

EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF *LEUCAS ASPERA* HYDROALCOHOLIC LEAF EXTRACT DURING EXPOSURE TO LEAD ACETATE IN MALE ALBINO WISTAR RATS

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ABSTRACT

The objective of the present study is to evaluate hepatoprotective activity of hydroalcoholic leaf extract of *Leucas aspera* on male albino wistar rats. Hepatoprotective activity of hydroalcoholic leaf extract of *Leucas aspera* on male albino wistar rats at a dose of 400 mg/kg was evaluated during exposure to lead acetate at a oral dose of 50 mg/kg. Male albino wistar rats of 6 numbers in each group was undertaken for study and evaluated by liver function test. A dose of 400 mg/kg hydroalcoholic leaf extract of *Leucas aspera* showed a significant reduction in the liver enzymes ($P < 0.05$) in the dose dependent manner. Hence the hydroalcoholic leaf extract of *Leucas aspera* have shown hepatoprotective activity.

Keywords: *Leucas aspera* leaf, Hepatoprotective activity, Liver function test, Lead acetate.

INTRODUCTION

Metals, a major category of globally-distributed pollutants, are natural elements that have been extracted from the earth and harnessed for human industry and products for millennia. Metals are notable for their wide environmental dispersion from such activity; their tendency to accumulate in selected tissues of the human body; and their overall potential to be toxic even at relatively minor levels of exposure¹.

Lead is a cumulative poison and it affects every organ and system in the body². It accumulates in bone, brain, muscle, liver, kidney, hematopoietic system, central nervous system and gastrointestinal system^{3,4,5} results in various behavioral and physiological disorders⁵. Lead-induced oxidative stress in blood and other soft tissues has been postulated to be one of the possible mechanisms of lead-induced toxic effects⁶. Disruption of pro-oxidant/antioxidant balance might lead to the tissue injury⁷.

The liver is a large and complex vital organ that functions as a centre of metabolism for nutrients such as carbohydrates, proteins, lipids and excretion of waste metabolites. Additionally, it also handles the metabolism and excretion of drugs and other xenobiotics from the body, thereby providing protection against foreign substances by detoxifying and eliminating them⁸. It plays a central role in toxicology, exposed to the ingested chemical and consequently, its potential for injury by the chemical is greater⁹.

Recently, there is a greater global interest in non synthetic, natural drugs derived from herbal sources due to their better tolerance and minimum adverse drug reactions¹⁰. *Leucas aspera* (Family: *Lamiaceae*) commonly known as 'Thumbai' is one such medicinal plant which is being used traditionally as an antipyretic and insecticide. Parts of the plant is also being used for many disorders like rheumatism, psoriasis, chronic skin eruptions, stimulant, expectorant, aperient, diaphoretic and emmenagogue. Leaves are found to possess hepatoprotectivity and hypoglycemic activities. Crushed leaves are applied locally in snake bites¹¹.

Since, there is no scientific validation for this activity, this study was undertaken to evaluate the hepatoprotective activity of *Leucas aspera*.

MATERIALS AND METHODS

Chemicals

Bovine serum albumin purchased from Sigma Chemical Co. USA. DL-aspartic acid and Lead acetate purchased from Merck, Germany. DL-alanine, Sodium pyruvate, Phenol, Folin & Ciocalteu reagent were obtained from SRL, India. α -Ketoglutaric acid was obtained from SD fine chem. Ltd., Biosar.

Sample preparation

Collection of plant material

Fresh leaves of *Leucas aspera* have been collected from Dr. ALM PG institute of basic medical sciences campus, Taramani, Chennai 600 113. After collection the leaves were dried under shade, powdered and subjected to extraction using Soxhlet apparatus.

Extraction of plant material

Pulverised powder (1 kg) was extracted with hydroalcoholic solvent (70:30) 2 litres by cold extraction process. The sample was stirred continuously and was kept for almost 72 hrs. The extract was filtered off and evaporated to dryness by rotary evaporator. The nature of the extract was dark green and with semisolid consistency. The extract was stored in a refrigerator at 4° C till further use.

