ANTIOXIDANTIVE EFFECT OF BAMBOO LEAVES EXTRACT AND DL-\(\alpha\)-LIPIOIC ACID ALONE OR AS COMBINED THERAPY ON LEAD INDUCED NEPHRITIC AND NEURONAL OXIDATIVE IMPAIRMENT

SOOD, P. P., \(^1\) CHIRAGINI, H. M. \(^2\) AND KALIA, K. \(^2\)

\(^1\)Department of Biochemistry, Saurashtra University, Rajkot 356 005; \(^2\)BRD School of Biosciences, Sardar Patel University, Vallabh Vidyanagar 388 120. E. Mail: kirankalia_in@yahoo.com

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Abstract: The present study has been carried out to investigate the in vivo protective role of \(\alpha\)-lipoic acid and bamboo leaves methanolic extract (BME) against lead-induced nephritic- and neuronal- oxidative damage in albino Wistar rats. Lead acetate at a dose of 12.5 mg/ kg body weight (b.w.) per day was preferred as the source of lead in our study. Various oxidative stress markers like reduced glutathione (GSH) content, total thiol (TSH) level, lipid peroxidation, as a parameters to measure degree of oxidative damage and healing due to \(\alpha\)-lipoic acid and bamboo leaves extract were studied along with activities of enzymes, like gamma-glutamyl transpeptidase (\(\gamma\)-GT), catalase (CAT), delta-amino levulinic acid dehydratase (\(\delta\)-ALAD) and glutathione peroxidase (GPx), responsible for antioxidant defense system. Bamboo leaves extract and \(\alpha\)-lipoic acid given at the doses of 250 mg/ kg body wt and 100 mg/ kg body wt for fifteen days after lead treatment overcome the lead induced oxidative stress both in brain and kidney. Both antioxidants alone and in combination eliminate the effect of lead induced oxidative stress by being potent free radical scavengers.

Key words: \(\alpha\)-Lipoic acid, Bamboo leaves extract, Lead

INTRODUCTION

The industrialization and pollution proceed hand in hand. The best way to balance the two is to reduce the extent of pollution and develop some cure for the effective individuals. Lead, a non-essential heavy metal recognized as a general metabolic poison, is ubiquitously found in the environment as a result of high practice of leaded petrol, paints, water pipes, ceramic products, ammunition and roofing, batteries and soldered food cans, variety of medical, scientific and military equipments [1]. Although usage of lead in manufacturing some of the products have been banned since last few decades, even low level exposure of this metal is also very much harmful to all living organisms including humans. To day lead has become omnipotent as it exist in all components of environment (air, soil and all water bodies), in animal and plants or even mother’s milk and sperm [1]. The fruits, vegetables, grains, canned food, soft drinks, cigarettes are good source of lead to which, and man is exposed.

Lead enters in body by breathing, food or by skin. Once lead enters in blood it deposits in soft tissues (liver, kidney, brain, spleen muscles and heart). In adults 94% of lead is stored in bones and teeth. The lead that is not stored in hard tissues leaves the body through urine and feces.

Lead exposure may cause memory loss, weakness, blood pressure, anemia, damage the brain, kidney and testis, causes miscarriage and effect thyroid functions. Along with this lead causes immunological, biochemical, histopathological changes, decrease macro- and micro-nutrients. Unborn children are exposed to lead through mother and newly born through mother’s milk [1].
Till date direct mechanism of lead toxicity on organs or systems is unrevealed [2,3] but still reports suggest its contribution in developing various physiological dysfunctions as a result of production of oxidative stress and impairment in a range of metabolic and biochemical pathway [4]. Studies conducted in vivo on animals and epidemiological studies on human population suggested that lead exerts its effect by disturbing cell’s pro-oxidant-antioxidant status which results in the production of free oxygen radicals, a key player which cause cell damage and also damage to cellular organelles [5,6].

Lead interrupt mainly three systems i.e. hematopoietic, renal and nervous system [7, 8]. To overcome this problem of scavenging free radicals produced by heavy metal intoxication, antioxidant therapy is the best suggested therapy. Treatment of antioxidants of different synthetic and natural origin is the best recommended way to conquer the problem of lead toxicity. To our knowledge, very limited work has been done on the use of antioxidants in reduction of oxidative stress induced by lead in the animal model. For this purpose, in the present study we have intended to check the effect of synthetic antioxidant “α-lipoic acid (LA)” in combination with a herbal antioxidant i.e. “bamboo leaves methanolic extract (BME)” on lead induced oxidative stress on albino rats. Several reports suggested the use of many thiol containing antioxidants to nullify the effect of metal induced oxidative stress and tissue damage [9,10].

