

AN *IN VITRO* INVESTIGATION OF AMELIORATIVE EFFECT OF SYNTHETIC AND HERBAL ANTIOXIDANTS ON LEAD INDUCED NEUROTOXICITY

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ABSTRACT

Lead is one of the ubiquitous and dangerous occupational as well as environmental toxicants of global concern. Due to its capacity to act as potent systemic toxicant, it is the present need to investigate different ameliorative techniques for prevention of lead toxicity through proper understanding of molecular mechanisms. Present study deals with *in vitro* system of investigation of ameliorative effect of specific synthetic antioxidants especially, N-acetyl cysteine (5.5 mM/kg/day), Ascorbic acid (200 mg/kg/day), Tocopheryl acetate 160 mg/kg/day) and Thiamine (30 mg/kg/day) as novel combinational therapy approach as well as *Bacopa monnieri* (10 mg/kg/day) as herbal antioxidant therapy against lead induced neurotoxicity. The current synergistic study

involves culturing of goat brain in DMEM: F12 (1:1 mixture) culture media containing 1 ppm lead acetate along with administration of selective antioxidants at prescribed dosage for 6-hrs. Selective biochemical parameters such as Lipid Peroxidation, Protein Levels, Alkaline Phosphatase, Acid Phosphatase, Succinate Dehydrogenase, Adenosine Triphosphatase and Superoxide Dismutase activities were analyzed and observed for protection against lead intoxication. Results indicate significant alteration in all biochemical parameters studied in lead exposed cultures as compared to control. Total and Soluble Proteins, Alkaline Phosphatase, Succinate Dehydrogenase, and Adenosine Triphosphatase showed significant reduction while Lipid Peroxidation, Acid Phosphatase and Superoxide Dismutase activities increased significantly in lead exposed cultures as compared to control. Results also emphasized that simultaneous administration of prescribed antioxidants and lead in cultures manifested maintenance of all biochemical parameters studied and strongly suggest that

synthetic and herbal antioxidants therapy can confer protection against lead induced neurotoxicity in mammals.

KEYWORDS: Lead Toxicity, Synthetic Antioxidants, Herbal Antioxidant, *Bacopa monnieri* extract, Neurotoxicity, Oxidative Stress.

1. INTRODUCTION

Lead is one of the most cumulative and hazardous heavy metal toxicants of environmental and industrial origin. In terms of occurrence frequency, toxicity and the potential for human exposure, lead is second on the list of the 'Top twenty hazardous substances' according to the Agency for Toxic Substances and Disease Registry.^[1] Standard for acceptable exposure to lead has steadily declined in current century and is the subject of much remediation research. Lead is an abundant and frequently encountered toxic contaminant of global environment due to its significant role in modern industry and it is still being used recklessly. Lead occurs in a variety of organic and inorganic compounds with a multitude of additional uses in the manufacture of protective paints for iron and steel, explosives, rodenticides, batteries, cosmetics, pigment industries, medicines, pipes, radiation shields, ammunition and in more recent times in alloys for welding storage materials for chemical reagents.^[2] Environmental contamination of lead is a serious problem worldwide due to its bioaccumulation in food chain and continued persistence in ecosystem. A soft, grey-blue heavy metal lead is a common cause of poisoning in domestic animals throughout the world.^[3]

Lead poisoning (also known as plumbism, colica pictonium, saturnism, Devon colic, or painter's colic) is a medical condition caused by increased levels of lead in the body.^[4] Lead is a prevalent environmental toxin with no beneficial biological role. Lead metal is a highly neurotoxic agent that particularly affects the developing nervous system.^[5,6] Lead is a potent toxicant even at very low level of exposure.^[7] Exposure to lower levels of lead is associated with behavioral abnormalities, learning disabilities, hearing dysfunctions and impaired cognitive functions in experimental animals and humans.^[8,9,10,11,12]

The pathogenesis of lead toxicity is multi-factorial, as lead directly interrupts enzyme activation, competitively inhibits trace mineral absorption, binds to sulfhydryl of proteins and interrupts structural protein synthesis, alters calcium homeostasis, and lowers the level of available sulfhydryl antioxidant reserves in the body.^[13] Therefore, it is extremely essential to find protective measures against adverse exposure of this toxic metallic element.

