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Research Article

AMELIORATIVE ACTION OF SYNTHETIC AND HERBAL ANTIOXIDANTS ON LEAD INDUCED HEPATOTOXICITY: AN *IN VITRO* STUDY

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ABSTRACT

Objective: A lead is one of the most hazardous and persistent environmental toxicants of global concern today. The lead has propensity to act as a potent mammalian systemic toxicant. Therefore, investigation of effective ameliorative techniques against lead toxicity through proper exploration of molecular mechanisms is the main objective of the current study.

Methods: Present *in vitro* study deals with the investigation of ameliorative effect of specific synthetic antioxidants in a mixture 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 hepatotoxicity. The current synergistic study involves culturing of goat liver in Dulbecco's Modified Eagle Medium:F12 (1:1 mixture) culture media containing 1 ppm lead acetate along with co-administration of selective antioxidants at prescribed dosage for 6 hrs. Selective biochemical parameters such as lipid peroxidation (LPO), protein levels, alkaline phosphatase (ALPase), acid phosphatase (ACPase), succinate dehydrogenase (SDH), adenosine triphosphatase (ATPase), and superoxide dismutase (SOD) activities were analyzed and observed for protection against lead intoxication.

Results: Results indicate significant alterations in all biochemical parameters studied in lead exposed cultures as compared to control. Total and soluble proteins, ALPase, SDH, and ATPase showed significant reduction while LPO, ACPase, and SOD activities increased significantly in lead exposed cultures as compared to control. The results also emphasized that simultaneous administration of prescribed antioxidants and lead in cultures manifested maintenance of all biochemical parameters studied nearest to control group.

Conclusion: Synthetic and herbal antioxidants therapy have protective role against lead induced hepatotoxicity.

Keywords: Lead toxicity, Synthetic antioxidants, Herbal antioxidant, *Bacopa monnieri* extract, Hepatotoxicity, Oxidative stress, Plumbism, Hepatoprotective activity, Lipid peroxidation, Superoxide dismutase.

INTRODUCTION

A lead is one of the most ubiquitous and cumulative heavy metal toxicants of environmental and industrial origin. Agency for toxic substances and disease registry has placed lead on the second position in the list of "Top 20 hazardous substances" [1]. A standard for acceptable exposure to lead has steadily declined in the current century which has aroused serious concerns for researchers and remained the subject of much remediation research.

Non-essential and pervasive toxic metal lead has been used recklessly in the modern industry in manufacturing of lead-acid batteries, cosmetics, protective paints for iron and steel, pencil, explosives, weights, rodenticides, toys, kitchen utensils, fusible alloys, ayurvedic medicines, pipes, radiation shields, and ammunition [2].

Environmental contamination of lead is a serious problem worldwide due to its bioaccumulation in food chain and continued persistence in ecosystem. Lead has been detected in all phases of biological systems [3]. It poses serious health problems to susceptible populations, such as children and occupationally exposed people. A soft, grey-blue metal lead is a common cause of poisoning in domestic animals throughout the world [4].

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 [5]. Lead poisoning is known to induce a large spectrum of physiological, biochemical and behavioral dysfunctions in laboratory animals and humans [6]. Lead is universal environmental pollutant having undesired effects on growth and development [7], nervous system [8], hemopoietic system [9], cardiovascular system [10], excretory system [11], hepatic system [12,13], reproductive system [14-16], and immunological functions [17].

Lead has been proved to be a potent toxicant even at very low level of exposure [18]. Lead exposure mainly occurs through the respiratory and gastrointestinal systems. Absorbed lead is conjugated in the liver and passed to the kidney, where a small quantity is excreted in the urine, and the rest accumulates in the soft tissues affecting various biological activities at the molecular, cellular and intracellular levels resulting into morphological alterations that can remain even after the lead levels have fallen [6,19]. Evaluation of lead exposed humans revealed that liver tissue is the largest repository of lead per g of wet tissue (33%) among the soft tissues followed by kidney cortex and medulla [20].

