Research Article

# Hepatoprotective Role of *Abutilon indicum* on Lead Induced Liver Injury in *Wistar* Rats

**R. Santhosh Kumar[1], J. Arul Daniel[1], S. Annie Jeyachristy[2], S. Asha Devi[1]***
[1]School of Bioscience and Technology, VIT University Vellore, Tamil Nadu, India.
[2]Unit of Biochemistry, AMIST University, Semeling, Malaysia.
***Corresponding author's E-mail:** ashaselvaraj74@gmail.com

**Accepted on:** 17-07-2016; **Finalized on:** 30-09-2016.

**ABSTRACT**

*Abutilon indicum* is an indigenous shrub, traditionally used as folk medicine for various diseases. The aim of our study was to investigate the pharmacological activity of *A.indicum* against hepatotoxicity induced by lead acetate. For the study animals were divided into four groups each consisting of six animals. Group I animals were treated with saline, group II with lead acetate (0.15%) and group III & IV with 300 and 500 mg/kg b.wt of *A.indicum* aqueous extract (AE). The study was carried out for 14 days following which rats were sacrificed and processed for histopathological and antioxidant enzymatic assays. The study provides evidence that administration of *A. indicum* (AE) at a higher dosage of 500 mg/kg bt wt helps in reducing the toxicity by increased antioxidant activity such as glutathione peroxidase, superoxide dismutase, catalase and reduced levels of lipid peroxidation thereby demonstrating that *A.indicum* (AE) does have a protective effect on lead acetate induced hepatotoxicity.

**Keywords:** lead acetate, *Abutilon indicum,* hepatoprotective, antioxidant, catalase.

## INTRODUCTION

Lead is a toxic metal and exposure can majorly occur through drinking water, industrial processes like smelting, through battery recycling, and paints. Lead does not have any biological function so even in low levels exposure can affect multiple clinical functions in humans.

Lead exhibits its effect on the oxidative stress mechanism, wherein antioxidants like glutathione within the cell protect from cellular damage induced by the reactive oxygen species (ROS). High level of lead exposure increase amounts of ROS and reduce effect of antioxidants.[1]

Lead exhibits its toxicity by replacing the monovalent and bivalent ions like $Na^{+}$, $Ca^{+}$, and $Mg^{+2}$. This disturbs metabolism and also changes various other processes like cell adhesion, cell signaling, apoptosis, and release of neurotransmitters.[2] The adverse effects of lead on different body systems can be counteracted with treatment of antioxidant compounds.[3]

The toxic effects of various toxicants like heavy metals can be reduced by supplementation with certain plants components.[4] *Abutilon indicum* (Malvaceae) is an under-shrub, grows in tropical regions of America, Malaysia, Sri Lanka and India. It is known as Country mallow, Indian mallow (English), Kanghi, Kakahi (Hindi) Tutti, Paniara, Thuththi (Tamil), and aerial parts of the plants have been used traditionally in various ailments.

The roots of the plant are considered as demulcent, diuretic in chest infection and urethritis, prescribed in fevers as a cooling medicine and is considered useful in strangury, haematuria and in leprosy. In ancient medicine like Ayurveda, the root of the plant is known to increase the male reproductive health. The roots contain biological compounds such as linoleic, oleic, stearic, palmatic, sitosterol.[5,6]

## MATERIALS AND METHODS

### Plant Material and Extract Preparation

The roots of *Abuitulon indicum* were collected from Vellore district, Tamil Nadu in the month of January. The plant was authenticated at the Horticulture Research Station, Yercaud. Fresh roots of *A. indicum* were dried and powdered in a mixture grinder, 10 g of root was weighed and made up to 100 ml with distilled water and kept in a boiling water-bath for 30 mins at 90°C. The solution was kept for cooling and filtered using Whatman filter paper No. 1 and the filtrate stored at 4°C for further use.

