Research Article

# Effect of *Aloe emodin* Against Lead Induced Hepatotoxicity

**Ananya Bhattacharjee*, Venkatrao H Kulkarni, Prasanna V Habbu**

Research Scholar, Pharmacology Department, Soniya Education Trust's College of Pharmacy, S.R. Nagar, Dharwad, Karnataka, India-580002.
Professor, Pharmacology Department, Soniya Education Trust's College of Pharmacy S.R. Nagar, Dharwad, Karnataka, India-580002.
Professor, Pharmacognosy Department, Soniya Education Trust's College of Pharmacy, S.R. Nagar, Dharwad, Karnataka, India-580002.
***Corresponding author's E-mail:** mouroland@gmail.com

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**ABSTRACT**

The liver is the critical organ. Long-term occupational or environmental exposure as well as excessive exposure to lead may cause severe hepatotoxic effects. The widespread use of lead has led to manifold rise in the occurrence of free lead in biological systems and the inert environment. The lead induced hepatotoxicity study was carried out in adult male wister albino rats. Rat doses of Aloe emodin were selected as 100 mg/kg and 200 mg/kg through oral route. After acclimatization, the animals were randomly divided into 4 groups of 8 animals in each and received normal saline, lead acetate, high and low doses of Aloe emodin along with lead acetate respectively for 28 days. Serum enzymes such as AST, ALT, ALP, total bilirubin and lipid levels were measured by semi-autoanalyser. Antioxidants like SOD, Catalase, TBARS and GSH activity were measured in liver tissue homogenate. Remaining livers were subjected for histological examination. Observed results suggested dose dependent beneficial effects for Aloe emodin against lead acetate induced hepatotoxicity and it was concluded that Aloe emodin exhibited dose-dependent protection against lead induced hepatoxicity.

**Keywords:** Lead acetate, hepatotoxicity, Aloe emodin, heavy metal, liver toxicity.

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## INTRODUCTION

Heavy metal Contaminations in the environment are major public health concern ecologically and globally. Lead is one of the earliest heavy metals. Ductility, resistance to corrosion, low melting point, softness, malleability - these properties of lead was responsible for its widespread usage in different industries such as ceramics, automobiles, paint, plastics, *etc*. Chronic exposure to lead causes life threatening detrimental effects on brain, liver, and other major organs such as heart, kidney, bones, reproductive organs etc. [1]

The liver is composed of highly active metabolic tissue containing a huge complement of detoxification machinery referred to as phase I and phase II enzyme systems that ideally serve to guard other physiological systems from the toxic effects of xenobiotic compounds. Through portal vein liver is exposed to nutrients and other xenobiotics. In different studies it has been observed that acute and chronic intoxication of lead is responsible for alterations in hepatic xenobiotic metabolism, cholesterol metabolism, liver cell proliferation and DNA synthesis. It has been reported that after lead exposure a huge amount approximately 33% accumulated in soft tissues like liver. Lead exposure is responsible for detrimental effect on hepatic microsomal cytochrome P-450 and associated enzymatic activities in rat liver. [2]

Herbal medicines are getting popularity throughout the world due to their potency and apparent safety profile. Polyphenolic compounds have many phenolic groups, are widely present in different plants, fruits and vegetables. Their beneficial effect against many diseased condition affecting different major organs already has been well established in heavy metal toxicity. [3]

Polyphenols were also reported for detoxification and removal of heavy metals. [4]

The probable reason by which it may show the protection is scavenging of reactive oxygen species, generated by lead and other heavy metals. It is reported that they are also responsible for detoxification by removal of accumulated heavy metals from major organs. Polyphenols also attenuated ROS-mediated inflammatory cytokines secretion through ERK/JNK/p38 pathways resulting protection against lead induced inflammatory reactions. [5, 6]

One of the potential anthraquinone derivatives Aloe-emodin is well known for several medicinal properties. Aloe-emodin is chief bioactive components of rhubarb (Rheum palmatum), which has been used in traditional Chinese medicine. Apart from that Aloe-emodin is also abundant in the leaves of the common plant Aloe vera. [7]

It is proved to have potential antioxidant, hepato-protective, Neuro protective, antidiabetic, Anti

inflammatory, Anticancer and anti-anginal activity, Antibacterial and protection against retinal ganglion activities. [8-16] So the present study has been designed to evaluate protective effect of Aloe emodin against lead induced hepatotoxicity.