The liver is actively involved in many metabolic functions and frequent target organ for number of toxicants [21]. It plays vital role in detoxification and elimination of biotoxicants [22]. Lead induced hepatotoxicity was reported to be associated with the distortion of liver structure as well as functions [23]. Lead is known to promote oxidative damage in liver by enhancing peroxidation of membrane lipids [24]. Lead caused lymphocyte infiltration and cirrhosis of liver cells [25]. Hepatic disorders due to lead exposure were noticed in rats as manifested by increased activities of liver enzymes [26].

The pathogenesis of lead toxicity is multi-factorial. The lead toxicity is closely related to its interference with the essential bio-elements that hamper several physiological processes [27]. Lead directly interrupts enzyme activation, competitively inhibits trace mineral absorption, binds to sulfhydryl groups and interrupts protein synthesis and alters calcium homeostasis [28]. Lead potentially induces oxidative stress due to its propensity to catalyze oxidative reactions and generation

of reactive oxygen species (ROS). These ROS reduce the antioxidant defense system reserve of cells via depleting glutathione and inhibiting the production of sulfhydryl antioxidants, impair heme production, damage nucleic acids, and inhibit DNA repair [17,29,30]. Lead initiates lipid peroxidation (LPO) in cellular membranes resulting into alteration of cell membrane integrity and fatty acid composition [31]. Keeping this scenario in view, it is extremely essential to investigate protective measures against adverse exposure of this toxic metallic element.

The major objective of the present *in vitro* study was to elucidate underlying mechanism of action of lead intoxication through alteration in LPO, enzyme activities and protein levels in goat liver to avoid ethical concern of involving experimental animals. Besides this, search for ameliorative agents which could help in the eradication of lead toxicity is essential to be worked out.

Activation of free radical processes and impairment of the antioxidant defense system are likely to be few of the basic mechanisms responsible for hepatic damage during prolonged lead intoxication [32]. Knowledge of lead's capacity to disrupt the pro-oxidant/antioxidant balance within mammalian tissues suggests that definitive therapy for chronic lead poisoning should encompass both chelating and antioxidant actions. Antioxidants have long been known to reduce free radical-mediated oxidative stress and attracted widespread interest in clinical nutrition and medicinal research. Micronutrients such as vitamins and thiol containing N-acetyl cysteine (NAC) 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 [33] and scavengers of ROS [34]. As oxidative stress is the major cause of lead-induced hepatotoxicity, main focus of the study was to explore the therapeutic efficacy of specific synthetic antioxidants such as NAC, 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 antioxidants with minimum side effects as ameliorating agents against lead induced hepatotoxicity. There is a growing focus to follow systemic research methodology for evaluation of scientific basis for the therapeutic potential of traditional natural antioxidants which can reduce free radical-induced tissue injury [35]. *Bacopa monnieri* is a creeping, glabrous, succulent, traditional ayurvedic herb with interesting antioxidant properties, as expressed by its capacity to scavenge superoxides, peroxides and hydroxyl radicals [36,37] as well as modulating expression of enzymes involved in generation and scavenging of ROS [38]. Keeping this perspective in mind, another major objective of present study was to evaluate therapeutic potential of medicinal plant *B. monnieri* as an effective hepatoprotective agent in counteracting lead toxicity.

METHODS

Experimental design

In the present *in vitro* study, the experimental protocol was divided into two separate phases. During the first phase, the prescribed dose of lead acetate (1 ppm) selected on the basis of LD_{50} and reported literature based on previous studies was added in goat liver cultures for specific period of time to investigate the lead-induced alterations in various biochemical parameters *in vitro*. The second phase involves simultaneous addition of synthetic antioxidants as well as *B. 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 hepatotoxicity.

Chemicals

The AR grade lead acetate trihydrate, ascorbic acid, NAC, vitamin B_1 (Thiamine), vitamin E (Tocopheryl acetate), and other chemicals having 99% purity used, in the present study, were obtained from HIMEDIA, SIGMA, and MERCK Laboratory Pvt. Ltd., India.

