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ORIGINAL ARTICLE

Protective Effect of Aloe vera extract in Lead Nitrate Hepatotoxicity in Swiss Albino Mice

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ABSTRACT
The study was planned to evaluate the efficacy of Aloe vera (AV) leaf extract in preventing lead induced oxidative stress in liver of albino mice. Mice randomly divided into 4 groups were treated with and without lead nitrate and AV alone or in combination for 35 days. The oxidative stress was measured by LPO level, reduced glutathione content, total protein level and by enzymatic activities of SOD, CAT, GSH and GST in liver tissue homogenate. Lead nitrate enhanced lipid peroxidation with concomitant reduction in SOD, CAT, GST, GSH and total protein content. Treatment of mice with AV resulted in marked improvement in most of the studied parameters. On the basis of above results it can be hypothesized that AV; a natural product can protect lead nitrate mediated hepatic toxicity.
Keywords: *Aloe vera*, hepatotoxicity, oxidative stress, lead.

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INTRODUCTION

In the modern era, living organisms are frequently exposed to eco-friendly pollutants such as heavy metals that have many harmful genetic impacts. Heavy metals are powerful metabolic inhibitors, due to these metals and their salts establish a very important group of ecological pollutants. The toxic chemicals discharged into air, water and soil get into food chain from the environment. By entering into biological system they disturb the biochemical processes leading to health abnormalities, in some cases to deadly consequences. Lead (Pb) is a ubiquitous heavy metal. Exposure of lead mainly occurs through the respiratory and gastrointestinal systems. Absorbed lead (whether inhaled or ingested) is stored in soft tissues. Several studies indicate that among all the tissues liver is the largest repository (33%) of Pb. It can cause liver damage and may disturb the normal biochemical process. Oxidative damage can be evaluated by several parameters such as glutathione (GSH) and glutathione disulphide (GSSG) and antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR) [1]. It has been reported that Cadmium (Cd) and lead (Pb) are toxic heavy metals that origin adverse health belongings in humans and animals [2].Medicinal plants are commonly used for the treatment of various ailments, as they are considered to have advantages over the conventionally used drugs that are much expensive and known to have harmful side effects. The *Aloe vera* plant, *Aloe barbadensis* Miller, family Liliaceae (Lily of the desert) is the most investigated and used of more than 300 species of *Aloe* is an important herb in Ayurvedic and indigenous medical systems for centuries in India. *Aloe vera* contains 75 potentially active constituents that include vitamins, enzymes, minerals, sugars, lignin, saponins, salicylic acids and amino acids[3]. Many investigators have shown that *Aloe vera* extract, induces hepatoprotective effects [4]; protects against heavy metals induce oxidative stress [5]. Given these considerations, the present study was carried out to evaluate the curative effect of *aloe vera* extract against radiation-induced some biochemical parameters in albino mice.

MATERIAL AND METHODS

Chemicals

Lead nitrate was purchased from Central Drug House (India). All other chemicals used in the study were of analytical reagent and obtained from HIMEDIA (India).

Preparation of ethanolic AV leaf extract

The *Aloe vera* leaf was collected locally. The specimen was placed at Herbarium, Department of Botany, University of Rajasthan, and Jaipur. The voucher number is RUBL-19886. Extract of fresh, shade dried and powdered leaves of *Aloe* were prepared in ethyl alcohol. It was dissolved in distilled water whenever needed for experiment.

Experimental animal

Male Swiss albino mice weighing approximately 24–26 g were used for experimental purpose. All surgical and experimental procedure was performed by the recommendations found in the Guide for the Care and approved by the institutional Animal House and Use Committee of the University of Rajasthan, Department of Zoology; Jaipur. Experiments were conducted on adult male albino mice weighing 25-30g. They were housed in polypropylene cages in an air-conditioned room with temperature maintained at 250C±30C. The mice were provided with a nutritionally adequate chow diet (Hindustan lever Limited, India) and drinking water ad libitum throughout the study.