α-lipoic acid, a low molecular weight dithiol that possesses metal chelating capabilities, found in most of the eukaryotic and prokaryotic cells. It can readily be absorbed through gut, crosses blood brain barrier and reaches to brain. α-lipoic acid and its reduced form dihydroxy lipoic acid both are important cofactors in the mitochondria and strong antioxidants. However, antioxidants derived from plant products (or dietary antioxidants) have been proposed as excellent replacements to compensate the natural antioxidant of body when ever reduced during stressed conditions. As for example bamboo leaves have been used as an herbal remedy from the ancient time in Asia [11-13]. Fu et al. [14] found that antioxidant of bamboo leaves was capable of blocking chain reactions of lipid auto oxidation, chelating metal ions of transient state, scavenging nitrite compounds and blocking the synthetic reaction of nitrosamine.

Since lead is a potent stressor agent, its concentration in all environments is increasing day by day and ultimately effecting the human population either directly or through food chain, it is necessary to search proper and meaningful therapy. For this purpose, mix therapy including herbal, natural and/or synthetic antioxidants is considered to be quite advantageous as in the case of mercury and methylmercury detoxication [15,16] or as protector against lead [17]. Inspired from these investigations therapy to lead intoxicated subjects with α-lipoic acid and bamboo leaves extracts, either alone or in combinations has been applied and desired results are obtained.

MATERIALS AND METHODS

Chemicals and reagents: Lead acetate, glycyl glycine, 2-thiobarbituric acid, 5, 5′-dithiobis (2-nitrobenzoic acid), reduced glutathione and α-lipoic acid were procured from himedia. δ-aminolevulinic acid (ALA) was purchased from Sigma (St. Louis, MO, USA). L-γ-glutamyl-p-nitroanilidine was obtained from SRL. All other analytical grade laboratory chemicals were purchased from sigma, Himedia and SRL.

Collection and extraction of bamboo leaves: The mature leaves of bamboo were collected in autumn season from Botanical garden, Department of Bioscience Sardar Patel University. The specimens (leaves of Bambusa arundinacea) were botanically identified with the help of available literature. The collected leaves were washed 4-5 times with D/W to remove dust particles, shade dried and prepared for extraction. The dried leaves were ground into uniform powder using mixture grinder and stored in airtight bottle in refrigerator till required for analysis and extraction by methanol by continuous shaking in electronic shaker for 24 hours. The solvent was selected on the basis of the results obtained in our previous study which showed in vitro highest antioxidant and metal chelating ability of BME [18]. After every 24 hours solvent was changed. The extraction solvents were filtered using whatman filter paper no. 1. The solvent evaporated on hot plate at 50 °C. The dried extract was suspended in distilled water.

Animals and experimental design: Male albino Wistar rats (weighing 150 ± 10 g) were acclimatized for 7 days prior to lead treatment and housed under standard condition of temperature 24 ± 1 °C and 55 ± 5% relative humidity with regular 12 hour light: dark cycle and allowed free access to standard laboratory food (Pranav agro industries, Vadodara)
and water *ad libitum*. Animals were maintained as per the guidelines of National Institute of Nutrition Indian Council of Medical Research (Hyderabad, India) and protocol was approved by research committee of Sardar Patel University, V. V. Nagar.

The animals were divided into 5 groups (n 6). The selected dose of lead (12.5 mg/kg b.w.) was 1/11 of daily human environmental exposure of lead (140 mg /kg b.w.) Rats were treated for a period of 45 days.

**Group I (control):** The animals received only vehicle.

**Group II.** Rest of the animals (4 groups n 5) received a daily dose of 12.5 mg/kg b.w. lead acetate in normal saline by oral gavages (20 animals). After 45 days, lead exposed animals were divide further in four group of 5 rats each and given following treatment consecutively for 15 days.