Preparation of herbal (*B. monnieri*) extract

B. monnieri plant was collected from botanical garden of school of sciences, Gujarat University, Ahmedabad, Gujarat, 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 plants of *B. monnieri* were thoroughly washed with double-distilled water, cut into small pieces and dried in shade in dust free condition for 1 week at room temperature before being ground to coarse powder. Powdered whole plant material (10 g) was subjected to soxhlet extraction procedure at 78°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.

Preparation of lead acetate solution

About 0.010 g 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 concentration of 1 ppm lead acetate.

Sample collection

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

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 B_1 - 30 mg/kg/day) (co-administration) exposed group
III. Herbal antioxidant (*B. 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 (*B. monnieri* extract) (co-administration) exposed group.

In vitro study

All the experimental groups were cultured in Dulbecco's Modified Eagle Medium:F12 (1:1 mixture) media supplemented with 0.5% fetal bovine serum, 1% streptomycin and L-glutamine along with HEPES in the culture petri plates at same conditions in biological oxygen demand incubator containing 5% CO_2 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.

Biochemical analysis

To analyze free radical induced cell injury by lead acetate and its protection by antioxidants, the levels of malondialdehyde (MDA) were measured in cultured liver homogenates. The estimation of LPO as a part of oxidative indices in the liver cultures was done by the method of Ohkawa *et al.* [39] As a representative of oxidative stress marker, the activity of superoxide dismutase (SOD) was analyzed by the method of Kakkar *et al.* [40]. To study the impact of lead acetate on protein metabolism; levels of total proteins, soluble proteins, and insoluble proteins were determined in liver homogenate by the method of Lowry *et al.* [41]. At the end of lead acetate exposure as well as antioxidants treatment, certain specific parameters of goat liver such as enzyme activities of alkaline phosphatase (ALPase) and acid phosphatase

(ACPase) were also assayed by the method of Bessey *et al.* [42]. To understand alteration in energy metabolism, activities of succinate dehydrogenase (SDH) and adenosine triphosphatase (ATPase) were estimated by methods of Beatty *et al.* [43] as well as Quinn and White [44], respectively.

Statistical analysis

A student's "t-test" was used for the statistical analysis of the data. For each parameter (n=5), the data were expressed as mean±standard error of mean after subjecting to Student's 't-test' using GraphPad software for the interpretation of results. The significance difference was statistically considered at the level of $p<0.05$.

RESULTS

LPO

In vitro study results of the LPO in the goat liver cultures exposed to lead acetate (1 ppm) alone and along with synthetic antioxidants mixture or herbal antioxidant *B. monnieri* and their related controls 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 and with reference to lead exposed group are given in Table 2. Lead acetate exposure to liver cultures for 6 hrs was found to increase production of thiobarbituric acid reactive substances as marked by highly significant elevation ($p<0.0001$) in LPO with respect to control as represented by 10.52%. However, simultaneous supplementation of mixture of synthetic antioxidants as ameliorative agent reduced (5.95%) lead induced LPO as compared to lead exposed group (Table 2) and provided statistically significant protection against lead intoxication. Simultaneous addition of lead acetate and *B. monnieri* extract in goat liver cultures reduced lead induced LPO by 7.14% as compared to lead exposed group (Table 2) and exerted protection against lead-induced oxidative stress.

SOD activity (E.C.1.15.1.1)

Results of the SOD activity in the goat liver cultures exposed to lead acetate (1 ppm) alone and along with selected antioxidants as well as their respective controls 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 are given in Table 2. Lead acetate exposure for 6 hrs to goat liver in a culture medium was found to increase oxidative stress which can be marked by statistically extremely significant elevation (67.33%) in SOD activity with respect to control as represented by $p<0.0001$. Simultaneous addition of lead acetate and synthetic antioxidants in goat liver cultures significantly reduced (18.40%) lead induced increase in SOD activity as compared to control (Table 2). Co-administration of lead acetate and *B. monnieri* extract in liver cultures brought about significant reduction in SOD activity (8.76%) with respect to control as given in Table 2. Thus, supplementation of prescribed antioxidants imparted significant maintenance of enzyme activity against lead induced oxidative damage.