The main objective in undertaking this *in vitro* study was to elucidate the mechanism of lead toxicity through alteration in lipid peroxidation, enzyme activities and protein levels in goat brain to avoid ethical concern of involving experimental animals. Besides this, the search for agents which could help in the amelioration of lead toxicity is essential to be worked out. Experimental evidence suggests that cellular damage mediated by free radicals can be involved in the pathology associated with lead intoxication. Micronutrients such as vitamins and thiol containing N-acetyl cysteine have been proved to possess antioxidative property and lie functionally at the heart of protective mechanism involving mobilization of metal from soft as well as hard tissues. In addition to modifying the metal toxicity, these micronutrients can also act as metal chelating agents^[14] and scavengers of reactive oxygen species.^[15] As oxidative stress is the main mechanism in lead neurotoxicity, the real focus of the current study was to explore the beneficial role of specific synthetic antioxidants such as N-acetyl cysteine, Ascorbic acid, Tocopheryl acetate and Thiamine in a novel combination against lead intoxication which was not worked out earlier. In addition, there was meager information available regarding cost effective herbal products with minimum side effects as ameliorating agents against lead induced neurotoxicity. *Bacopa monnieri* is a well known nerve tonic reported to enhance memory development, learning behavior and cognitive ability in humans.^[16] *Bacopa monnieri* extract was found to exhibit interesting antioxidant properties, expressed by its capacity to scavenge superoxides, peroxides and hydroxyl radicals^[17,18] as well as modulating the expression of enzymes involved in generation and scavenging of reactive oxygen species.^[19] Keeping in view, another major aim of present study was to evaluate therapeutic potential of traditional ayurvedic medicinal plant *Bacopa monnieri* as an alternative nutraceutical in counteracting lead toxicity.

2. MATERIALS AND METHODS

Experimental Design

In the present *in vitro* study, experimental protocol was divided into two separate phases. During the first phase, the prescribed dose of lead acetate (1 ppm) selected on basis of LD₅₀ and reported literature based on previous studies was added in goat brain cultures for specific period of time to investigate the lead induced alteration in various biochemical parameters *in vitro*. The second phase involves simultaneous addition of synthetic antioxidants as well as *Bacopa monnieri* extract as herbal antioxidant separately in lead acetate containing cultures for specific time duration to investigate ameliorative effect of the antidotes against lead induced neurotoxicity.

2.1 Chemicals

The AR grade Lead acetate trihydrate, Ascorbic acid, N-Acetyl Cysteine (NAC), Vitamin-B₁ (Thiamine), Vitamin-E (Tocopheryl acetate) and other chemicals having 99% purity used in the present study were obtained from HI-MEDIA, SIGMA, and MERCK Laboratory Pvt. Ltd., India.

2.2 Preparation of herbal (*Bacopa monnieri*) extract

Bacopa monnieri plant was collected from botanical garden of School of Sciences, Gujarat University, Ahmedabad, India in the month of December. The plant material was identified and authenticated by Botany Department of the university with voucher specimen submitted to the herbarium. The whole plant of *Bacopa monnieri* was thoroughly washed with double distilled water, cut into small pieces and dried in shade in dust free condition for one week at room temperature before being ground to a coarse powder. The powdered whole plant material (10 g) was subjected to soxhlet extraction procedure at 70°C for 11 - hrs using 90% ethanol (100 ml) as a solvent. The resultant crude extract was concentrated, air-dried and stored at -20°C in a dark bottle until use.

2.3 Preparation of lead acetate solution

0.010 gm of lead acetate was dissolved in 100 ml of double distilled water to prepare the stock solution of 100 ppm. A definite volume of this stock solution was used in a final volume of the reaction mixture, so as to get the required concentrations of 1 ppm lead acetate.

2.4 Sample collection

In the present experimental study, goat brain was used as a mammalian vital organ. Brain sample of healthy adult goat (*Capra hircus*) was obtained from approved local slaughter house. After sacrificing the animal, fresh brain tissue was brought to laboratory under frozen condition and used immediately. The appearance of fresh tissue can be described as a whitish grey color. The brain tissue was washed in normal saline, blotted dry by pressing between 2-3 folds of filter paper, cut into pieces of appropriate weight and divided into different experimental groups.