### Animals

Healthy adult male Wistar rats (8 weeks old) were chosen for the study. Wistar rats were issued from the animal house facility, VIT University, India. Different groups of rats were maintained in different cages with food and water *ad libitum* condition. All the animals were maintained in the animal house at 24±1°C with a 12 h light-dark cycle. During the time period of experiments, rats were kept free from any pathogenic infection.

### Experimental Design

After 1 week of acclimatisation, animals were divided randomly into four groups (n=6). Group I served as control was treated saline via gavage once daily for 14 days. Group II received 0.15% lead acetate; group III and group IV animals were treated with 0.15% lead acetate along with aqueous extract of *A. indicum* at doses of 300 and 500 mg/kg b.wt via gavage once daily respectively for


© Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

14 days. 24 hrs after the last dosage, animals were weighed and sacrificed by decapitation and liver tissue was excised carefully, washed in ice cold normal saline to remove the blood stains and other fat depositions.

Tissue samples were then used for antioxidant and histopathological studies.

### Determination of Body Weight

Body weight of each animal was checked on the first day before the start of experiment, which was recorded as the initial body weight. On 15th day before sacrifice, weight was checked and recorded as the final body weight.

The percentage increase of body weight was calculated from the data obtained.

$$\%\, Increase\ of\ Body\ Weight = \frac{Final - Initial\ b.wt}{Initial\ b.wt} \times 100$$

### Homogenate Preparation

Liver homogenate was prepared in ice cold 0.1 M phosphate buffer (pH 7.4) containing 1.17% KCl. A part of this homogenate was used to measure lipid peroxidation. Remaining homogenate was centrifuged at 10,000 rpm for 10 min at 4°C. The supernatant obtained was used for performing enzymatic antioxidant assays such as Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx) and protein estimation.

### Estimation of Lipid Peroxidation, Proteins and Antioxidant Assays

The lipid peroxidation assay was performed according to the method of Okhawa[7] and the result obtained was expressed as nmoles MDA formed/mg protein.

Pyrogallol autooxidation method by Marklund and Marklund[8] was followed to determine the superoxide dismutase enzyme activity in liver homogenate.

The Glutathione Peroxidase (GPx) assay was performed according to the method described by Rotruck and the results were expressed in terms of µg GSH utilized/min mg protein.[9] The catalase assay was carried out using the protocol described by Sinha and result was expressed as µ mole of $H_2O_2$ consumed/ min mg protein.[10] Total protein was estimated according to the standard protocol by Lowry method.[11]

### Histological Examination

The liver removed from each group was stored in 10 % neutral buffered formalin solution. Using an automated tissue processor liver tissue sections were prepared. The sections were cut to a thickness of 5 µm using Leica microtome RM 2155 and stained with Hematoxylin and Eosin (H&E). The stained slides were observed under light microscope.

### Statistical Analysis

All the results obtained were expressed as mean ± SEM for six rats in each group.

Statistical analysis was performed by ANOVA followed by Dunnett's comparison test. $p \leq 0.05$ was considered statistically significant when compared with normal and vehicle control group.

All the statistics were carried out in Graphpad Instat software Inc., V. 3.06, San Digeo, USA.

## RESULTS AND DISCUSSION

### Body and Organ Weigh

Table 1 indicates the variations in body weight gain and changes on administering lead and *A.indicum* AE on lead induced toxic rats.

Body weight was significantly reduced ($p < 0.01$) in group II, lead treated animals by 24.48 % when compared to group I normal control.

Group III animals treated with lead acetate with 300 mg/kg b.wt of *A. Indicum* AE also showed decrease in body weigh by 18.19 % in comparison to group I animals whereas rats treated with lead Acetate with 500 mg/kg b.wt of *A.Indicum* AE showed significant increase ($p < 0.01$) in body weight gain by 65.18% when compared to the group II rats.

Reduced body weight in group II animals may be due to the toxic effects induced by the lead.