**Group IIA:** These lead intoxicated animals received no further treatment.

**Group IIB:** The animals of this group received 250 mg/ kg wt BME once daily via same route for 15 days.

**Group IIC:** Lead intoxicated animals of this group received a daily dose of 100 mg/kg bw α-lipoic acid.

**Group IID:** The animals of 4th group were given daily doses of 250 mg/ kg wt BME and 100 mg/kg wt α-lipoic acid.

After 48 hours of the last dose, the animals were sacrificed under light ether anesthesia. Blood was collected in heparinized tubes and brain and kidney were removed, washed with normal saline and weighed. 10% tissue homogenate (w/v) was prepared in 50 mM phosphate buffer (pH 7.4).

**Estimation of reduced glutathione:** Kidney and brain GSH estimation were performed as described by Ellman [19] modified by Jollow [20]. Briefly, 0.4 ml of 10% tissue homogenate was added to 0.5 ml 10% sulfosalicylic acid and the volume was made to 1.5 ml with distilled water (d/ W). Then it was centrifuged at 3000 rpm for 10 min. 0.5 ml of supernatant and 4.5 ml tris (pH 8.23) were mixed with 0.5 ml of 10 mm DTNB in 0.1M phosphate buffer and incubated at 37 °C for 5 min. Absorbance was recorded at 412 nm by UV spectrophotometer.

**Total sulphydryl (TSH) estimation:** Estimation was carried out by the method of Sedlack and Lindsley [21] In this method, 10 µl aliquots of homogenate was mixed with 0.2 M tris (pH 8.2), 0.02 M EDTA and 0.01 M DTNB. Final volume was made up to 1 ml with methanol. Reaction mixture was incubated for 15 min at 37 °C and centrifuged at 4500 rpm for 15 min. Absorbance was recorded at 412 nm.

**Glutathione peroxidase:** The activity of glutathione peroxidase was measured by the method of Rotruck [22]. Assay mixture containing 0.4 M sodium phosphate buffer, 10 mM sodium azide, 2 mM GSH, 2.5 mM H₂O₂ and 0.5 ml enzyme source was incubated at 37 °C exactly for 1 min interval. Reaction was stopped by adding 10 % TCA (in control tube enzyme source was added after TCA). Centrifugation was then carried out at 3000 rpm for 10 min. 1 ml supernatant was then mixed with 0.04% DTNB reagent and read at 412 nm. Activity was expressed as µM GSH oxidized per min per mg protein.

**γ-glutamyl transpeptidase (γ--GGT):** GT hydrolytic activity was determined according to method of Grisk [23]. Briefly, homogenate was mixed with 0.2 ml of 100 mM Tris-HCl (pH 8.0), 2.5 mM L-γ-glutamyl-p-nitroanilidine, in the presence or absence of 20 mM glycyl-glycine. The reaction was ceased by adding 1.8 ml 1 M sodium acetate (pH 4.0). The reaction mixture was then centrifuged at 4500 rpm for 15 min. p-nitroaniline produced was measured at 405 nm by spectrophotometer. The unit enzyme activity was calculated using molar extinction coefficient of 11,300 for p-nitroaniline formed.

**Catalase activity;** Catalase activity was measured by the method of Sinha [24]. Briefly, 0.5 ml 0.01 M phosphate buffer (pH 7.1) was incubated with 0.1 ml homogenate (only in test) at 37 °C for 10 min. Then 0.4 ml of 0.2 M H₂O₂ solution is added exactly one minute before addition of 2 ml of 5% dichromate solution. After that 0.1 ml enzyme was added to control tube. All the test and control tubes were kept in boiling water bath for 10 min and absorbance was measured at 570 nm.

**Thiobarbituric acid reactive substances level estimation (TBARs):** TBARs level represents the state of lipid peroxidation in tissue. Its activity was measured by the method of Onkawa [25]. In this method 0.4 ml tissue homogenate (only in test) at 37 °C for 10 min. Then 0.4 ml of 0.2 M H₂O₂ solution is added exactly one minute before addition of 2 ml of 5% dichromate solution. After that 0.1 ml enzyme was added to control tube. All the test and control tubes were kept in boiling water bath for 10 min and absorbance was measured at 570 nm.
mixture (15:1 v/v) and centrifuged the reaction mixture at 3000 rpm for 10 min. Absorbance was measured at 532 nm by taking n-butanol: pyridine mixture as a blank.