Experimental design

In the present study 16 male Swiss albino mice weighing 25-30g (3-4 months old) were used for hepatic biochemical parameters. For these parameters 4 groups with 4 mice in each group were taken and are treated by oral administration once daily as follows:

Group-1: Administered 1ml distilled water; served as control.

Group-2: Administered lead nitrate (10 mg/ kg body weight/day) dissolved in distilled water

Group-3: Administered ethanolic AV leaf extract at a dose of 100 mg/kg, body weight/per day, respectively.

Group-4: Administered lead nitrate at a dose of 10mg/kg body weight/per day along with a dose of ethanolic AV leaf extract at a dose of 100 mg/kg, body weight/per day.

The dose for lead was decided and selected on the basis of previously published [4, 5].

Hepatic oxidative stress Parameters

After 35 days of duration the mice were fasted overnight and then sacrificed under light ether anesthesia. Liver lobules were dissected out, washed immediately with icecold saline to remove blood, and the wet weight was noted and then stored at -80°C for various biochemical assays. Half of each liver was processed for biochemical analysis and the other half was preserved for further Histopathological/histological examination.

Biochemical analysis

Liver was perfused with chilled saline 84.61% NaCl and removed immediately after scarification of animals for biochemical and histopathological study. Small pieces taken from all lobes were crushed in masticator. Homogenates were prepared in TrisKCl, DDW, acetate buffer, DDW, 30 % KOH and glacial acetic acid for estimation of Lipid peroxidation (LPO) [6], Superoxide dismutase (SOD)[7], Catalase (CAT) [8], Glutathione S-Transferase (GST) [9], Reduced Glutathione (GSH) [10], and total Protein content [11] in various groups of mice.

Histological examination

Liver fragments removed from the mice were fixed in Bovins solution, dehydrated in an ethanol series, and embedded in paraffin wax for histological procedure. Liver was cut to obtain representative section of all liver lobules.

Statistical analysis

Results were presented as mean and standard error (Mean ± S.E). The statistical significance between the control and each of the treated groups were determined by SPSS- 20 after one-way ANOVA. The level of significance was set at $P < 0.05$.

RESULT

Table: 1-Antioxidant Effect of Aloe vera in LN toxicity in albino rats

Groups	LPO (n mole MDA/g fresh wet tissue)	SOD (Units/mg protein)	CAT (µmol.H2O2/ min/ mg protein)	GST (nmol CDNB formed/min/ mg/ protein)	GSH (nmol GSH/gm tissue)	Protein (mg/g fresh wt of tissue)
Control (I)	84.26±1.03	6.45±0.49	39.54±1.46	198.00±2.09	232.65±2.21	39.24±1.08
Lead nitrate (LN)-(II)	145.63±1.28[a]	3.11±0.26 [a]	18.35±0.38 [a]	107.32±1.34 [a]	109.75±1.14 [a]	22.08±0.89 [b]
AV (III)	88.25±0.77	6.44±0.15	48.23±0.62	194.36±2.16	258.36±1.18[a]	35.64±0.55
LN+AV (IV)	76.18±1.11*	4.98±0.14**	27.48±0.83*	154.08±1.64*	197.04±1.33*	28.35±0.08**

Values are Mean±S.E.M; n= 6; aP<0.001 compared to normal animal; bP<0.01 compared to normal animals; cP<0.02 compared to normal animals; *P<0.001 compared to lead exposed animals; **P<0.01 compared to lead exposed animals;

Abbreviations- LN: Lead nitrate; AV: *Aloe ver*a, TBARS: Thiobarbituric acid reactive substances ((n mole MDA/g fresh wet tissue); SOD: Superoxide dismutase ((Units/mg protein)) ;Catalase (µmol.H2O2/min/mg protein); GST: Glutathione S-transferase (nmol CDNB formed/min/mg/ protein ; GSH: Reduced Glutathione (nmol GSH/gm tissue); Protein (mg/g fresh wt of tissue); Values are Mean± S.E; n= 6.