**Table 1:** Effects of *Abutilon indicum* (AE) on Body and Testis Weight in Lead Acetate Treated Male Wistar Rats.

| Groups | Body Weight | | % Increase |
|---|---|---|---|
| | Initial (gms) | Final (gms) | |
| **Group I (Normal Control)** | 250 ± 0.256 | 283 ± 0.368 | 12.213 ± 0.563 |
| **Group II (Lead Acetate alone)** | 256 ± 0.56 | 280 ± 0.68 | 9.245 ± 0.537 a** |
| **Group III (Lead Acetate with 300mg/kg b.wt of *A.Indicum* AE)** | 223 ± 0.365 | 245 ± 0.295 | 9.991 ± 0.410 a** |
| **Group IV (Lead Acetate with 500mg/kg b.wt of *A.Indicum* AE)** | 255 ± 0.402 | 295 ± 0.51 | 15.271 ± 0.720 a** b** |

Results are Mean ± SEM, n=6. *p<0.05, **p < 0.01 considered statistically significant. Comparisons are as follows: a-group II when compared with normal group. b-group III &IV when compared with negative control group II. Statistical analysis by ANOVA followed by Dunnett's comparison test.

© Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

**Table 2:** Effect of *A. indicum* Administration on Antioxidant Status in Rat Liver.

| Parameters | Lipid Peroxidation nM MDA formed/mg protein | Catalase (U/mg protein) | Superoxide dismutase (U/mg protein) | Glutathione peroxidase (µg of GSH utilized/min mg protein) |
|---|---|---|---|---|
| **Group I Normal control** | 8.853 ± 0.287 | 2.468 ± 0.034 | 10.47 ± 0.247 | 3.033 ± 0.351 |
| **Group II Negative Control** | 17.496 ± 0.264 a** | 1.426 ± 0.030 a** | 2.098 ± 0.121 a** | 1.16 ± 0.165 a** |
| **Group III (Lead Acetate with 300 mg/kg b.wt of *A.Indicum* AE)** | 13.081 ± 0.191 a**b** | 1.903 ± 0.013 | 6.567 ± 0.345 a**b** | 1.902 ± 0.05 a**b* |
| **Group IV (Lead Acetate with 500 mg/kg b.wt of *A.Indicum* AE)** | 11.331 ± 0.280 a**b** | 2.012 ± 0.060 | 8.203 ± 0.200 a**b** | 2.485 ± 0.158 b** |

Results are Mean ± SEM, n=6, *p<0.05, **p < 0.01 considered statistically significant. Comparisons are as follows: a-group II when compared with normal group. b-group III &IV when compared with negative control group II. Statistical analysis by ANOVA followed by Dunnett's comparison test.