2.5 Experimental groups

Experimental design includes six experimental groups:

(I) Control Group

(II) Synthetic Antioxidants Mixture (NAC-5.5 mM/kg/day, Vitamin C-200 mg/kg/day, Vitamin E-160 mg/kg/day and Vitamin B₁-30 mg/kg/day) (co-administration) Exposed Group

(III) Herbal Antioxidant (*Bacopa monnieri* extract – 10 mg/kg/day) Exposed Group

(IV) Lead Acetate (1 ppm) Exposed Group

(V) Lead Acetate and Synthetic Antioxidants Mixture (co-administration) Exposed Group and

(VI) Lead Acetate and Herbal Antioxidant (*Bacopa monnieri* extract) (co-administration) Exposed Group

2.6 *In vitro* study

All the experimental groups were cultured in DMEM: F12 (1:1 mixture) media supplemented with 0.5% FBS, 1% Streptomycin and L-Glutamine along with HEPES in the culture petri plates at same conditions in BOD incubator containing 5% CO₂ at 35°C-37°C for 6-hrs duration and subjected to various biochemical analysis after the process of proper homogenization with constant pace and speed under suitable condition of 4°C in chilled glass mortar pestle in order to maintain its viability. The cultures were subjected to cell viability test using trypan blue for investigating ameliorative effect of the antidotes against lead toxicity.

2.7 Biochemical analysis

To analyze free radical induced cell injury by lead acetate and its protection by antioxidants, the levels of MDA were measured in cultured brain homogenates. The estimation of Lipid Peroxidation (LPO) as a part of oxidative indices in the brain cultures was done by the method of Ohkawa *et al.*^[20] To study the impact of lead acetate on protein metabolism; levels of Total Proteins, Soluble Proteins and Insoluble Proteins were determined in brain homogenate by the method of Lowry *et al.*^[21] At the end of lead acetate exposure as well as antioxidants treatment, certain specific parameters of goat brain such as enzyme activities of Alkaline Phosphatase (ALPase) and Acid Phosphatase (ACPase) were also assayed by the method of Bessey *et al.*^[22] To understand alteration in energy metabolism, activities of Succinate Dehydrogenase (SDH) and Adenosine Triphosphatase (ATPase) were estimated by methods of Beatty *et al.*^[23] as well as Quinn and White^[24] respectively. As a representative of oxidative stress marker, the activity of Superoxide Dismutase (SOD) was analyzed by the method of Kakkar *et al.*^[25]

2.8 Statistical analysis

Student's 't – test' was used for the statistical analysis of the data. For each parameter (n=5), the data were expressed as mean \pm SEM after subjecting to Student's 't – test' for the interpretation of results.

3. RESULTS

3.1 Lipid peroxidation

Results of the lipid peroxidation in the goat brain cultures exposed to Lead acetate (1ppm), Synthetic antioxidants mixture, Herbal antioxidant *Bacopa monnieri* and their related control *in vitro* are given in the Table-1. The percentage of difference, due to the lead acetate and synthetic as well as herbal antioxidants exposure with respect to control group as well as lead exposed group, is given in Table-2. The lead acetate exposure for 6-hrs was found to increase production of thiobarbituric acid reactive substances (TBARS) as marked by highly significant elevation in lipid peroxidation with respect to control as represented by 23.53 % ($p < 0.0001$).

Addition of prescribed dosage of antioxidants to goat brain cultures did not cause any significant effect. However, simultaneous addition of lead acetate and synthetic antioxidants in goat brain cultures significantly reduced (11.76 %) lead induced lipid peroxidation (Table-2). Supplementation of mixture of synthetic antioxidants as ameliorative agent provides statistically very significant protection against lead intoxication ($p < 0.0021$). Simultaneous addition of lead acetate and *Bacopa monnieri* extract in goat brain cultures significantly reduced lead induced lipid peroxidation (Table-2). Supplementation of herbal antioxidant as ameliorative agent exerted statistically extremely significant (19.08%) protection against lead induced oxidative stress ($p < 0.0001$) as compared to lead exposed group.