## MATERIALS AND METHODS

### Chemicals

All chemicals used were of analytical grade and purchased from standard companies. Lead acetate was purchased from Loba Chemicals, Mumbai. Biochemical kits were procured from Crest Biosystems (Goa, India).

### Phyto-chemicals

Aloe emodin sample was obtained from Yucca Enterprises (Mumbai, India).

### Experimental Animals

Healthy adult male Wistar albino rats weighing 170-200 g were housed in polypropylene cages, maintained under standardized condition (12 h L:D cycles, 25° ± 5°C) with paddy husk bedding at the Central Animal House of the institution, were provided with standard pellet food and had free access to purified drinking water. The guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India were followed and prior permission was sought from the Institutional Animal Ethics Committee for conducting the study.

### Dose selection of Aloe emodin

Acute oral toxicity was performed according to OECD 425 guidelines. As per the limit test, male wistar albino rats were fasted overnight and given 2000 mg/kg of Aloe-emodin orally. Animals were observed for 48 hours, with special attention during the first 4 hours and daily thereafter for a period of 14 days, for any signs of toxicity or mortality. In the similar manner five animals were dosed and observed one followed by other. All the animals were found to be safe so $1/10^{th}$ & $1/20^{th}$ of found safe dose i.e. 200 mg/kg & 100 mg/kg through oral route were selected as high and low dose respectively. [17]

### Experimental design

After one week of acclimatization, the animals were randomly divided into 4 groups of 8 animals in each. Group I Served as normal control and received normal saline 2 ml/kg body weight through oral route. Group II Served as toxic control and animals were treated with lead acetate (60 mg/kg) p.o. for 28 days. Group III & IV animals received Aloe emodin 200 and 100 mg/kg p.o. respectively for 28 days along with that lead acetate was administered as in group II. [18]

Twenty four hours after the last treatments, the animals were anesthetised with light ether anesthesia and blood was withdrawn by retro-orbital puncture. Serum was separated by centrifugation for the estimation of enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkanine phosphate (ALP), total bilirubin and lipid levels such as total cholesterol (TC), trigycerides (TG), low density lipoprotein (LDL), high density lipoprotein (HDL) levels by semi-autoanalyser (Robonik, Mumbai). Thereafter the animals were sacrificed; livers were used for the preparation of homogenate to estimate antioxidants like super oxide dismutase (SOD), catalase, thio-barbituric acid reactive substances (TBARS) and reduced glutathione (GSH). Remaining livers were embedded in formaline in saline solution (10%) for histological examination. [19,20]

### Preparation of Liver Tissue Homogenate:

The livers removed were gently rinsed with ice cold physiological saline solution (0.9% NaCl) to remove blood, mucus and other debris adhering them. The sliced liver was immediately homogenized in ice-cold o.1 M sodium phosphate buffer (pH 7.4) at 1-4 C to give a 10% (w/v) homogenate. The homogenates were centrifuged twice at 10000 rpm for 15 minutes at 4°C. The supernatants were used for the estimation of SOD, Catalase, GSH and TBARS.[21]

### Histological analysis

Liver sections were prepared from the remaining half of the liver samples in each group, stained with Hematoxylin and Eosin (H&E) and changes in histology were observed.

### Statistical analysis

Results were expressed as mean +/- SEM. Statistical significance was assessed using One-way Analysis of variance (ANOVA) followed by Tukey-Karmer multiple comparison tests. $P<0.05$ was considered significant.

## RESULTS AND DISCUSSION

### Effect on serum enzymes

#### *Effect on AST, ALT, ALP and Bilirubin (Table 1)*

Toxic control (only lead acetate treated) group demonstrated extremely significant ($P <0.001$) increase in serum AST, ALT, ALP and bilirubin levels compared to normal control.

Treatment group such as AE200 showed extremely significant ($P <0.001$) decrease in AST, ALT, ALP levels where as AE100 showed significant ($P <0.05$) decrease in AST, ALT, ALP levels compared to toxic control. AE200 and AE100 both groups showed extremely significant ($P <0.001$) decrease in bilirubin levels.

#### *Effect on TC, TG, HDL, LDL (Table 2)*

Toxic control (only lead acetate treated) group demonstrated extremely significant ($P <0.001$) increase in serum TC, TG, LDL values compared to normal control, whereas extremely significant ($P <0.001$) decrease in HDL values compared to normal control.

