and Z.officinalae supplement alleviates lipid peroxidation in arsenic-lead treated animals19. Administration of the antioxidants is useful in promoting the recovery from arsenic-lead iatrogenic toxicity, maybe by augmentation of glutathione system; its involvement in detoxification method would possibly facilitate to delay the lipid peroxidation rate24. within the gift study, the E.officinalis and Z.officinalae administration has enhanced the body's inhibitor munitions that decreases the tissue injury that is caused because of radical generation by arsenic and lead toxicity, that supports earlier reports.

CONCLUSION

The Brain and sex gland Antioxidants (SOD, CAT, GPx, GST, and GR,) were found to be altered because of Arsenic-Lead iatrogenic toxicity. Treatment with the effective dose (120mg/kg b.wt) of E.officinalis and Z.officinale leaf extract considerably improved the altered levels of the organic chemistry profiles. the current findings area unit the primary to report back to show the protecting role of E.officinalis and Z.officinale leaf extract potentials arsenic-lead iatrogenic Brain and sex gland toxicity. Hence, it's terminated from the current findings that E.officinalis and Z.officinale exhibit protecting role through the restoration of inhibitor enzymes within the Brain and sex gland of

arsenic-lead iatrogenic toxicity in male rats. More studies area unit required to elucidate the precise mechanisms of protecting role of E.officinalis and Z.officinale to determine its potential.

ACKNOWLEDGEMENTS

The authors wish to thank the Department of Bio-chemistry, Saveetha Dental College & Hospitals, Biomedical Research Unit and Lab Animal Center
(BRULAC), Saveetha Institute of Medical & Technical Sciences, Chennai – 600 077. India, for providing laboratory facilities.

References:

1. Adonaylo VN, Oteiza PI. Lead intoxication: Antioxidant defenses and oxidative damage in rat brain. Toxicology 1999;135:77-85.
2. Bates MN, Smith AH, Hopenhayn-Rich C. Arsenic ingestion and internal cancers: A review. Am J Epidemiol1992;135:462-76.
3. Brochin R, Leone S, Phillips D, Shepard N, Zisa D, Angerio A. The cellular eff ect of lead poisoning and its clinical picture. GU J Health Sci 2008;5:1-8.
4. Chen YC, Lin-Shiau SY, Lin JK. Involvement of reactive oxygen species and caspase 3 activation in arsenite- induced apoptosis. J Cell Physiol1998;177:324-33.
5. Bhatt ID, Rawat S, Badhani A, Rawal RS. Nutraceutical potential of selected wild edible fruits of the indianhimalayan region. Food Chem 2017;215:84-91.
6. Wang CC, Yuan JR, Wang CF, Yang N, Chen J, Liu D, *et al*. Anti-inflammatory effects of phyllanthus emblica l on benzopyrene-induced precancerous lung lesion by regulating the il-1beta/mir-101/lin28b signaling pathway. Integr Cancer Ther 2017; 16:505-15.
7. De A, Powers B, De A, Zhou J, Sharma S, Van Veldhuizen P, *et al*. *Emblica officinalis* extract downregulates pro-angiogenic molecules via up regulation of cellular and exosomal mir-375 in human ovarian cancer cells. Oncotarget 2016;7:31484-500.
8. Susanti SK, Santoso IS, Murwanti R, Suzery M, Oku H. Comparison on the cancer specific cytotoxicity of three gingers (*Zingiber officinale*) leaves varieties from indonesia. Int J Pharm Phytochem Res 2017;9:129-34.
9. Devasagayam TP, Tarachand U. Decreased lipid peroxidation in the rat kidney during gestation. BiochemBiophys Res Commun1987;145:134-8
10. Pick E, Keisari Y. Superoxide anion and hydrogen peroxide production by chemically elicited peritoneal macrophages-induction by multiple nonphagocytic stimuli. Cell Immunol 1981;59:301-18
11. Puntarulo S, Cederbaum AI. Comparison of the ability of ferric complexes to catalyze microsomal chemiluminescence, lipid peroxidation, and hydroxyl radical generation. Arch BiochemBiophys1988;264:482- 91.
12. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem1974;47:469-74.
13. Sinha AK. Colorimetric assay of catalase. Anal Biochem1972;47:389-94.
14. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG, *et al*. Selenium: Biochemical role as a component of glutathione peroxidase. Science 1973;179:588-90.
15. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem 1974;249:7130-9.
16. Staal GE, Visser J, Veeger C. Purification and properties of glutathione reductase of human erythrocytes. BiochimBiophys Acta 1969;185:39-48.
17. Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione S- transferase activities in rat lung and liver. BiochimBiophys Acta 1979;582:67-78.

18. Valko, M, Morris, H., Cronin, M.T. Metals, toxicity and oxidative stress. *Curr. Med. Chem.*, 2005, **12(10)**:1161-208. doi: 10.2174/0929867053764635
19. Ahmed, R. S., Seth, V., & Banerjee, B. D. (2000). Influence of dietary ginger on antioxidant defence system in rat: Comparison with ascorbic acid.*Indian Journal of Experimental Biology*, *38*, 604–606.