δ-Aminolevulinic acid dehydratase (δ-ALAD) activity: The δ-ALAD activity was assayed essentially by method of Berlin and Schaller modified by Semionova [26,27]. After incubation at 37 °C for 10 min, phosphate buffer pH 6.4 and 5-aminolevulinic acid were added to heparinized blood sample. Incubations were carried out for 1 h at 37 °C. Reaction was terminated by addition of HgCl₂. The reaction product porphobilinogen was determined using modified Ehrlichs reagent at 555 nm with a molar absorption coefficient of $6.1 \times 10^4$ M⁻¹ for the Ehrlich-porphobilinogen salt.

Statistical analysis: Data are expressed as Means ± S.D. Data were compared using one way analysis of variance (ANOVA) followed by Turkey’s post test with least significance difference to compare means between the different treatment groups. Difference with a p value < 0.05 was considered significant.

RESULTS

δ-aminolevulinic acid dehydratase: Lead exerts its toxic effect on erythropoietic system by acting upon one of its important enzyme, δ-aminolevulinic acid dehydratase, involved in heme biosynthetic pathway. In the present study reported 31.08% decline in its activity after lead treated as compared to control group. The 81.61%, 83.71% restoration of δ-aminolevulinic acid dehydratase activity was observed after LA and BME therapy, whereas, groups received combination of both restored its activity to 90.04%. The studied antioxidant therapy has shown 18.42%, 21.47% and 30.64% recoveries in order of BME< LA< BME + LA as compared to lead treatment.

Reduced glutathione (GSH) and total thiol: Similar mechanism of lead toxicity can be applied to critical molecules like reduced glutathione (GSH) and total thiol as they also possess ‘-SH’ group at their functional site. Depletion in GSH content in brain (43.28 %) and kidney (43.61 %) was observed in group treated only with lead compared to control. Kidney GSH level was recovered by 24.45 % and 33.88 % and in brain recovery was 68.53 % and 72.54 % in groups treated with BME and LA respectively (p ≤ 0.001). However, antioxidant therapy of BME, LA and BME+LA combination has shown higher restoration of GSH content in the brain (90.53%, 92.68% and 98.97%) in comparison with kidney (70.18%, 75.5% and 86.43%) as compared to control group.

Kidney and brain showed 55.57% and 50.80% significant (p ≤ 0.001) depletion in their total thiol content in lead intoxicated group. This lose was restored by 61.34%, 73.99% and 83.92% in kidney and 65.48%, 71.38%, 89.94% in brain in BME, LA and combination treatment groups respectively compared to control. If we focused on recovery, brain recovers its 33.14%, 45.14% and 82.88% total thiol back and the level of recovery was 41.56%, 70.75% and 95.98% compared to lead treated group with less than 0.001 significance in order BME< LA< BME + LA. In both organs no significant differences have been observed between control and combined treatment group, which pointed, combined therapy as the best strategy.

Glutathione peroxidase (GPx): It is a SH dependent enzyme, also showed significant (p ≤ 0.001) 46.18% decrease in the kidney and 50.32 % decrease in brain of the groups treated with lead. The reason why this enzyme become inactive lied in the fact that it is a selenium dependent enzyme and lead can replace selenium as it possess comparatively higher affinity towards enzyme active site. In spite of, the activity was restored upon treatment with BME, LA and LA + BME which was 32.65 %, 39.69 %, 57.85 % in kidney and brain recovered 56.4 %, 64.07 % and 84.53 % activity respectively compare to control (p ≤ 0.001). Loss of activity was restored by70.32%, 75.18%, 84.96% in kidney and 77.7%, 81.51% and 91.68% in brain after treated with BME, LA, BME + LA respectively as compare to control.

Catalase: Antioxidative enzyme, catalase, also showed negative co-relation with lead exposure. Significant loss in catalase activity was also noticed in the kidney (43.22%) and brain (58.9%), which was recovered by antioxidant treatment in kidney by 25.78 %, 33.4 % and 54.04 % and in the brain by 73.97 %, 84.89 % and 111.55 % compared to lead intoxicated group. Restoration was noticed almost similar in both the organs i.e. 71.41 %, 75.74 % and 87.46 % restoration in kidney and 71.5 %, 75.99 %, 86.95 % restoration in the brain after treated with BME, LA, BME + LA respectively as compare to control.
Fig. 1 represents δ-ALAD activity in blood during lead intoxication as well as during α-lipoic acid (α-LA), bamboo leaves methanolic extract (BME) and α-lipoic acid, bamboo leaves methanolic extract (α-LA+ BME) therapies. Value represents a Mean ± S.D. for 5 rats. * indicates 