Protein levels

Lead acetate exposure caused significant decline in the total protein and soluble protein levels in goat liver cultures as compared to control (Table 1). The decrease in the total protein and soluble protein at 1 ppm lead exposure was represented as 0.69% ($p<0.0001$) and 27.27% ($p<0.0001$), 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 liver cultures significantly increased reduction in total protein (0.46%) and soluble protein (28.13%) levels as compared to the lead exposed group (Table 2). Supplementation of *B. monnieri* extract to lead exposed tissue as a therapeutic agent resulted in extremely significant maintenance of total protein and soluble protein levels nearest to control group. Insoluble protein levels also showed decline due to synthetic antioxidants (1.79%) and herbal antioxidant (2.04%) exposure as compared to lead exposed group.

ALPase activity (E.C.3.1.3.1)

Lead acetate exposure to goat liver culture for 6 hrs duration resulted in significant alteration in the ALPase activity. Results revealed that ALPase activity was markedly declined (26%) at 1 ppm lead exposure as compared to control as represented by $p<0.0001$ (Table 3). The addition of lead acetate and synthetic antioxidants simultaneously in goat liver cultures provided significant protection against reduction in enzyme activity (0.30%) as compared to control (Table 4). Exposure of herbal antioxidant to lead exposed cultures acts as an effective ameliorative agent and maintained reduction in ALPase activity by 34.74% as compared to lead exposed tissue.

Table 1: Oxidative stress indices and protein content of control and exposed liver tissues *in vitro* (n=5, mean±SEM)

Biochemical Parameters	Group I Control	Group II Synthetic Antioxidants	Group III Herbal Antioxidant	Group IV Lead acetate (1 ppm)	Group V Lead acetate+ Synthetic Antioxidants	Group VI Lead acetate+ Herbal Antioxidant
LPO[a]	2435.8±10.135	2499.9±10.127**	2467.85±10.135^{NS}	2692.2±10.14****	2531.95±10.14**	2499.9±10.14**
SOD[b]	1.359±0.021	1.611±0.051**	1.514±0.023***	2.274±0.058****	1.609±0.024****	1.478±0.023**
Total protein[c]	23.161±0.017	23.135±0.008^{NS}	23.189±0.016^{NS}	23.00±0.008****	23.108±0.017^{NS}	23.135±0.008^{NS}
Soluble protein[d]	2.332±0.017	2.279±0.008*	2.385±0.016^{NS}	1.696±0.008****	2.173±0.014**	2.253±0.008**
Insoluble protein[e]	20.299±0.019	20.326±0.019^{NS}	20.273±0.008^{NS}	20.776±0.02****	20.405±0.019**	20.352±0.008*

*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. NS: Non significant versus control group, [a]Nano moles of MDA/100 mg tissue weight/60 minutes, [b]milliunits/mg protein, [c]mg total protein/100 mg fresh tissue weight, [d]mg soluble protein/100 mg fresh tissue weight, [e]mg insoluble protein/100 mg fresh tissue weight. LPO: Lipid peroxidation, SOD: Superoxide dismutase, SEM: Standard error of mean, MDA: Malondialdehyde

Table 2: Gross effect of lead and antioxidants on oxidative stress indices and protein content of goat liver cultures *in vitro*

Biochemical Parameters	Group IV Lead acetate (1 ppm)	Relative to control		Relative to Group IV	
		Group V Lead acetate+synthetic Antioxidants	Group VI Lead acetate+herbal Antioxidants	Group V Lead acetate+synthetic Antioxidants	Group VI Lead acetate+herbal Antioxidants
Lipid peroxidation	10.52*	3.95*	2.63*	5.95	7.14
Superoxide dismutase	67.33*	18.40*	8.76*	29.24	35
Total proteins	0.69	0.23	0.11	0.46*	0.58*
Soluble proteins	27.27	6.82	3.39	28.13*	32.84*
Insoluble proteins	2.35*	0.52*	0.26*	1.79	2.04