ESTIMATION OF HEPATOPROTECTIVE ACTIVITY

Estimation of serum aspartate amino transferase¹²

Serum sample of volume 0.1 ml and standard pyruvate (0.2 to 0.6 micromoles) in 18x150 mm tubes were incubated with 1ml of Serum Glutamate Oxaloacetate Transaminase substrate at 37°C for 30 min. The reaction was arrested by adding 1 ml of Dinitro Phenylhydrazine and incubated at room temperature for 20 min. Blank was processed similarly, but the serum added after arresting the reaction. Absorbance of the colour developed by adding 10 ml of 0.4 N Sodium hydroxide was measured at 520nm against the blank in the spectronic 20 spectrophotometer. The serum enzyme activity expressed as IU/L.

Estimation of serum alanine aminotransferase

Serum sample of volume 0.1 ml and standard pyruvate (0.2 to 0.6 micromoles) in 18x150 mm tubes were incubated with 1ml of Serum Glutamate Pyruvate Transaminase substrate at 37°C for 30 min. The reaction was arrested by adding 1 ml of Dinitrophenyl hydrazine and incubated at room temperature for 20 min. Blank was processed similarly, but the serum added after arresting the reaction. Absorbance of the colour developed by adding 10 ml of 0.4 N Sodium hydroxide was measured at 520nm against the blank in the spectronic 20 spectrophotometer.

Estimation of serum alkaline phosphatase¹³

To 0.05 ml of serum and standard phenol (0.05- 0.2 micro mole) in 15x 150 mm test tubes, 1.5 ml of sodium carbonate - sodium bicarbonate buffer (PH 10), 1 ml of disodium phenyl phosphate and 0.1 ml of magnesium chloride were added and vortexed well. The final volume was adjusted to 3ml with distilled water, after incubation at 37°C for 15 min, 1 ml of Folin's reagent was added to arrest the reaction. Blank was processed similarly but the serum

added after arresting the reaction. The precipitate formed after addition of Folin's reagent was separated by centrifugation at 1000x g for 10 min. To the supernatant 1 ml of 20% sodium carbonate was added and left for 20 min. The intensity of blue colour was measured at 640nm.

Estimation of total protein¹⁴

Serum sample was diluted 100 times (1:100 dilution double distilled water). To 0.1ml of diluted serum sample, 5ml of Alkaline Copper Reagent was added, mixed well and after 10 minutes ,0.5ml of Folin's phenol reagent was added. The serum test blank taken separately contained the Alkaline Copper reagent & Folin's Phenol reagent was incubated on par with the test. In this tube 0.1 ml of serum was added after addition of 0.5 ml of Folin's Phenol reagent.

Reagent blank with 0.1ml of distilled water and standard with 0.1ml of Bovine Serum Albumin solution were processed similar to the sample. 30 minutes after adding Folin's phenol reagent (reagent D), the optical density was measured at 620nm using spectrophotometer. The values are expressed as grams/decilitre.

RESULTS

Estimation of serum aspartate amino transferase (AST)

When compared with day 0, Group I & III showed an increase in the mean serum AST on 21st day. Statistically analysis did not show any significance. When compared with day 0, Group II showed a statistically significant increase in the mean serum AST on 14th & 21st day (Table-I, Figure 1).

Table-I :Shows Aspartate Aminotransferase (Ast) (Iu/L)

Group	Day 0	Day 7	Day 14	Day 21
GI	108.88±4.90	106.10±7.79	100.58±7.79	110.76±5.60
GII	108.23±5.23	114.49±12.95	133.03*±9.55	146.71*±10.45
GIII	113.47±2.91	115.17±3.28	111.38±9.57	116.10±7.79

n=6, Mean ± S.d, * =P<0.05 (within groups)

Group I - *Leucas aspera* hydroalcoholic extract.