Fig. 2 represents reduced glutathione level in kidney and brain tissues during lead intoxication as well as during α-lipoic acid (α-LA), bamboo leaves methanolic extract (BME) and α-lipoic acid, bamboo leaves methanolic extract (α-LA+ BME) therapies. Value represents a Mean ± S.D. for 5 rats. * indicates 

Fig. 3 represents total thiol level in kidney and brain tissues during lead intoxication as well as during α-lipoic acid (α-LA), bamboo leaves methanolic extract (BME) and α-lipoic acid, bamboo leaves methanolic extract (α-LA+ BME) therapies. Value represents a Mean ± S.D. for 5 rats. * indicates 

Fig. 4 represents Glutathione peroxidase activity in kidney and brain tissues during lead intoxication as well as during α-lipoic acid (α-LA), bamboo leaves methanolic extract (BME) and α-lipoic acid, bamboo leaves methanolic extract (α-LA+ BME) therapies. Value represents a Mean ± S.D. for 5 rats. * indicates
Fig. 5 represents catalase activity in kidney and brain tissues during lead intoxication as well as during α-lipoic acid (α-LA), bamboo leaves methanolic extract (BME) and α-lipoic acid, bamboo leaves methanolic extract (α-LA+BME) therapies. Value represents a Mean ± S.D. for 5 rats. * indicates p ≤ 0.05 compared to control group, † indicates p ≤ 0.05 compared to lead treated group.

Fig. 6 represents γ-glutamyl transpeptidase activity in kidney and brain tissues during lead intoxication as well as during α-lipoic acid (α-LA), bamboo leaves methanolic extract (BME) and α-lipoic acid, bamboo leaves methanolic extract (α-LA+BME) therapies. Value represents a Mean ± S.D. for 5 rats. * indicates p ≤ 0.05 compared to control group, † indicates p ≤ 0.05 compared to lead treated group.

Fig. 7 represents lipid peroxidation level in kidney and brain tissues during lead intoxication as well as during α-lipoic acid (α-LA), bamboo leaves methanolic extract (BME) and α-lipoic acid, bamboo leaves methanolic extract (α-LA+BME) therapies. Value represents a Mean ± S.D. for 5 rats. * indicates p ≤ 0.05 compared to control group, † indicates p ≤ 0.05 compared to lead treated group.

γ-glutamyl transpeptidase (γ-GGT): One more parameters showing positive co-relation with lead dose was also measured in the present study i.e. γ-GGT. Higher γ-GGT activity is one of the reasons behind depletion in cellular GSH level; both showed inverse relationship with each other. In the groups treated only with the lead 205.76 % and 236.19 % rise was seen in the kidney and the brain respectively (p ≤ 0.001). γ-GGT activity was restored 179.92 %, 160.23 % and 142.24 % in the kidney and restoration reported in the brain was 277.9 %, 227.61 %, 218.48 % in the groups treated with BME, LA and BME + LA respectively compare to control group. Groups treated with BME and LA alone and with combine therapy showed 12.56 %, 22.12 %, 30.87 % decreased activity in the kidney and 17.34 %, 32.29 % and 35.01 % decreased activity in the brain respectively compare to the group exposed only to lead.

Thiobarbituric acid reactive substances level (TBARs): Degree of lipid peroxidation was measured by level of TBARs. Significant (p ≤ 0.001) rise in the TBARs level was reported 101.38 % and 106.53 % in the kidney and in the brain respectively compare to control group. Restoration of peroxidation damage was observed by 108.65 %, 104.5 % and 106.23 % in the kidney and 122.95 %, 115.16 % and
100.21% in the groups treated with BME, LA and BME + LA respectively compare to control group. Decrease in the TBARs level was 46.5%, 48.11%, 47.25% in the kidney and in the brain recovery was 40.47%, 44.24%, 51.48% in the groups of BME, LA and BME + LA respectively compared to lead treated group. No significant difference have been observed between groups treated with combined therapy and control group which indicated that this treatment succeeded to reverse cell membrane tissue damage occurred due to lead toxicity.

**DISCUSSION**

Out of total circulating lead, 99% is present in the erythrocyte [28] which also interferes in most of the reactions of haem biosynthesis. Due to direct the binding of metal to -SH group, many enzymes containing sulfydryl group at its active site becomes inactive [29]. Out of 99% lead binds only to a sulfydryl containing enzyme δ-aminolevulinic acid dehydratase as a result of this its substrate can’t be converted to product, porphobilinogen, a precursor of haem synthesis pathway and δ-aminolevulinic acid accumulates in the erythrocytes and oxidized. This oxidized substrate further form free oxy radicals to cause progressive tissue damage. In our study both the source of antioxidants succeed to prevail over effect of lead to significant extent but their combined therapy is more effective than their effect alone.

