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EFFICACY OF *EMBLICA OFFICINALIS* FRUITS IN LPO AND ANTIOXIDANTS ON LEAD TREATED RATS

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ABSTRACT

Emblica officinalis (*EO*) a native plant of India is used in a variety of conditions and to treat a host of ailments besides being a rich source of Vitamin C. The efficacy of this fruit powder administered orally in lead induced toxicity was investigated in rats. Lipid peroxidation and antioxidant enzymes of lead induced and treated rats were studied in liver and kidney. The changes observed in toxic conditions were reverted to near normal values in the tissues under study thereby confirming the potential antioxidant effect of *EO* fruits against lead induced toxicity.

INTRODUCTION: In view of the long history of lead's toxicity, extensive literature reports and the ban imposed on it worldwide, one would think that lead exposure is under control and its poisoning was merely a historical entity. Unfortunately, this is not the case, as lead is widely used in industries such as smelting and battery manufacture, that are on the increase due to rapid industrialization and exponential growth of automobile use by ever expanding population [1]. The exposure to lead takes place from poorly glazed ceramic ware, lead solder in the food canning industry, lead in drinking water, lead compounds in paint and cosmetics and by deposition on crops and dust from industrial and motor vehicle sources [2].

However, there are still industries in many countries (particularly the demolition industry) in which the clinical lead poisoning still occurs [3]. Studies have also shown that considerable exposure can occur in occupations that are not normally considered to be at risk, significant lead levels were seen in blood of workers involved in erecting and dismantling during the renovation of lead painted structures [4]. In addition, organic lead poisoning is occasionally seen in tank cleaners who clean petrol storage tanks that have contained leaded petrol [2].

Emblica officinalis (*EO*) Gaertn. (Indian Gooseberry or Amla) is a medium size deciduous tree belonging to *Euphorbiceae* family. It is distributed in the wild or cultivated throughout India. It is often cultivated in the gardens and home yards. Its fruits are half to one inch in diameter, fleshly obscurely six lobbed containing six trigonous seeds. *EO* has been found to posses antibacterial, anti-viral and anabolic activities [5, 6, 7]; analgesic and anti-pyretic activity [8]; anabolic activity and immunomodulatory properties [9] and antioxidant activity in chronic stress induced changes in rat brain [10]. Natural antioxidants particularly in plants demonstrate a wide range of biochemical and pharmacological activities in animals and humans such as anti-inflammatory, anti-hyperlipidemic, anti-ulcer, anti-proliferative, immunomodulatory, neuro, cardio and hepato- protective effects [11]. In addition, plants and their anatomical parts are used as antidotes in case of poisoning as well as prophylaxis to overcome the toxic effect. These materials can also be taken for a long time as food or food supplements.

Traditional plants have been the starting point for the discovery of many important drugs and this fact has led to chemical and pharmacological investigation of plants and undertaking of general biological screening of plants worldwide [12]. Of late, there is an upsurge in utilizing the plant compounds as antioxidants. Keeping in view the reported beneficial effects of Vitamin C in providing recovery in lead induced toxicity, it was considered worthwhile to study this vitamin present in naturally occurring and easily accessible plant *Emblica officinalis* in lead exposed rats.

METERIAL AND METHODS:

Chemicals: Commercially available lead (Pb) metal powder was procured from S. D. Fine Chemicals, Mumbai, India.

Plant material: *E. officinalis* fruits were procured from the local market and the pulp was collected by removing the seeds, cut into small pieces and air dried indoor under subdued light and with good ventilation, ground well with mortar and pestle and sieved to get a fine powder.

Animals and treatment: Adult male albino rats of Wistar strain (180-190 g) were obtained from Tamil Nadu University of Veterinary and Animal Sciences, Chennai, India and maintained according to the principle and guidelines of CPCSEA (No. 324) under the supervision of Animal Ethical Committee. The animals were housed in standard polypropylene

laboratory cages containing 5cm deep layer of sawdust bedding, kept under normal photoperiod (12 h light and 12 h dark). They were fed with commercial pelleted chow supplied by Poultry Research Station, Chennai, India and water was provided *ad libitum*.