3.2 Protein levels

Lead acetate exposure caused significant decline in the total protein and soluble protein levels in goat brain cultures as compared to control (Table-1). The decrease in the total protein and soluble protein at 1 ppm lead exposure was represented as 2.88% ($p < 0.0001$) and 29.97% ($p < 0.0001$) respectively while statistically extremely significant ($p < 0.0001$) increase in the insoluble protein was observed in lead exposed cultures.

However, co-administration of lead acetate and synthetic antioxidants in goat brain cultures significantly increased total protein (2.04 %) and soluble protein levels (5.71%) (Table-2).

Supplementation of *Bacopa monnieri* extract to lead exposed tissue as a therapeutic agent resulted in extremely significant ($p < 0.0001$) maintenance of total protein and soluble protein levels nearest to control group. Insoluble protein levels also showed decline due to synthetic antioxidants (1.32%) and herbal antioxidant (2.38%) exposure as compared to control group.

3.3 Alkaline phosphatase activity (E.C.3.1.3.1)

Lead acetate exposure to goat brain cultures for 6-hrs brought about a significant alteration in the alkaline phosphatase activity. Results revealed that alkaline phosphatase activity markedly decline as compared to control (Table-1). The reduction in the enzyme activity at 1 ppm exposure was represented as 25.10 % ($p < 0.0001$).

Addition of lead acetate and synthetic antioxidants simultaneously in goat brain cultures provided significant protection (6.38 %) in enzyme activity against lead induced alterations as compared to control (Table-2). Addition of prescribed dosage of *Bacopa monnieri* to goat brain cultures did not cause any significant effect. Exposure of herbal antioxidant to lead exposed cultures worked as an effective ameliorative agent and maintained alkaline phosphatase activity (33.51%) as compared to lead exposed tissue.

3.4 Acid phosphatase activity (E.C.3.1.3.2)

Results revealed that acid phosphatase activity significantly increased in lead acetate exposed goat brain cultures as compared to control group (Table-1). Statistically extremely significant elevation of ($p < 0.0001$) (7.78 %) of enzyme activity was observed at 1 ppm concentration of lead acetate exposure.

However, administration of synthetic antioxidants as well as herbal antioxidant in lead exposed goat brain cultures significantly ($p < 0.0001$) restored (2.60 %) and (1.57%) enzyme activity respectively (Table-2) which suggests therapeutic efficiency of antioxidants against lead toxicity.

3.5 Succinate dehydrogenase activity (E.C.1.3.99.1)

Statistically significant decline was found in the Succinate dehydrogenase activity in the brain cultures exposed to lead acetate as compared to control group (Table-1). The reduction in enzyme activity at low dosage (1 ppm) was represented as 29.07 % ($p < 0.0001$). However, co-administration of lead acetate and synthetic antioxidants as well as lead acetate and *Bacopa monnieri* extract in goat brain cultures significantly ($p < 0.0001$) maintained (4.72 %) and

(7.07%) enzyme activity respectively with respect to control and (34.34%) and (31.02%) with respect to lead exposed group respectively (Table-2) which suggests the ameliorative potential of the selected antioxidants.

3.6 Adenosine triphosphatase activity (E.C.3.6.1.3)

Lead acetate exposure to goat brain cultures for 6-hrs brought about a significant alteration in the adenosine triphosphatase activity. Results revealed that enzyme activity markedly declined as compared to control group (Table-1). The reduction in the enzyme activity at 1 ppm exposure was represented as 31.16 % ($p < 0.0001$). Addition of lead acetate and synthetic antioxidants simultaneously in goat brain cultures exerted significant protection (25.13 %) against enzyme activity alteration (Table-2). Addition of prescribed dosage of *Bacopa monnieri* to goat brain cultures acts as an effective ameliorative agent and prevented alteration in ATPase activity (26.63%) in the lead intoxicated tissue (Table-2).