There was a significant increase (P<0.001) noticed in hepatic LPO in lead exposure induced as comparison to untreated animals. However, AV leaf extract treatment significantly reduced the lead induced increase in LPO level when compared to lead treated animals. Some major components were also determined involved in the deregulation of substances formed during oxidative stress such as SOD, catalase, GST, and GSH respectively. Conspicuously, (P<0.001) these substances were significantly less regulated by lead treatment, and the effects were largely prevented by AV leaf extract treatment as compared to LN treated animals.

Histological examination of hepatic tissue section reveals that lead caused a severe inflammatory response of the liver, as indicated by inflammatory cellular infiltration as well as cytoplasmic vacuolation and degeneration of hepatocytes. Disintegration of nuclear membrane, few enucleated hepatocytes and mild shrinkage of nuclei were noticed in liver of control mice group (Fig 1). Cord like arrangement of hepatocytes was found to be completely distorted. Mild fatty degeneration, and several enucleated hepatocytes, severe degranulation and vacuolization of cytoplasm, crenated and shrunken nuclei were evidently seen. Mononucleated and binucleated edematous giant hepatocytes were noticed in LN treated animals.

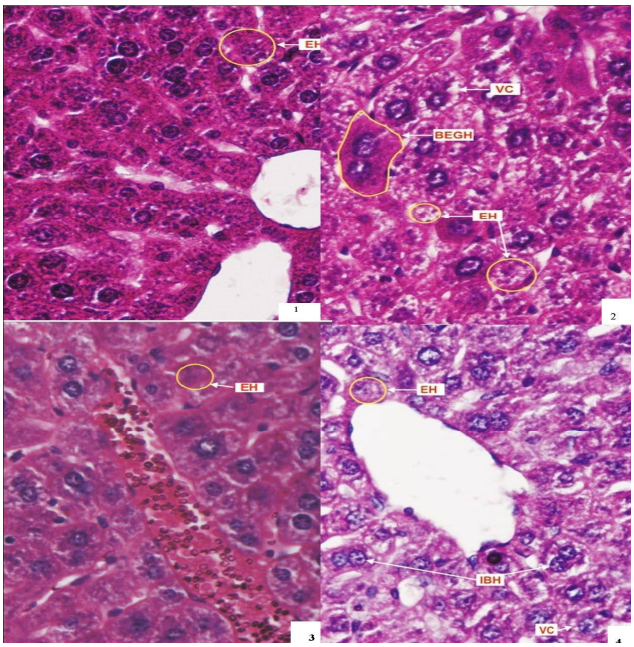

Fig 1: Treatment with AV only was largely prevented. Mice exhibited almost normal structure but mild lymphocytic infiltration, fatty degeneration and few enucleated hepatocytes were still noticed [1,2]. LN and AV treated mice also showed distorted hepatic architecture, mild fatty degeneration[3], and increased number of binucleated hepatocytes, few enucleated hepatocytes, degranulated and vacuolated cytoplasm, shrunken and crenated nuclei. Numbers of normal hepatocytes seem to be higher [4].

DISCUSSION

"Oxidative stress" resulted due to imbalance between Reactive oxygen species (ROS) metabolites and antioxidant defense. Reactive oxygen species (ROS), generated through radiolysis of water molecules caused most of the damage. This might be one of the reasons for significant alteration in LPO and significant changes in the activity of antioxidant enzymes. Lipid peroxidation LPO is a free radical chain reaction and mainly involves three distinct steps i.e. Initiation, propagation and termination [12]. It results in a loss of biochemical and structural architecture of cellular organelles and therefore, it is a highly destructive process. Antioxidants like vitamins A β-carotene, C and E [13], glutathione peroxidase [14], several isozymes of superoxide dismutase[15], and minerals such as selenium [14] present in *Aloe* seem to be responsible for inhibiting lipid peroxidation level in liver. These results suggest that the basic cause of lipid peroxidation is not only the free radicals but also the low level of antioxidants in a biological system, which removes them.