% of difference with respect to their control as well as lead exposed cultures. All values are expressed in % of decrease or *increase

Table 3: Biochemical parameters of control and exposed liver tissues *in vitro* (n=5, mean±SEM)

Biochemical Parameters	Group I Control	Group II Synthetic Antioxidants	Group III Herbal Antioxidant	Group IV Lead acetate (1 ppm)	Group V Lead acetate+synthetic Antioxidants	Group VI Lead acetate+herbal Antioxidant
ALPase[f]	2.334±0.002	2.230±0.005**	2.275±0.005**	1.727±0.005**	2.327±0.005NS	2.327±0.005[NS]
ACPase[g]	12.523±0.012	12.449±0.013*	12.412±0.006**	14.635±0.01**	13.079±0.006**	13.041±0.01**
SDH[h]	280.18±0.601	266.86±1.203**	270.66±1.346*	194.56±1.81**	240.23±1.3456**	242.13±1.04**
ATPase[i]	0.304±0.001	0.299±0.001*	0.304±0.001[NS]	0.271±0.001**	0.275±0.001**	0.300±0.00*

*p<0.001, **p<0.0001. NS: Non significant versus control group. [f,g]μ moles of p-nitrophenol released/30 minutes/100 mg tissue weight, [h]μg formazan formed/15 minutes/100 mg tissue weight, [i]μ moles of ip released/hr/mg tissue weight. SEM: Standard error of mean, ALPase: Alkaline phosphatase, ACPase: Acid phosphatase, SDH: Succinate dehydrogenase, ATPase: Adenosine triphosphatase

Table 4: Gross effect of lead and antioxidants on biochemical parameters of goat liver cultures *in vitro*

Biochemical Parameters	Group IV Lead acetate (1 ppm)	Relative to control		Relative to Group IV	
		Group V Lead acetate+synthetic Antioxidants	Group VI Lead acetate+herbal Antioxidants	Group V Lead acetate+synthetic Antioxidants	Group VI Lead acetate+herbal Antioxidants
Alkaline phosphatase	26	0.30	0.30	34.74*	34.74*
Acid phosphatase	16.86*	4.44*	4.14*	10.63	10.89
Succinate dehydrogenase	30.56	14.26	13.58	23.47*	24.45*
Adenosine triphosphatase	10.86	9.54	1.32	1.48*	10.70*

% of difference with respect to their control as well as lead exposed cultures. All values are expressed in % of decrease or *increase

ACPase activity (E.C.3.1.3.2)

Results revealed that ACPase activity significantly increased in lead acetate exposed goat liver cultures as compared to control group (Table 3). Statistically extremely significant elevation (p<0.0001) of enzyme activity was observed at 1 ppm concentration of lead acetate exposure as marked by 16.86 %. However, administration of synthetic antioxidants as well as herbal antioxidant in lead exposed goat liver cultures significantly maintained (10.63%) and (10.89%) enzyme activity respectively as compared to lead exposed group (Table 4) which suggests therapeutic efficiency of antioxidants against lead toxicity.

SDH activity (E.C.1.3.99.1)

Statistically extremely significant decline was found in the SDH activity in the liver cultures exposed to lead acetate as compared to control group (Table 3). The reduction in enzyme activity at low dosage (1 ppm) was represented as 30.56% (p<0.0001). However, co-administration of lead acetate and synthetic antioxidants as well as lead acetate and *B. monnieri* extract in goat liver cultures significantly ameliorated (23.47%) and (24.45%) enzyme activity as compared to the lead exposed group, respectively, (Table 4) which suggests the ameliorative potential of the selected antioxidants.

ATPase activity (E.C.3.6.1.3)

Lead acetate exposure to goat liver cultures for 6 hrs duration brought about a significant alteration in the ATPase activity. Results revealed that enzyme activity was markedly declined at 1 ppm lead exposure as compared to control group (Table 3). The reduction in the enzyme activity was represented by 10.86% (p<0.0001). The addition of lead acetate and synthetic antioxidants simultaneously in goat liver cultures exerted significant protection (1.48%) against enzyme activity alteration as compared to lead intoxicated tissue (Table 4). The addition of prescribed dosage of *B. monnieri* to goat liver cultures acts as an effective ameliorative agent and prevented alteration in ATPase activity (1.32%) with respect to control group (Table 4).