Treatment groups such as AE200 showed extremely significant ($P <0.001$) decrease in serum TC, TG, LDL values, compared to toxic control. AE100 showed significant (P

<0.05) decrease in serum TC, TG, LDL values, compared to toxic control. All the treatment groups showed extremely significant (P <0.001) increase in serum HDL values.

**Effect on antioxidants in Liver tissue homogenate (HTH)**

***Effect on SOD, Catalase and GSH (Table 3)***

Toxic control group reported extremely significant (P <0.001) decrease in SOD, Catalase and GSH activity compared to normal control.

Experimental group AE 200 it was found to be moderately significant (P <0.01) and AE 100 treated group showed significant (P <0.05) increase in SOD, Catalase and GSH values compared to toxic control group.

***Effect on TBARS (Table 3)***

Toxic control group demonstrated extremely significant (P <0.001) increase in TBARS activity compared to normal control.

Treatment groups such as AE 200, AE 100 treated groups it was found to be moderately significant (P <0.01) decrease in TBARS activity compared to toxic control group.

The aim of the present study was to investigate the protective effect of Aloe emodin against lead acetate induced hepatotoxicity. Observed results suggested dose dependent beneficial effects of Aloe emodin against lead acetate induced hepatotoxicity.

Lead exposure is responsible for generation of huge amount of free radicals which is responsible for development of oxidative stress. Enormous amount of oxidative stress associated with lead exposure is responsible for decline in intracellular ATP, oxidative DNA damage and the apoptosis of hepatocytes.[22]

**Table 1:** Effect on serum enzymes against lead acetate induced hepatotoxicity

| Treatment | Blood serum level (U/L) | | | |
|---|---|---|---|---|
| | AST | ALT | ALP | Bilirubin |
| Normal control | 69.63±0.28 | 32.66±0.56 | 93.64±0.68 | 1.34±0.34 |
| Toxic control | 129.64±0.34$^{***}$ | 97.32±0.46$^{***}$ | 179.73±0.72$^{***}$ | 2.03±0.64$^{***}$ |
| AE200 | 88.39±0.94$^{###}$ | 61.44±0.57$^{###}$ | 133.54±0.47$^{###}$ | 1.52±0.87$^{###}$ |
| AE100 | 108.33±0.81$^{#}$ | 82.37±0.84$^{#}$ | 167.45±0.87$^{#}$ | 1.89±0.61$^{###}$ |

All values are mean ± SEM, n=8, $^{***}$P <0.01 when compared to normal control; $^{###}$P <0.001, compared to Toxic control group.

**Table 2:** Effect on serum enzymes against lead acetate induced hepatotoxicity

| Treatment | Blood serum level (U/L) | | | |
|---|---|---|---|---|
| | TC | TG | HDL | LDL |
| Normal control | 120.4±8.34 | 39.6±6.31 | 49.6±8.81 | 71.8±5.93 |
| Toxic control | 165.3±10.41$^{***}$ | 74.9±8.21$^{***}$ | 24.8±6.49$^{***}$ | 126.7±9.38$^{***}$ |
| AE 200 | 139.2±12.81$^{###}$ | 52.8±5.84$^{###}$ | 39.4±5.53$^{###}$ | 93.7±8.28$^{###}$ |
| AE 100 | 153.4±9.98$^{#}$ | 65.5±8.17$^{#}$ | 38.7±3.69$^{###}$ | 115.7±8.53$^{#}$ |

All values are mean ± SEM, n=8, ***P <0.01 when compared to normal control; $^{###}$P <0.001, $^{#}$P <0.05 compared to Toxic control group.

**Table 3:** Effect on antioxidants in HTH and histological score against lead acetate induced hepatotoxicity

| Treatment | Liver Tissue Homogenate (Units/mg of protein) | | | |
|---|---|---|---|---|
| | SOD | CATALASE | TBARS | GSH |
| Normal control | 13.83±0.53 | 44.34±0.76 | 29.83±0.38 | 9.53 ±0.31 |
| Toxic control | 03.43±0.67$^{***}$ | 19.78±0.39$^{***}$ | 56.32±0.59$^{***}$ | 2.19 ±0.62$^{***}$ |
| AE 200 | 07.20±0.72$^{##}$ | 29.25±0.54$^{##}$ | 38.33±0.60$^{##}$ | 6.19 ±0.39$^{##}$ |
| AE 100 | 05.23±0.54$^{#}$ | 23.87±0.63$^{#}$ | 48.71±0.18$^{##}$ | 4.91 ±0.22$^{#}$ |

All values are mean ± SEM, n=8, ***P <0.001, **P <0.01 when compared to normal control; ###P <0.001, ##P <0.01, #P <0.05 compared to Toxic control group.