Experimental protocol: Animals were randomly segregated into two groups. Group- I with six animals served as control. The remaining twenty four animals received lead metal as fine powder of 100 ppm suspended in coconut oil, administered orally once daily for a period of 30 days. The dose was based on previous work by Ding *et al.,* [13] but Pb metal was administered orally suspended in coconut oil instead of drinking water. On day 31, the lead exposed animals were further divided into four groups of six animals each and treated as follows for seven days.

Group I- Control
Group II- Lead exposed (100 ppm)
Group III- Pb+*EO* (50mg/kg)
Group IV- Pb+*EO* (100 mg/kg)
Group V- Pb+*EO* (200mg/kg)

On day eight, the over night fasted animals were sacrificed under light ether anesthesia by cervical dislocation and organs *viz.* liver and kidney were removed, weighed separately and stored at 20°C.

LPO and Antioxidant studies: A portion of the tissue of known weight was minced into small pieces with sharp scissors and then homogenized in the cold room with 0.01M Tris HCL buffer, pH 7.4 using a high speed potter- Elevehjem Teflon Homogenizer to obtain a 10% homogenate [14]. Aliquots of this homogenate were used for estimating Lipid Peroxidation (LPO)[15], *Total R*educed Glutathione (TRG)[16], Vit. C [17], Superoxide Dismutase (SOD)[18], Glutathione Peroxidase (GPx)[19], Catalase (CAT)[20] and Protein [21] following standard procedures. The enzyme assays were completed within 24 hours of sacrifice of rats.

Statistical Analysis: The data obtained from the studies were subjected to statistical analysis using SPSS package. The values are expressed as the mean±SD and the differences between groups were statistically analyzed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT).

RESULTS: Lipid peroxide and antioxidant enzymes mentioned above were estimated in liver and kidney of control, lead induced toxicity and *EO* treated rats. In liver an increase of LPO, SOD, GPx, CAT and decrease of Vitamin C, TRG were observed on lead treated rats. Treatment with *EO* (50, 100 and 200 mg/kg) showed a reverse in the values in LPO, TRG, Vitamin C, SOD and CAT dose dependently towards that of control rats. In the case of GPx there was a significant decrease in the values of 50 and 100 mg/kg of *EO* but within the group there was an increase with 200 mg/kg dose than lead induced rats (**Table 1**).

In kidney LPO, SOD and GPx increased significantly while TRG, Vitamin C and CAT decreased on lead induced rats. On subsequent treatment with *EO* with different doses, the LPO in 50 mg/kg dose showed a significant decrease when compared to the control and there was a further significant decline in its value on 100 and 200 mg/kg doses. TRG, Vitamin C and SOD showed a reverse in its value towards that of the control rats in a dose dependent manner. In case of GPx there was a significant decrease in the values of 50 mg/kg dose but on 100 and 200 mg/kg doses the value significantly increased with 200 mg/kg dose showing higher value than that of the lead induced rats. CAT showed an excellent recuperation on 50 mg/dl dose but further increased dose dependently (**Table 2**).

TABLE 1: EFFICACY OF *EMBLICA OFFICINALIS* ON LPO AND ANTIOXIDANT ENZYMES IN LIVER OF LEAD TREATED RATS

Parameters	LPO	TRG	Vitamin C	SOD	GPx	CAT
Control	0.17±0.025	15.00±0.190	4.89±0.152	0.85±0.041	8.51±0.050	98.07±1.353
Pb exposed	3.86±0.050^{a***}	8.50±0.038^{a***}	1.72±0.065^{a***}	3.75±0.041^{a***}	19.27±0.057^{a***}	246.52±0.062^{a***}
Pb+EO (50 mg/kg)	0.50±0.038^{b***}	4.15±0.038^{b***}	2.54±0.039^{b***}	2.04±0.037^{b***}	11.43±0.053^{b***}	127.23±0.502^{b***}
Pb+EO (100 mg/kg)	0.45±0.041^{b***}	6.75±0.041^{b***}	2.65±0.060^{b***}	1.80±0.038^{b***}	16.65±0.045^{b***}	85.35±0.041^{b***}
Pb+EO (200 mg/kg)	0.42±0.044^{b***}	9.25±0.041^{b***}	2.80±0.038^{b***}	1.20±0.038^{b***}	20.72±0.031^{b***}	60.45±0.041^{b***}