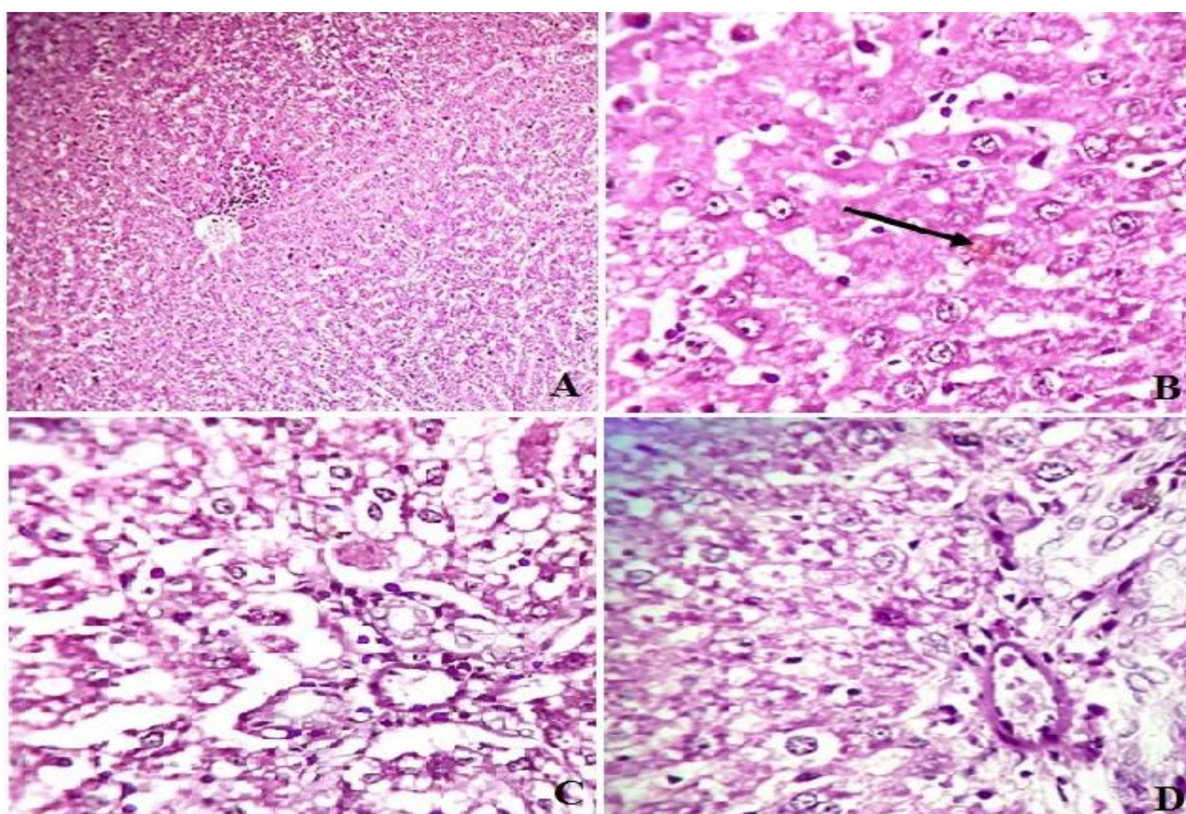


**Figure 1:** Photograph of sections of the liver showing (A) Group 1 control rat with normal architecture (B) Group II negative control showing Biliary plugging (arrow), periportal inflammation, increased karyolysis and pyknosis of nuclei (C) Group III (Lead Acetate with 300 mg/kg b.wt of *A.Indicum* AE) showing minimal inflammation. (D) Group IV (Lead Acetate with 500 mg/kg b.wt of *A.Indicum* AE) relatively normal features with usual arrangement of cells with minimal inflammation.

**Lipid Peroxidation Assay**

Group II animals treated with lead showed a significant increase in lipid peroxidation level ($p < 0.01$) in comparison to normal control group where as group III and IV animals treated with 300 mg/kg and 500 mg/kg b.wt of *A.indicum* AE along with lead acetate significantly decreased ($p < 0.01$) in lipid peroxidation levels when compared with negative control (Table 2).

**Catalase Assay**

*Abutilon indicum* AE treated rat groups showed significant increase in liver antioxidant levels when compared to negative control group II (Table 2).


© Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

Lead acetate treated rats, group II showed a significant decrease ($p < 0.01$) in the catalase activity by 42.22 % in liver homogenate when compared to normal control group I. *A.indicum* AE treated groups III and IV 300 mg/kg and 500 mg/kg b.wt resulted in increased levels of catalase activity by 33.45 % and 41.09 % respectively when compared to negative control group II animal.

### Superoxide Dismutase Assay

Superoxide dismutase (SOD) levels significantly reduced ($p < 0.01$) in group II by 79.96%, group III by 37.27% and group IV by 21.65 % in comparison to normal control group (Table 2).

However, groups III and IV; 300 mg/kg b.wt and 500 mg/kg b.wt co-administered with *A.indicum* AE and lead acetate rats showed an increased ($p < 0.01$) in SOD by 213.01 % and 290.99 % respectively when compared to negative control animals.

### Glutathione Peroxidase Assay

Glutathione peroxidise levels was significantly reduced ($p < 0.01$) in negative group II (61.71%) when compared to normal control group I.