3.7 Superoxide dismutase activity (E.C.1.15.1.1)

Results of the SOD activity in the goat brain cultures exposed to Lead acetate (1ppm), Synthetic antioxidants mixture, Herbal antioxidant *Bacopa monnieri* and their related control *in vitro* are given in Table-1. The percentage of difference, due to the lead acetate and synthetic as well as herbal antioxidants exposure with respect to control group as well as lead exposed group, is given in Table-2. Lead acetate exposure for 6-hrs to brain cultures was found to increase oxidative stress which can be marked by highly significant elevation in superoxide dismutase activity with respect to control as represented by $p < 0.0001$.

Simultaneous addition of lead acetate and synthetic antioxidants in goat brain cultures significantly reduced (52.78 %) lead induced increase in SOD activity as compared to control and (62.66%) as compared to lead exposed group (Table-2). Supplementation of mixture of synthetic antioxidants as ameliorative agent exerted statistically extremely significant ($p < 0.001$) maintenance of enzyme activity against lead induced oxidative damage. Simultaneous addition of lead acetate and *Bacopa monnieri* extract in goat brain cultures significantly reduced SOD activity (36.46%) with respect to control and (66.65%) with respect to lead exposed group as given in Table-2. Supplementation of herbal antioxidant as ameliorative agent imparted statistically extremely significant ($p < 0.0001$) protection in lead intoxicated brain cultures.

Table – 1: Biochemical parameters of control and exposed brain tissue (n=5, mean ± SEM)

BIOCHEM. PARAMET.	CONTROL	SYNTHETIC ANTIOXIDANTS	HERBAL ANTIOXIDANT	LEAD ACETATE	LEAD ACETATE + SYNTHETIC ANTIOXIDANTS	LEAD ACETATE + HERBAL ANTIOXIDANT
LPO ^a	544.85 ± 10.135	576.90 ± 20.270 ^{NS}	544.85 ± 10.135 ^{NS}	673.05±22.662 ^E	608.95 ± 10.135 ^G	544.85 ± 10.135
Total Protein ^b	11.872 ± 0.022	11.63 ± 0.016 ^A	11.63 ± 0.008 ^A	11.53 ± 0.019 ^A	11.63 ± 0.008 ^A	11.63 ± 0.008 ^A
Soluble Protein ^c	1.855 ± 0.014	1.776 ± 0.008 ^F	1.855 ± 0.008 ^{NS}	1.299 ± 0.008 ^A	1.749 ± 0.014 ^E	1.855 ± 0.022
Insoluble Protein ^d	10.017 ± 0.022	9.858 ± 0.017 ^C	9.779 ± 0.008 ^A	10.229 ± 0.019 ^A	9.885 ± 0.008 ^D	9.778 ± 0.022 ^A
ALPase ^e	1.741 ± 0.005	1.675 ± 0.002 ^A	1.749 ± 0.002 ^{NS}	1.304±0.006 ^A	1.630 ± 0.002 ^A	1.741 ± 0.006
ACPase ^f	3.575 ± 0.006	3.668 ± 0.010 ^A	3.427 ± 0.006 ^A	3.853±0.010 ^A	3.668 ± 0.006 ^A	3.631 ± 0.006 ^D
SDH ^g	242.13 ± 0.598	236.43 ± 0.614 ^B	238.33 ± 0.601 ^G	171.73±1.346 ^A	230.714± 0.604 ^A	225 ± 1.186 ^A
ATPase ^h	0.199 ± 0.001	0.182 ± 0.001 ^A	0.170 ± 0.001 ^A	0.137±0.001 ^A	0.149±0.001 ^A	0.146 ± 0.00
SOD ⁱ	2.359 ± 0.037	3.024 ± 0.151 ^G	2.895 ± 0.049 ^A	9.651±0.228 ^A	3.604 ± 0.062 ^A	3.219 ± 0.055 ^A

p – Values: A = < 0.0001; B = 0.0002; C = 0.0004; D = 0.0005; E = 0.0009; F = 0.0014; G = 0.002