In present study lead induced toxicity might result in decreased tissue activities of enzymatic antioxidants like SOD and CAT. The decrease of SOD and CAT activities might influence the liver of mice to oxidative stress which provide a clear indication of cytotoxic damage in hepatic tissue.

Lead exposure inhibits the activities of these antioxidants led to increased oxidative modifications of cellular membrane and intracellular molecules. Earlier findings suggest the mechanism of alterations in these oxidative enzymes [16].

Reduced Glutathione (GSH) concentration in the present study suggests the utilization of glutathione by glutathione peroxidase. GSH is essential for the protection of the cells against reactive oxygen species and free radicals produced even in normal metabolism [17, 18]. The GPx catalyses the oxidation of GSH to GSSG and this oxidation reaction occurs at the expense of hydrogen peroxide (H_2O_2). Direct coupling of lead to GSH, results in the formation of a GSH- lead complex that is subsequently excreted in the bile, has been demonstrated in vivo[19]. In this study the decrease in GST activity after the exposure to lead could be caused by Pb-induced changes in the enzyme structure as well as by the lack or insufficient amount of GSH, being a substrate for this enzyme [20].

The pathogenesis of lead toxicity is responsible for the of protein observed in the present study. Lead is multifactorial and directly interrupts enzyme activation, competitively inhibits trace mineral absorption, binds to sulfhydryl proteins (interrupting structural protein synthesis), alters calcium homeostasis, and lowers the level of available sulfhydryl antioxidant reserves in the body. Administration of *Aloe vera* leaf extract alone had slight effect on LPO, SOD, CAT, GST and GSH activity but no effect of plant extract was seen on protein content. However, treatment with plant leaf extract along with lead; decreased the lipid peroxidation in hepatic tissue as compared with lead treated animals, thus indicating protective role of this plant extract in lead intoxication.

In the present study, lead exposure produced pronounced histological alternations in liver including by inflammatory cellular infiltration as well as cytoplasmic vacuolation and degeneration of hepatocytes, disintegration of nuclear membrane, few enucleated hepatocytes and mild shrinkage of nuclei, dilation of central vein and sinusoids. Findings are supported by [21].

Lead is well known toxic metal for inducing hepatic injury. The pathological changes alter the function of liver which interferes with the secretion of plasma proteins. Results also showed a remarkable cellular infiltration in the hepatic tissue. Cellular infiltration in the hepatic tissue suggest abundance of leucocytes, in general, and lymphocytes, in particular, that are a prominent response of body tissues facing any injurious impacts.When AV extract along with lead administered, it retained hepatic architecture and was able to diminish the fibrosis, congestion, hepatocyte vacuolation, swelling, leucocyticinfilteration, pykonotic nuclei, dilation of central vein and sinusoids. This might be due to the presence of flavonoids, alkanoids and ascorbic acid [22].

Hence, the mechanism by which the *Aloe vera* exerts a hepatoprotective effect could be attributed to (i) presence of natural antioxidants, (ii) its free radical scavenging and antioxidant properties. The exact underlying mechanism is still unclear and further research is needed to clarify antioxidant role of this plant. It is thus concluded that ethanolic leaf extract of *Aloe vera* may provide protective effect against lead intoxication.

CONCLUSION

Results of the present study conclude that treatment of mice with *Aloe* extract with lead exhibit less toxic effects in the liver at biochemical as well as histopathological levels.