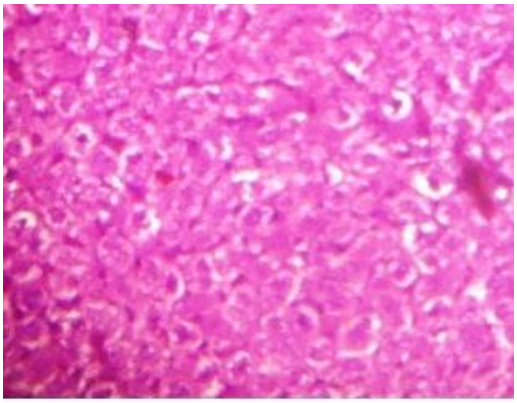

**Figure- 1a:** (H&E) (x400) stained microscopic section of normal control
1. Normal texture of cells

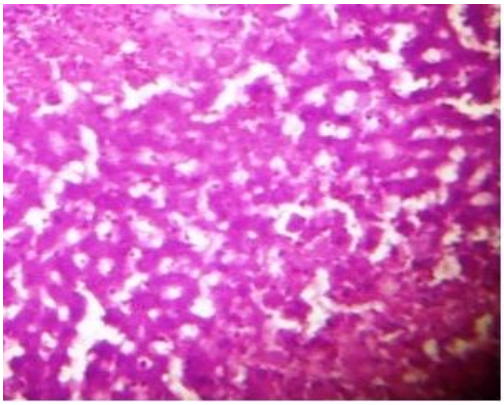

**Figure- 1b:** (H&E) (x400) stained microscopic section of toxic control.
**1.** vacuolization, 2. leucocyte infilteration, 3. pyknotic cells and 4. loss of radial arrangement of hepatocytes

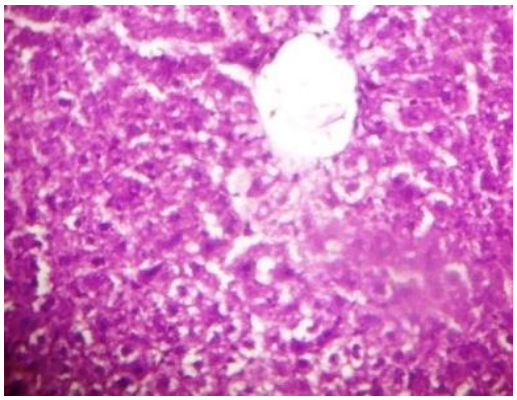

**Figure- 1c:** (H&E) (x400) stained microscopic section of AE 200.
1. Restoration of normal hepatic arrangement of the hepatocytes

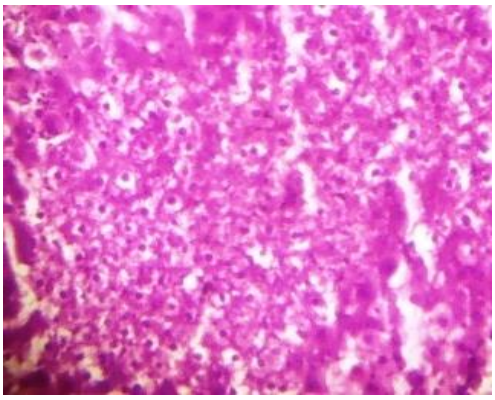

**Figure- 1d:** (H&E) (x400) stained microscopic section of AE 100.
1. diminished fibrosis 2. restoration of normal hepatic arrangement of the hepatocytes

**Figure 1:** Haematoxylin and eosin (H&E) stained section of liver in lead acetate induced liver damage. Photographed at magnification 400X

Reactive oxygen species (ROS) are effectively neutralized by the antioxidant defense system. But during patho-physiological conditions such as lead exposure results in enormous increased production of ROS and/or impaired antioxidant capacity, which culminate in oxidative stress.[23]

Results of this study also witnessed extremely significant decrease in superoxide dismutase (SOD), catalase, reduced glutathione (GSH) and increase in thio barbituric acid reactive substances (TBARS) in liver tissue. This may be due to excessive damage produced by lead exposure.