NS = Non significant v/s control group

a = nano moles of MDA / 100 mg tissue weight / 60 minutes

b = mg Total Protein / 100 mg fresh tissue weight

c = mg Soluble Protein / 100 mg fresh tissue weight

d = mg Insoluble Protein / 100 mg fresh tissue weight

e = μ moles of p-nitrophenol released / 30 minutes / 100 mg tissue weight

f = μ moles of p-nitrophenol released / 30 minutes / 100 mg tissue weight

g = μ g formazan formed / 15 minutes / 100 mg tissue weight

h = μ moles of ip released / hr / mg tissue weight

i = milliunits / mg protein

Table – 2 Gross Effect of Lead and Antioxidants on Goat Brain Cultures *in vitro* (% of difference with respect to their control as well as lead exposed cultures)

SR.NO.	BIOCHEMICAL PARAMETERS	Group IV Lead (1 ppm)	Group V Lead + Synthetic Antioxidants (Relative to Control)	Group VI Lead + Herbal Antioxidants (Relative to Control)	Group V Lead + Synthetic Antioxidants (Relative to Group IV)	Group VI Lead + Herbal Antioxidants (Relative to Group IV)
1.	Lipid Peroxidation	23.53*	11.76*	0.00	9.52	19.08
2.	Total Proteins	2.88	2.04	2.04	0.87*	0.87*
3.	Soluble Proteins	29.97	5.71	0.00	34.64*	42.80*
4.	Insoluble Proteins	2.12*	1.32	2.38	3.36	4.40
5.	Alkaline Phosphatase	25.10	6.38	0.00	25*	33.51*
6.	Acid Phosphatase	7.78*	2.60*	1.57*	4.80	5.76
7.	Succinate Dehydrogenase	29.07	4.72	7.07	34.34*	31.02*
8.	Adenosine Triphosphatase	31.16	25.13	26.63	8.76*	6.57*
9.	Superoxide Dismutase	309.11*	52.78*	36.46*	62.66	66.65

All values are expressed in % of decrease or *increase

4. DISCUSSION

Current findings showed that MDA levels were increased in lead exposed group compared to control group. The difference between two groups was statistically significant. Elevated levels might be due to formation of free radicals. Several studies suggested the primary involvement of increased amount of reactive oxygen species (ROS) in lead-exposed animals in support of our data.^[26] Heavy metal lead exposure enhances lipid peroxidation in RBC, liver and brain. Most important consequence includes the peroxidation of membrane lipids, with an increase in permeability of the cell membrane.^[27] Lead may impair the production of free radical scavengers such as glutathione in the brain thereby, an increase occurred in generation of lipid peroxides and thus, rendering the tissue susceptible to free radical injury which highlights role of free radicals in lead toxicity. In the present study, the results emphasized that co-administration of synthetic antioxidants as well as *Bacopa monnieri* extract to lead exposed cultures separately, significantly ameliorates lead acetate-induced lipid peroxidation by inhibiting the chain reaction and lowering free radical levels.

Decline in total protein content might be due to the fact that lead reacts with the sulfhydryl groups in certain proteins and thus interferes with a number of enzyme systems essential to cellular metabolism. In the present study, reduction in protein levels could be attributed to their damage by singlet oxygen, often due to oxidation of essential amino acids. Further malondialdehyde (MDA) formed during lipid peroxidation could react with –SH groups of proteins to damage them, thus inhibiting enzymes requiring –SH groups for their activities.^[28] The other factors responsible for alteration in protein metabolism might be the inhibition of biosynthesis of proteins due to lead acetate treatment, which could be due to the impairment of peptide chain initiation. The decrease in proteins might be as a result of increased proteolysis and the reduced incorporation of amino acids into proteins.^[29] Thus, one of the reasons for neurotoxicity in the current study might be the lack of available proteins necessary for growth and differentiation of tissues and various enzyme systems. Increase in insoluble proteins may be due to conversion of soluble proteins into insoluble proteins as a result of lead intoxication.

The selected antioxidants are able to prevent cell injury by maintaining sulfhydryl groups of membrane binding proteins. The neuroprotective and membrane stabilizing properties of the prescribed antioxidants can be attributed to their metal chelating and quenching action,

reduction in free radicals, reduction in cell protein necrosis as well as glutathione depletion reduction potential.