A significant reduction ($p < 0.05$) was also observed in group III animals by 59.46 %.

However, significant increases ($p < 0.01$) in GST level were resulted by 63.96 % and 114.22 % respectively for group III and group IV *A.indicum* AE treated rats (Table 2).

### Histopathological Examinations

Figure 1 represents the histological variations on the effect of lead treatment. Normal control (Figure 1A) indicates a normal histology of liver tissue.

Liver tissue received lead acetate alone showed high level of damage in hepatocytes. Biliary plugging, periportal inflammation, congestion of blood vessels and dilated central vein, increased karyolysis and pyknosis of nuclei (sign of hepatocellular injury) were also observed in group II animals treated with lead alone.

In Group III animal periportal inflammation with more of normal hepatocytes were observed (Figure 1C).

In group IV almost normal liver architecture with minimal inflammation around portal vessels (Figure 1D) were observed.

This study was intended to assess the activity of *A. indicum* root extract on lead acetate induced liver oxidative stress in Wistar male rats. Lead poisoning is majorly due to the oxidative stress level.

The main activity of oxidative stress damage, with lead exposure implies that antioxidants may increase the efficacy of treatment deliberate to attenuate lead-induced toxicity.[12] Our finding revealed that co-administration of *A.indicum* extract reduces the lead induced liver toxicity and enhances the antioxidant parameters in rats.

## CONCLUSION

The present study provides evidence of toxicity of lead in the liver, by significant reduction in antioxidant enzymes. But, it is observed that administration of *A.indicum* at a higher dosage of 500 mg/kg bt wt helps in reducing the toxicity by increasing antioxidant enzymes such as glutathione peroxidase, superoxide dismutase and catalase and reduced levels of lipid peroxidation.

Thus it can be concluded that *A.indicum* has protective effect on lead acetate induced liver toxicity. However the molecular mechanism of the study needs to be carried out.

## REFERENCES

1. Jaishankar M, Tseten T, Anbalagan N, Mathew BB, Beeregowda KN. Toxicity, mechanism and health effects of some heavy metals, Interdiscip. Toxicol., 7(2), 2014, 60–72.
2. Flora SJS, Mittal M, Mehta A. Heavy metal induced oxidative stress & it's possible reversal by chelation therapy, Indian J. Med. Res., 128, 2008, 501–523.
3. Mishra M, Acharya UR. Protective action of vitamins on the spermatogenesis in lead-treated Swiss mice, J Trace Elem Med Biol, 18, 2004, 173–178.
4. Nandi P, Talukder G, Sharma A. Dietary factor in cancer chemoprevention, The Nucleus, 40, 1997, 128–144.
5. Rajakaruna N, Cory S, Harris, Towers GHN. Antimicrobial activity of plants collected from Serpentine outcrops in Sri Lanka, Pharmaceutical Biology, 40, 2002, 235–244.
6. Archna S, Sharma RA, Hemlata S. Phytochemical and Pharmacological Profile of *Abutilon Indicum* L. Sweet : A Review, Int. J. Pharm. Sci. Rev. Res., 20, 2013, 120-127.
7. Ohkawa H., Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction, Ana.l Biochem., 95, 1979, 351-58.
8. Marklund SL, Marklund G. Involvement of superoxide anion radical in the autooxidation of pyrogallol and a convenient assay for superoxide dismutase, Eur J Biochem., 47, 1974, 469-74.
9. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. Selenium: Biochemical role as a component of glutathione peroxidase, Science, 179, 1973, 588-90.
10. Sinha AK. Colorimetric assay of catalase, Anal. Biochem, 47, 1972, 389-94.
11. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent*, J. Biol. Chem.,* 193, 1951, 265-75.
12. Hermes-Lima M, Pereira B, Bechara EJH. Are free radicals involved in lead poisoning, Xenobiotica, 21, 1991, 1085–1090.

**Source of Support:** Nil, **Conflict of Interest:** None.

© Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.