AE, an Anthraquinones, is a natural phenolic compound, has been reported for strong antioxidant activity. [24-28]

The antioxidant effect associated with Aloe emodin may be due to direct action on free radical scavenging and indirect action through the induction of antioxidant enzymes.[29]

In our present study also aloe emodin showed significant decrease in serum AST, ALT, ALP, total bilirubin levels which reflect protection against lead induced hepatotoxicity. Moreover the treated groups showed significant increase in SOD, catalase, GSH and decrease in TBARS levels.

Lead intoxication is responsible for impaired hepatic cholesterol metabolism which is responsible for increase in both liver and serum total cholesterol levels. [30]

In our present study also it has been evident that in the toxic group there is significant increase in TC, TG, LDL levels and significant decrease in beneficial HDL level. Aloe emodin showed significant decrease in TC, TG, LDL levels and significant increase in HDL levels.

Only lead exposed group caused enormous changes in the liver cell associated with degeneration of liver tissue, focal necrosis with hepatocyte vacuolation, pykonotic nuclei, swelling, dilation of central vein and sinusoids, infiltration of inflammatory cells. Treatment with aloe emodin dose dependently inhibited lead induced damage by restoration of reticular fibres and inflammation. [31,32]

## CONCLUSION

It can be concluded from the present study that aloe emodin exhibited dose-dependent protection against lead induced hepatotoxicity. Findings of the present study can be important for those who are chronically exposed to high level lead. Aloe emodin in the dietary source or in the form of formulation can keep their liver healthy and safe. Future studies can be carried out to establish the fact clinically.