Values represent mean ± SD of six animals. a denoted parameters are compared to control rats, b - denoted parameters are compared to lead treated rats *P< 0.05, **P<0.01, ***P<0.001, ^{NS} -Non-significant LPO - Lipid peroxidation (nmol / mg protein); TRG - Total reduced glutathione (mg / g of tissue); Vitamin C (µg/g of tissue); SOD - Superoxide dismutase (U^B / mg protein); GPx - Glutathione peroxidase (U^C / mg protein); CAT - Catalase (U^A / mg protein) U^A - µmoles of H_2O_2 utilized/sec/mg protein; U^B - Amount of enzyme required to give 50% inhibition of NBT reduction; U^C - µmoles of GSH utilized/minute

TABLE 2. EFFICACY OF *EMBLICA OFFICINALIS* ON LPO AND ANTIOXIDANT ENZYMES IN KIDNEY OF LEAD TREATED RATS

Parameters	LPO	TRG	Vitamin C	SOD	GPx	CAT
Control	0.98 ± 0.025	10.50±0.078	2.87±0.120	1.00±0.047	9.30±0.254	72.31±0.496
Lead exposed	2.24±0.317^{a***}	6.32±0.215^{a***}	0.85±0.041^{a***}	2.50±0.273^{a***}	26.50±0.158^{a***}	39.25±0.573^{a***}
Pb+EO (50 mg/kg)	0.44±0.055^{b***}	5.50±0.055^{b***}	1.08±0.098^{b***}	1.95±0.025^{b**}	19.99±0.025^{b***}	74.56±0.033^{b***}
Pb+EO (100 mg/kg)	0.32±0.022^{b***}	7.10±0.015^{b***}	1.25±0.035^{b***}	1.50±0.024^{b***}	25.12±0.016^{b***}	80.15±0.024^{b***}
Pb+EO (200 mg/kg)	0.18±0.025^{b***}	8.85±0.045^{b***}	1.32±0.081^{b***}	1.10±0.055^{b***}	29.63±0.025^{b***}	86.86±0.045^{b***}

Values represent mean ± SD of six animals. a – denoted parameters are compared to control rats, b - denoted parameters are compared to lead treated rats *P< 0.05, **P<0.01, ***P<0.001; ^{NS} -Non-significant LPO - Lipid peroxidation (nmol / mg protein); TRG - Total reduced glutathione (mg / g of tissue); Vitamin C (µg/g of tissue); SOD - Superoxide dismutase (U^B / mg protein); GPx - Glutathione peroxidase (U^C / mg protein); CAT - Catalase (U^A / mg protein) U^A - µmoles of H_2O_2 utilized/sec/mg protein; U^B - Amount of enzyme required to give 50% inhibition of NBT reduction; U^C - µmoles of GSH utilized/minute

DISCUSSION: In the present study, the increase of LPO on lead induced toxicity in both liver and kidney was observed supporting the fact that generation of highly reactive oxygen species (ROS), such as superoxide radicals (O_2^+), hydrogen peroxide (H_2O_2), hydroxyl radicals ($^-$OH) and lipid peroxides (LPO), in the aftermath of heavy metal ions toxicity damaging various cellular components including proteins, membrane lipids and nucleic acids [22].

On *EO* treatment in different doses LPO in both liver and kidney decreased significantly which was in accordance with Halliwell and Gutteridge [22] that vitamin C (ascorbic acid) being a low molecular mass antioxidant, scavenges the aqueous ROS by very rapid electron transfer that inhibits lipid peroxidation and same has been emphasized in liver and brain [23]. This is further supported by Dhawan [24] that the recovery from lead induced toxicity on treatment with ascorbic acid might occur via increased urinary elimination of lead and reduce hepatic and renal lead burden. Vitamin C supplementation in lead-exposed animals significantly reduced blood, liver, and renal lead levels indicating a significant protective action of vitamin C against toxic effects of lead on heme synthesis and drug metabolism [25].