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CONFLICT OF INTEREST: Nil

REFRENCE

1. Patra RC, Swarup D, Dwidedi SK. (2001). Antioxidant effects of α- tocopherol, ascorbic acid and L- Methionine on lead-induced oxidative stress of the liver, kidney and brain in rats. Toxicol; 162: 81-8.
2. QixiaoZhai, ArjanNarbad and Wei Chen. (2015). Dietary Strategies for the Treatment of Cadmium and Lead Toxicity. Nutrients, 7(1), pp. 552–571.
3. Shelton M (1991). *Aloe vera*, its chemical and therapeutic properties. International Journal of Dermatology 1991; 30(10): 679-683.
4. Chandan BK, Saxena AK, Shukla S, Sharma N, Gupta DK and Suri KA: (2007). Hepatoprotective potential of *Aloe barbadensis*Mill. against carbon tetrachloride induced hepatotoxicity, Journal of Ethnopharmacology ; 111(3): 560-566.
5. Gupta R and Flora SJ: (2005). Protective value of *Aloe vera* against some toxic effects of arsenic in rats. Phytotherapy Research; 19(1): 23-28.
6. Utley HC, Bernhein F, Hachslein P. (1967). Effect of sulphahydryl reagent on peroxidation in microsome. Arch BiochemBiophys; 260: 521-531.
7. Marklund S, Marklund G.(1974). Involvement of Superoxide anion radical in the autooxidation of pyrogallol and convenient assay for SOD. Eur J Biochem; 47: 469-74.
8. Aebi H. (1983). Catalase, In. Methods in enzymatic analysis. Bergmeyer H Ed. New York. Academic Press; 2: 76-80.
9. Jollow, DJ, Mitchell JR, Zampaglione N, Gillete JR. (1974). Bromobenzen-induced liver necrosis. Protective role of gluthione and evidence for 3, 4- bromobenzen oxide as the hepatotoxic metabolite. Pharmacol; 11: 151-169.
10. Habig WH, Pabst MJ, Jakoby WB. (1974). The first enzymatic step in mercapturic acid formation. The J BioloChem ; 219: 7130-39.
11. Lowry OH, Reserbroug N J, Farr AL, Randall RJ. (1951). Protein measurement with the folin phenol reagent. J. Biol Chem; 193-65.
12. Burchard C: (1959). Estimation of cholesterol. J Biol Chem; 167: 202.
13. Dadupanthi P: (2015). Effects of *Aloe vera* on the liver of Swiss albino mice against a sublethal dose of gamma radiation. IJPSR; 6(10): 4284-4289.
14. Frantz ID, Scheinder HS and Hineklemen BT: (1954). Suppression of hepatic cholesterol synthesis in the rat by cholesterol feeding. J Biol Chem; 206(1): 465-9.
15. Gould RC, Virgina B and Belly H: (1959). Stimulation of cholesterol biosynthesis from acetate in rat liver and adrenal by whole body X-irradiation. Am J Physiol; 196(6): 1231-1237.
16. Hsu PC, Guo YL. (2002). Antioxidant nutrients and lead toxicity. Toxicology; 180: 33-44.
17. Dixit VP, Agarwal M and Joshi SC:(1986). Role of cholesterium and elofibrate in correcting increased lipid levels. Ind J PharmacolSci; 48: 60.
18. Dadupanthi P: (2019). Protective effects of *Aloe vera* against radiation induced biochemical disorders in liver of Swiss albino mice. Int J Pharm Sci & Res ; 10(3): 1275-80. doi: 10.13040/IJPSR.0975-8232.10(3).1275-80.
19. Christie NJ, Costa M. (1984). In vitro assessment of the toxicity of metal compounds. IV. Disposition of metals in cells: interaction with membranes, glutathione, metallothionein, and DNA. Biol TraceElem Res; 6: 139–158.
20. Sivaprasad R, Nagaraj M, Varalakshmi P. (2004). Combined efficacies of lipoic acid and 2,3-dimercaptosuccinic acid against lead-induced lipid peroxidation in rat liver. J NutrBiochem ; 15: 18–23.
21. Sharma V, Pandey D.(2010). Protective Role of *Tinospora Cordifolia* against Lead-induced Hepatotoxicity Taxicol Intern; 17(1):12-17.
22. Sharma V, Sharma S. (2011): Lead induced hepatotoxicity in male swiss albino mice: the protective potential of the hydromethanolic extract W*ithania somnifera. International Journal of Pharmaceutical Sciences Review and Research*, 7(2): 116-121.