Group II - Lead acetate

Group III - Lead acetate + *Leucas aspera* hydroalcoholic extract

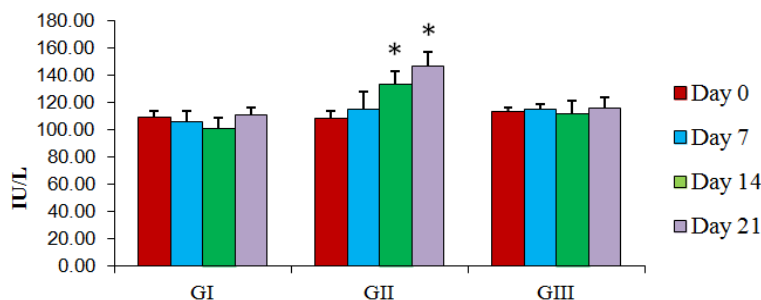


Figure 1: shows serum aspartate aminotransferase activity

Estimation of serum alanine aminotransferase (ALT)

When compared with day 0, there was an increase in the mean serum ALT in Group I at 21st day which was not statistically

significant. When compared with day 0, Group II showed a statistically significant increase in the mean serum ALT on 21st day. When compared with day 0, Group III did not show significant change in the mean serum ALT on 21st day (Table-II, Figure 2).

Table-II: Shows Alanine Aminotransferase (Alt) (Iu/L)

Group	Day 0	Day 7	Day 14	Day 21
GI	31.27±2.07	33.54±3.62	29.95±2.93	32.38±1.87
GII	32.64±2.04	33.18±1.78	33.97±1.75	43.17*±4.73
GIII	31.79±2.07	31.70±1.46	31.44±1.36	31.36±2.01

n=6, Mean ± S.d, * =P<0.05 (within groups)

Group I - *Leucas aspera* hydroalcoholic extract.

Group II - Lead acetate

Group III - Lead acetate + *Leucas aspera* hydroalcoholic extract

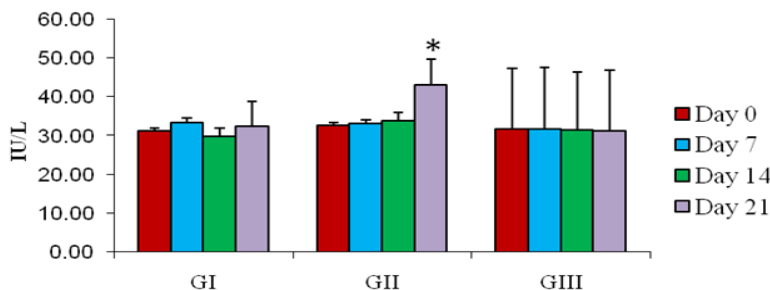


Figure 2: shows serum alanine aminotransferase activity

Estimation of serum alkaline phosphatase (ALP)

When compared with day 0, Group I showed a decrease in the mean serum ALP on 21st day which was not statistically significant. When

compared with day 0, Group II showed a statistically significant increase in the mean serum ALP on 21st day. When compared with day 0, Group III showed an increase in the mean serum ALP on 21st day which was not statistically significant (Table-III, Figure 3)

Table-III: Shows Alkaline Phosphatase (Alp) (Iu/L)

Group	Day 0	Day 7	Day 14	Day 21
GI	209.02±1.88	203.48±12.54	194.27±18.38	206.42±7.90
GII	205.06±16.90	212.68±21.06	214.26±18.82	237.61*±10.86
GIII	209.19±11.01	212.53±4.28	213.08±15.93	215.55±15.33

n=6, Mean ± S.d, * =P<0.05 (within groups)

Group I - *Leucas aspera* hydroalcoholic extract.

Group II - Lead acetate

Group III - Lead acetate + *Leucas aspera* hydroalcoholic extract.