GSH, the key player of antioxidative system and an intermediate of γ-GGT cycle, is highly susceptible to lead induced oxidative damage because lead have the tendency to bind ‘–SH’ group of protein and make them unavailable for their original functioning of buffering redox status and is also a substrate cofactor for many antioxidative enzymes. The same mechanism is applicable for the membrane bound and unbound total sulfydryl content. The glutathione system also plays role in neutralization of peroxides and maintenance of protein thiols in their reduced state. Any change in GSH or total thiol (decline or incline) is an indicative of antioxidative imbalance. Initially as oxidative stress produced in the cell GSH increases but on the progression of higher oxidative stress the level goes down. In the present study, damage and restoration related to reduced glutathione and total thiol in all the groups receiving different treatments were more in brain than in kidney. A plasma membrane bound enzyme, γ-glutamyl transpeptidase (γ-GGT) metabolizes extra cellular reduced glutathione. γ-GGT break GSH into γ-glutamyl and cysteinyl-glycine moieties whose thiol is much more reactive than the original compound. Because later lacks the α-carboxyl group of glutamate, which have been reported to prevent the interaction of cysteine–SH group with transition metal ions [30]. Also later one reacts with iron present freely in the system and gets oxidized. Reduced iron formed during this reaction reacts with molecular oxygen and at last form hydrogen peroxide. In the experimental animals exposed to lead have shown higher γ-GGT activity, which was further lowered down in the groups receiving LA or MBE alone and more efficiently by combined therapy. The decrease in GSH content in lead treated group [31] and its recovery in the animals given antioxidant therapy in both the organs might prevent oxidation of protein thiol and imprecise binding of lead to GSH. Also it has been proved that α-lipoic acid plays a very important role in replenishing body’s other antioxidant elements like ascorbic acid, α-tocoferol, reduced glutathione etc and scavenging reactive species [32].

High level of peroxides produced due to action of γ-GGT can be removed by GPx and CAT like antioxidative enzymes. The lead exposure has decreased the activities of antioxidative enzymes like glutathione peroxidase (GPx) and catalase (CAT) leads to the increased $\text{H}_2\text{O}_2$ production. GPx, a selenium dependent enzyme has shown decreased activity, as lead has the tendency to bind selenium [33,34] and also due to impairment of functional groups like GSH and NADPH.

Increase in thiobarbituric acid reactive substances (TBARs) level have been reported as a marker of endogenous lipid peroxidative damage and have also been studied extensively as a factor responsible in lead induced toxicity [35]. Lead exerts its effect on cell membrane lipids directly [36] or as a result of loss in reduced glutathione. High lipid peroxidation of membrane lipids leads to alteration in their integrity and deterioration of erythrocyte [2]. As a result cell membrane disruption occurs and essential components for enzyme activities and other antioxidative molecules of cells leached out. The lead induced lipid peroxidation was observed in both brain and kidney, as an indicative of peroxidative damage, which was almost restored by combined antioxidant therapy and to some extent LA and MBE therapy alone has also been proved beneficial. We have
observed that both the antioxidants (LA and MBE) were more effective in brain than in kidney and reaches almost near to control group.

From the present study, it has appeared that lead acetate caused significant oxidative stress in the kidney and brain tissues as a result of free radicals generated by oxidative stress, which was recognized by increased lipid peroxidation and \( \gamma \)-GGT activity; decreased level of antioxidants molecules like GSH and TSH and significantly diminished activities of antioxidative enzyme GPx and CAT. The lead exposed groups subjected to antioxidants treatment either alone or in combination has shown the beneficial effects where \( \alpha \)-lipoic acid proved it self a better antioxidant than bamboo leaves methanolic extract. Although bamboo leaves methanolic extract as a whole not exerted as good effect as \( \alpha \)-LA in the present study, it didn't prove it weak antioxidants because extracted material was not 100% pure. It may contain some impurity besides very important chemical constituents, previously isolated and proved beneficial as antioxidants. Still combined therapy showed best results than their therapies given alone.

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REFERENCES