Many products of lipid peroxidation such as hydro peroxides can inhibit protein synthesis and alter chemotactic and enzyme activity.^[30] The results elucidated that lead acetate treatment caused a significant depletion in activity of alkaline phosphatase in brain. Alkaline phosphatases are a group of enzymes, which hydrolyze phosphrate esters at alkaline pH and associated with many functions at cellular level. Alkaline phosphatase plays an important role in brain differentiation and the formation of synaptic connections.^[31] Reduction in alkaline phosphatase activity at low level lead exposure might be a consequence of oxidative stress generated alteration in permeability of plasma membrane in addition to alteration in the balance between synthesis and degradation of enzyme. Alteration in alkaline phosphatase activity is likely to create derangement in the transport of metabolites.

The data of current investigation also revealed statistically significant elevation in acid phosphatase activity in the brain because of lead acetate treatment. Acid phosphatase, a lysosomal enzyme is involved in a number of activities such as phagocytosis,^[32] autolysis, dissolution of tissue components, fat absorption in intestine, cellular differentiation and keratinization.^[33] The alteration in activity might be due to direct adverse effect of oxidative stress resulted due to accumulated lead in the brain. The effects of lead acetate treatment may cause imbalance between synthesis and degradation of acid phosphatase enzyme and thus affect lysosomal activity and phagocytosis.

SDH is an essential oxidative enzyme involved in Citric acid cycle. The results accumulated in the present biochemical analysis revealed a considerable loss of SDH activity in the lead acetate exposed brain cultures. Changes in the SDH activity of brain revealed alterations in its oxidative energy metabolism indicating lesions in TCA cycle. Decreased succinate dehydrogenase activity would affect the conversion of succinate to fumarate and cause a block in the Krebs's cycle. Moreover, since SDH is a mitochondrial enzyme, its decreased activity indicates a possible alteration in mitochondrial structural organization and function because of lead acetate treatment. The mitochondrial enzymes and tissue respiration may be altered due to accumulation of lead in mitochondria. Lead may cause a depression in the activity of SDH in various tissues and it may uncouple oxidative phosphorylation which may reflect on the slow rate of TCA cycle. This condition brought about a reduction in synthesis

of ATP. Thus, reduced activity of SDH reflects upon the disturbed state of oxidation and energy metabolism of a tissue.

The results of the present study revealed reduction in ATPase activity due to lead exposure in brain cultures which is suggestive of disturbance in energy metabolism. As ATPase enzyme possesses highly reactive -SH groups, the interaction of lead with these groups can be possible mechanism for interruption of enzyme activity. Degradation of proteins, reduction in GSH level and replacement of Ca^{+2} or Mg^{+2} by Pb^{+2} required for Ca-ATPase, Mg-ATPase or Na^{+} - K^{+} ATPase activities may also be plausible mechanism in lead intoxication. Similar type of result was also observed in the composition of RBC membrane Na^{+} - K^{+} ATPase by Hasan in 1971 in support of our data.^[34]

SOD forms the first line of endogenous antioxidative defense against reactive oxygen species generation in lead intoxication. SOD plays an important role in protecting the cells against toxic effects of superoxide radicals by catalyzing its dismutation reactions.^[35] Our findings showed increase in the SOD activity which is suggestive of oxidative insult in the tissue due to lead intoxication. Increased activity may be due to activation of compensatory mechanism in tissue in response to higher generation of superoxide radicals at low level lead exposure. Costa, Madiha and Soltaninejad also reported elevation of SOD activity in experimental animals treated with lead which corroborates with our data.^[36,37,38]

The results of the present study elucidated that the mixture of synthetic antioxidants and herbal extract both play protective role and manifested maintenance of Protein levels, alkaline phosphatase, acid phosphatase, succinate dehydrogenase, adenosine triphosphatase and superoxide dismutase activities against lead intoxication in brain.