## REFERENCES


1. Wittmann G. Toxic metals. In Metal pollution in the aquatic environment 1981 (pp. 3-70).
2. Lyn P. Lead toxicity, a review of the literature. Part I: exposure, evaluation and treatment. Altern Med Rev 2006;11:2-22.
3. Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Frontiers in pharmacology. 2014 Jan 10;4:177.
4. Pandey KB, Rizvi SI. Plant polyphenols as dietary antioxidants in human health and disease. Oxidative medicine and cellular longevity. 2009 Nov 1;2(5):270-8.
5. Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, Squadrito F, Altavilla D, Bitto A. Oxidative stress: harms and benefits for human health. Oxidative medicine and cellular longevity. 2017 Oct;2017.
6. Kurutas EB. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. Nutrition journal. 2015 Dec;15(1):1-22.
7. Sharma R, Tiku AB, Giri A. Pharmacological properties of emodin—anthraquinone derivatives. J Nat Prod Resour. 2017;3(1):97-101.
8. Arosio B, Gagliano N, Fusaro LM, Parmeggiani L, Tagliabue J, Galetti P, Castri D De, Moscheni C, Annoni G. Aloe-Emodin quinone pretreatment reduces acute liver injury induced by carbon tetrachloride. Pharmacol Toxicol 2000 Nov;87(5):229-33.
9. Tian B, Hua Y. Concentration-dependence of prooxidant and antioxidant effects of aloin and aloe-emodin on DNA. Food Chemistry 2005 July;91(3)413-18.
10. Arvindekar A, More T, Payghan PV, Laddha K, Ghoshal N, Arvindekar A. Evaluation of anti-diabetic and alpha glucosidase inhibitory action of anthraquinones from Rheum emodi. Food Funct 2015 Aug;6(8):2693-700.
11. Tao L, Xie J, Wang Y, Wang S, Wu S, Wang Q, Ding H. Protective effects of aloe-emodin on scopolamine-induced memory impairment in mice and $H_2O_2$-induced cytotoxicity in PC12 cells. Bioorg Med Chem Lett 2014 Dec 1;24(23):5385-9.
12. Lin HJ, Lai CC, Lee Chao PD, Fan SS, Tsai Y, Huang SY, Wan L, Tsai FJ. Aloe-emodin metabolites protected N-methyl-d-aspartate-treated retinal ganglion cells by Cu-Zn superoxide dismutase. J Ocul Pharmacol Ther 2007 Apr;23(2):152-71.
13. Park MY, Kwon HJ, Sung MK. Evaluation of aloin and *Aloe-emodin* as anti-inflammatory agents in aloe by using murine macrophages. Biosci Biotechnol Biochem 2009 Apr 23;73(4):828-32.
14. Coopoosamy RM, Magwa ML. Antibacterial activity of aloe emodin and aloin A isolated from Aloe excels. African Journal of Biotechnology 2006;5(11):1092-94.
15. Huang PH, Huang CY, Chen MC, Lee YT, Yue CH, Wang HY, Lin H. Emodin and Aloe-Emodin Suppress Breast Cancer Cell Proliferation through ER α Inhibition. Evid Based Complement Alternat Med 2013;13:376123.
16. Cárdenas C, Quesada AR, Medina MA. Evaluation of the anti-angiogenic effect of aloe-emodin. Cell Mol Life Sci 2006 Dec;63(24):3083-9.
17. Organization for Economic Cooperation and Development (OECD). OECD Guidelines for Testing of Chemicals. France: OECD Publishing; 2006 July 11.
18. Offor SJ, Mbagwu HOC and Orisakwe OE. Lead Induced Hepato-renal Damage in Male Albino Rats and Effects of Activated Charcoal. Front. Pharmacol. 2017;8:107.doi: 10.3389/fphar.2017.00107.
19. Chakraborty M, Kamath JV. Pharmacodynamic interaction of green tea extract with hydrochlorothiazide against ischemia-reperfusion injury-induced myocardial infarction. J Adv Pharm Technol Res 2014 Jul;5(3):134-9.
20. Chakraborty M, Kamath JV, Bhattacharjee A. Pharmacodynamic Interaction of Green Tea Extract with Hydrochlorothiazide against Cyclophosphamide-Induced Myocardial Damage. Toxicol Int 2014 May;21(2):196-202.
21. Ahmed E. Moneim A. Indigofera oblongifolia Prevents Lead Acetate-Induced Hepatotoxicity, Oxidative Stress, Fibrosis and Apoptosis in Rats. PLoS One 2016; 11(7): e0158965.
22. Milosevic N, Maier P. Lead stimulates intercellular signaling between hepatocytes and Kupffer cells. Eur J Pharmacol 2000;401:317-28.
23. Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O. Oxidative stress and antioxidant defense. World allergy organization journal. 2012 Dec;5(1):9-19.
24. Narayanan S, Jadhav AP, Kadam VJ. Forced Degradation Studies of Aloe Emodin and Emodin by HPTLC. Indian J Pharm Sci 2015;77(6):795-8.
25. Huang PH, Huang CY, Chen MC, Lee YT, Yue CH, Wang HY, Lin H. Emodin and Aloe-Emodin Suppress Breast Cancer Cell Proliferation through ERα Inhibition. Evid Based Complement Alternat Med 2013;2013:376123.
26. Wang HH, Chung JG. Emodin-induced inhibition of growth and DNA damage in the Helicobacter pylori.Curr Microbiol 1997 Nov;35(5):262-6.
27. Chang CH, Lin CC, Yang JJ, Namba T, Hattori M. Anti-inflammatory effects of emodin from *Ventilago leiocarpa*. Am J Chin Med 1996;24(2):139-42.
28. Cha TL, Qiu L, Chen CT, Wen Y, Hung MC. Emodin down-regulates androgen receptor and inhibits prostate cancer cell growth. Cancer Res 2005 Mar 15;65(6):2287-95.
29. Vargas F, Díaz Y, Carbonell K. Antioxidant and scavenging activity of emodin, aloe-emodin, and rhein on free-radical and reactive oxygen species. Pharmaceutical biology. 2004 Jan 1;42(4-5):342-8.
30. Kojima M, Nemoto K, Murai U, Yoshimura N, Ayabe Y, Degawa M. Altered gene expression of hepatic lanosterol

14xdemethylase (CYP51) in lead nitrate-treated rats. Arch Toxicol 2002;76:398-403.

31. Sharma V, Pandey D. Protective Role of *Tinospora cordifolia* against Lead-induced Hepatotoxicity. Taxicol Intern 2010;17(1):12-17.

32. Omotoso BR, Abiodun AA, Ijomone OM, Adewole SO. Lead-Induced Damage on Hepatocytes and Hepatic Reticular Fibres in Rats; Protective Role of Aqueous Extract of *Moringa oleifera* Leaves (Lam). Journal of Biosciences and Medicines 2015;3:27-35.

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