DISCUSSION

Findings of our study showed statistically extremely significant increase in MDA levels in lead exposed group as compared to control group. Many *in vitro* and *in vivo* studies have shown that MDA increases with lead exposure in liver, brain and RBCs [45-47]. Elevated levels might be due to the formation of free radicals. Several studies suggested primary involvement of increased amount of ROS in lead-exposed animals in support of our data [48]. Most important consequence includes the peroxidation of membrane lipids, with an increase in the cell membranepermeability [49]. All the products of LPO inactivate cell constituents by oxidation or cause oxidative stress by undergoing radical chain reaction ultimately leading to loss of membrane integrity [50,51]. Generation of highly ROS such as hydroxyl radical (OH^-), hydrogen peroxide (H_2O_2), superoxide anions (O_2^-), and lipid peroxides in the aftermath of lead exposure may result in systemic mobilization and depletion of cells intrinsic antioxidant defenses rendering the tissue susceptible to free radical injury which highlights the role of free radicals in lead toxicity. In the present study, the results emphasized that co-administration of synthetic antioxidants as well as *B. monnieri* extract to lead exposed cultures separately, significantly ameliorates lead acetate-induced LPO by inhibiting the chain reaction and lowering free radical levels.

SOD forms the first line of endogenous antioxidative defense against ROS generation in lead intoxication. SOD protects the cells against toxic effects of superoxide radicals by catalyzing its dismutation reactions [52]. Our findings showed increase in the SOD activity which is the suggestive of oxidative insult in the tissue due to lead intoxication. Increased activity may be due to activation of compensatory mechanism in liver 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 [53-55].

Decline in total protein content might be due to the fact that lead interferes with –SH groups of 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 MDA formed during LPO could react with –SH groups of proteins to damage them, thus inhibiting enzymes requiring –SH groups for their activities [56]. The other factors responsible for alteration in protein metabolism might be the inhibition of biosynthesis of proteins due to lead acetate exposure, which could be due to the impairment of peptide chain initiation, increased proteolysis and reduced incorporation of amino acids into proteins [57]. Thus, one of the reasons for hepatotoxicity 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 hepatoprotective 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.

LPO products such as hydroperoxides can inhibit protein synthesis and alter chemotactic and enzyme activity [58]. The results elucidated that lead exposure caused a significant depletion in ALPase activity in liver. ALPases hydrolyze phosphate esters at alkaline pH and associated with many functions at cellular level. Alteration in ALPase activity is likely to create derangement in the transport of metabolites. Reduction in ALPase activity at low-level lead exposure reflected on oxidative stress generated liver damage and increased membrane permeability or necrosis of hepatocytes in addition to alteration in the balance between synthesis and degradation of enzyme.

The data of current investigation also revealed statistically significant elevation in ACPase activity in lead exposed liver cultures. ACPase, a lysosomal enzyme is involved in phagocytosis [59], autolysis, fat absorption in intestine, cellular differentiation and keratinization [60]. Alteration in its activity may be due to direct adverse effect of oxidative stress resulted because of accumulation of lead in the liver and distortion of balance between synthesis and degradation of ACPase enzyme. ACPase activity may increase due to assimilation of fat in liver because of lead exposure and increased lysosomal imbalance due to destruction of intact membranes.

Data accumulated in the present biochemical analysis revealed a considerable loss of SDH activity in the lead acetate exposed liver cultures. Decreased SDH activity would affect conversion of succinate to fumarate and cause blockage in the Kreb's cycle. As SDH is a mitochondrial enzyme, its decreased activity indicates a possible alteration in mitochondrial structural organization and function because of lead acetate exposure. Mitochondrial enzyme activities and tissue respiration may be altered due to accumulation of lead in mitochondria. Lead exposure may uncouple oxidative phosphorylation which may reflect on the slow rate of tricarboxylic acid (TCA) cycle. This condition brought about a reduction in synthesis of ATP. Thus, reduced activity of SDH reflects on the disturbed state of oxidation and energy metabolism of a tissue indicating lesions in TCA cycle.