The significant changes in the antioxidant enzymes on lead exposure concurs with Chiba [26] who has observed that lead induced toxicity alter antioxidant activities by inhibiting functional SH groups in several enzymes such as SOD, CAT and GPx. Another observed mechanism for lead induced oxidative stress on the antioxidant defense systems of cells states that lead and other metals such as mercury and cadmium have a high affinity

for sulfhydryl (SH) groups, mercaptides are formed with the SH group of cysteine and less stable complexes with other amino acid side chains [27]. Increase in GPx maybe attributed to low level of TRG observed which is utilized in the removal of LPO through the GPx reaction.

Therefore, decreased level of LPO after concurrent Vitamin C treatment as is the case in this study may save GSH from its higher utilization in this reaction and may eventually be responsible for the restoration of GSH status. Such a protective effect may be of great importance in view of the reports that GSH offers a first line of defence against lead toxicity [28]. It is further supported by Hunaiti [29] where glutathione and glutathione mediated detoxification enzymes play an important role in the protection of cells against oxidative stress including lead toxicity and blood lead level is related to intracellular glutathione.

On lead exposure, the level of SOD and GPx showed a significant increase in both liver and kidney while CAT increased in the liver but showed a significant decrease in kidney which was in accordance of Gelman [30] who has reported that GPx, CAT, and SOD are potential targets for lead toxicity because these antioxidant enzymes depend on various essential trace elements for proper molecular structure and activity. On EO treatment SOD and CAT almost restored their levels towards that of normal rats dose-dependently in both liver and kidney and such a restoration of these antioxidant molecules are thought to play a crucial role in counteracting free radical-induced damage and found to heel the free radical-mediated cell damage [31], which has also been the case in the present work.

Additionally, administration of vitamin C significantly inhibited the lipid peroxidation levels of liver and brain and increased the catalase levels of kidney in lead-exposed rats, Patra [23] was also in support of the present study. Vitamin C has decreased significantly on lead exposure, this depletion might be due to the utilization of this antioxidant during oxidative stress. Treatment with *EO* normalized the vitamin C level in liver and kidney. As per earlier reports, Vitamin C directly reacts with reactive oxygen species, hydroxyl radicals and various lipid hydroperoxides, in addition, also restores the antioxidant property of oxidised Vitamin E [32] besides offering effective protection against plasma lipid peroxidation [33].

Some important naturally occurring vitamins, particularly Vitamin C fights off widespread environmental pollutants including carbon monooxide, hydrocarbons, pesticides and heavy metals by stimulating enzymes in the liver that detoxify the body [34]. *EO* which is a rich dietary source of vitamin C, minerals and amino acids and also contains a wide variety of phenolic compounds besides active constituents such as phyllembic acid, ellagic acid, gallic acid, tannoids [35] have been reported as powerful antioxidants and renders the fruit a valuable antiscorbutic in the fresh as well as in the dry condition.

The fruit extract has been reported to possess a variety of pharmacological activities and inhibits micronuclei formation, sister chromatid exchanges, clastogenicity and mutagenicity induced by metals such as lead, aluminum, cadmium, nickel and cesium [36]. All these phytochemicals maybe responsible for the reversal of LPO and antioxidant enzymes in lead intoxicated rats. It also contains hydrolysable tannins emblicanin A and B [37]. The prophylactic, curative and restorative effects of the fruits were thought to be mainly due to these factors, even though the actual mechanism has to be studied in detail.

CONCLUSION: *Emblica officinalis* maintains the endogenous antioxidants, thus reduces oxidative stress and alleviates the pathological changes

caused by lead in liver and kidney. *EO* fruit powder neutralize the oxidizing potentials of reactive oxygen species generated thereby maintaining cell membrane integrity and viability. The present study also indicated that *EO* in all the doses showed a significant revival affirming the vital role played by ascorbic acid in the reduction of the toxicity. Most of the parameters studied emphasizing the fact that the fruits of *EO* can be effectively utilized to combat lead induced toxicity as well as the clues from traditional use of plant materials can be employed to finding remedies for a wide spectrum of pollution related problems.

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