The mechanism of action of synthetic antioxidants seemed to be mainly by virtue of detoxification, because they are powerful reducing agents which participate in oxidation – reduction reactions. These antioxidants are very powerful free radical scavengers and can act as very effective neuroprotective agents against lead induced oxidative stress. N-acetyl cysteine has antioxidant capacity to lead, including oxidative stress via stimulating glutathione synthesis, thereby maintaining intracellular glutathione levels and scavenging reactive oxygen species.^[39] In addition, NAC also has some chelating action on lead.^[40] Nutritional factors are often mentioned as important modifiers of lead metabolism and lead toxicity.^[41] Growing evidence indicates that ascorbic acid also acts as an anti-oxidative

nutrient which is important for neurological functions.^[42] Chan in 1993 reported that vitamin C is a chain breaking antioxidant that stops the propagation of the peroxidation process.^[43] Vitamin C is widely known to restore and recycle the antioxidative properties of vitamin E and glutathione, when attacked by pro-oxidants.^[44] Vitamin E is nature's major lipid soluble chain breaking antioxidant, which is known to protect biological membranes and lipoproteins from oxidative stress.^[45] Thiamine, the endogenous -SH containing molecule, was recognized as protective agent for lead exposure.^[46] The proposed mechanism of thiamine in antagonizing lead toxicity might be attributed to formation of complexes between thiamine and lead followed by its excretion. Thiamine also has been found to protect against lead-induced lipid peroxidation in rat liver and kidney.^[47] It may scavenge O₂ and OH directly and thus affect the cellular response to oxidative stress.^[48] Anna and Wigło also reported that thiamine may act as a potent antioxidant as it scavenges free radicals.^[49] Thus, it can be interpreted that correct combination of selected synthetic antioxidants has capacity to completely eliminate lead induced neurotoxicity in mammals.

Bacopa monnieri (Brahmi) is a versatile, nootropic, indigenous herb with wide spectrum of medicinal properties. *Bacopa monnieri* has been shown to exert antioxidant effects through the chelating of metal ions, breaking oxidative chain reaction,^[50] improving the activities of antioxidative defense enzymes^[51] and scavenging the free radicals.^[52] By inhibition of acetylcholinesterase as antioxidant, brahmi can exert a neuroprotective effect that relieves neuronal oxidative stress.^[53] *Bacopa monnieri* can significantly recover endogenous antioxidants (SOD, CAT, GPx, GR and GSH) which protect neurons against reactive oxygen species along with improving acetylcholinesterase activity.^[54] *Bacopa monnieri* also confers protection against reduction in ATPase activity. Ethanolic extract of *Bacopa monnieri* contains a mixture of triterpenoids, steroids and saponins.^[55,56] The pharmacological properties of *Bacopa monnieri* were attributed mainly due to the presence of characteristic saponins called 'Bacopsides'.^[57,58] Total Phenolic and flavanoid components present in the extract may provide strong antioxidant property to plant by acting as free radical scavengers, metal chelators, reducing agents, hydrogen donors and singlet oxygen quenchers.

Thus, antioxidative potential of the selected antidotes helps in reducing the oxidative stress of lead exposure by improving the pro-oxidant/antioxidant balance of the cells and significantly ameliorates the lipid peroxidation which subsequently prevents reduction in protein levels as well as provides protection against alteration in enzyme activities in goat brain cultures.

5. CONCLUSION

In conclusion, the findings of present study clearly elucidated that heavy metal lead affects the anti-oxidative as well as other biochemical indices of goat brain possibly by inducing oxidative stress. From above study, it is concluded that lead metal appears to cause an imbalance in the antioxidant defense system by inhibiting some related enzymes, thereby enhancing the free radical mediated peroxidation of lipids. The results also revealed that lead acetate adversely affect the protein content as well as energy and oxidative metabolism of goat brain. Thus, from present *in vitro* study, it can be clearly concluded that lead exposure has definitely destructive effect on the structural, metabolic and functional status of brain even at low level.

The current synergistic study also emphasized that administration of the selected synthetic antioxidants as well as *Bacopa monnieri* extract to lead exposed cultures act as therapeutic eliminators of heavy metal and significantly exert protective effects against adverse effects of lead intoxication due to their well-known antioxidant and neuroprotective properties. Thus, it is clearly revealed that synthetic antioxidants combinational therapy as well as herbal treatment approach can play pivotal role in alleviating the lead toxicity. Hence, these antidotes can be proved to be very effective and beneficial ameliorating agents against lead induced neurotoxicity the world over.

The present investigation has elucidated the mechanism of action of lead induced toxicity as well as its mitigation through synthetic and herbal antioxidants and as such is a significant contribution in the field for amelioration of plumbism in endemic regions.

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