Reduction in ATPase activity due to lead exposure in liver cultures is suggestive of disturbance in energy metabolism. Interaction of lead with highly reactive –SH groups of ATPase can be a possible mechanism for interruption of enzyme activity. Degradation of proteins, reduction in glutathione 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. A 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 [61,62].

The present study elucidated that the mixture of synthetic antioxidants and herbal extract both play protective role and manifested maintenance of Protein levels, SOD, ALPase, ACPase, SDH, and ATPase activities against lead induced hepatotoxicity.

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 hepatoprotective agents against lead-induced oxidative stress. NAC has antioxidant capacity to lead, including oxidative stress via stimulating glutathione synthesis, thereby maintaining intracellular glutathione levels and scavenging ROS [63]. In addition, NAC also has some chelating action on lead [64]. Nutritional factors are often mentioned as important modifiers of lead metabolism and lead toxicity [65]. Chan in 1993 reported that vitamin C is a chain breaking antioxidant that stops the propagation of the peroxidation process [66]. Vitamin C is widely known to restore and recycle the antioxidative properties of vitamin E and glutathione, when attacked by pro-oxidants [67]. Vitamin E is nature's major lipid soluble chain breaking antioxidant, which is known to protect biological membranes and lipoproteins from oxidative stress [68]. Thiamine, the endogenous –SH containing molecule, was recognized as protective agent for lead exposure [69]. 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 LPO in rat liver and kidney [71]. It may scavenge O_2 and OH directly and thus affect the cellular response to oxidative stress [71]. Anna and Wiglo also reported that thiamine may act as a potent antioxidant as it scavenges free radicals [72]. Thus, it can be interpreted that correct combination of selected synthetic antioxidants has the capacity to completely eliminate lead induced hepatotoxicity in mammals.

B. monnieri (Brahmi) is a versatile, indigenous medicinal herb with a wide spectrum of pharmacological properties. *B. monnieri* has been shown to exert antioxidant effects through the chelating of metal ions, breaking oxidative chain reaction [73], improving the activities of antioxidative defense enzymes [74] and scavenging the free radicals [75]. *B. monnieri* also confers protection against reduction in ATPase activity. Ethanolic extract of *B. monnieri* contains a mixture of alkaloids, steroids and saponins [76,77]. A number of reports suggested that hepatoprotective and antioxidant properties of *B. monnieri* were attributed mainly due to the presence of its characteristic active constituent dammarane type triterpenoid saponin called "Bacopside-A" [78]. Total phenolic and flavonoid 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 lead-mediated oxidative stress and significantly ameliorates LPO by improving the pro-oxidant/antioxidant balance of the cells which subsequently helps in maintenance of protein levels as well as provides protection against alteration in enzyme activities in goat liver cultures.

CONCLUSION

In conclusion, the findings of present toxicological study clearly revealed that lead metal induces oxidative stress and alters biochemical indices of goat liver *in vitro*. Results of the present investigation clearly emphasized that heavy metal lead disrupts the antioxidant defense system by alteration of enzyme activities, thereby enhancing the free radical mediated LPO. The results also elucidated that lead acetate adversely affects the protein content as well as energy metabolism of goat liver. Thus, from current *in vitro* study, it can be clearly concluded that even low-level lead exposure has definitely destructive effect on the structural, metabolic and functional status of mammalian liver.

The present synergistic study also emphasized that administration of the selected synthetic antioxidants as well as ethanolic extract of *B. monnieri* 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 hepatoprotective properties. Hence, these antidotes can be proved to be very effective and beneficial ameliorating agents against lead induced hepatotoxicity the world over. 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.

The present investigation has not only elucidated the mechanism of action of lead-induced toxicity but also suggested its mitigation through synthetic and herbal antioxidants and as such is a significant application in the field of plumbism amelioration in endemic regions.

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