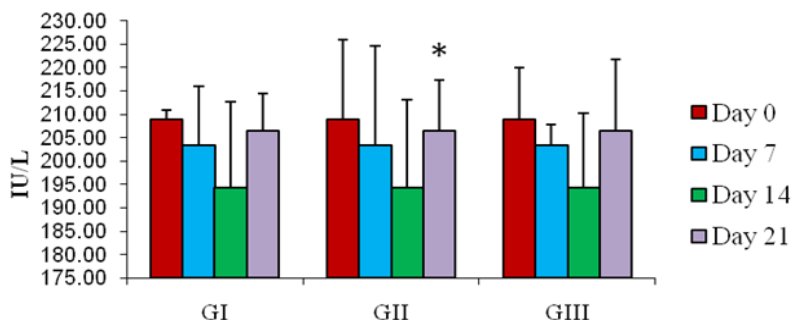


Figure 3:shows serum alkaline phosphatase activity

Estimation of total protein

When compared with day 0, Group II showed a statistically significant decrease in the mean serum Total protein on 14th & 21st

day. When compared with day 0, Group I & III showed a decrease in the mean serum total protein. Statistical analysis did not show any significant difference (Table-IV, Figure 4).

Table 4 :Shows Total Protein (G/Dl)

Groups	Day 0	Day 7	Day 14	Day 21
GI	7.91±1.21	7.33±0.48	7.17±1.65	7.3±0.99
GII	7.92±1.04	6.93±1.53	5.53±1.56	4.8±1.45
GIII	7.88±1.09	7.06±0.93	7.02±1.15	7.33±0.74

n=6, Mean ± S.d, * =P<0.05 (within groups)

Group I - *Leucas aspera* hydroalcoholic extract.

Group II - Lead acetate

Group III - Lead acetate + *Leucas aspera* hydroalcoholic extract

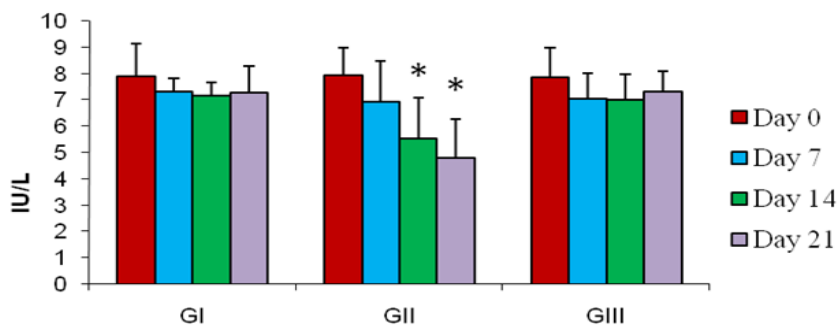


Figure 4 :shows serum total protein

DISCUSSION

Biochemical parameters carried out in the present investigation to evaluate the hepatic functions during exposure to lead acetate is clearly indicative of the metallic salt having an adverse effect.

Apart from these, lead acetate has been found to have an adverse influence on other tissues also as indicated by an increase in serum Aspartate aminotransferase (AST) which is found to increase in cardiac tissue compared to hepatic and other tissues. Exposure for 14-21 days has resulted in significant increase in serum AST. As the transaminase is an intracellular enzyme, the increase could be due to cellular damage in the cardiac tissue releasing this enzyme. Co-administration of hydroalcoholic leaf extract of *Leucas aspera* is found to suppress this effect of lead acetate as no significant increase was seen even up to 21 days of exposure to lead acetate.

Hepatic tissue seems to be more vulnerable to the toxic impact of lead acetate especially during continuous exposure for a longer time. This is exemplified by the observation of serum ALT it shows an increasing trend with the maximum activity recorded after 21 days of exposure. Similarly serum Alkaline phosphatase was also found to show a duration dependent increase during exposure to lead acetate with a maximum enzyme activity recorded after 21 days of exposure since an increase in serum ALT and ALP are regarded as indicators of hepatic damage, it can be inferred that exposure to lead acetate causes hepatic damage. As these two enzymes were also intracellular in nature.

Hydroalcoholic leaf extract of *Leucas aspera* seems to possess a protective effect on the toxic influence of lead acetate over hepatic tissue since there was no significant increase of these two enzymes

even after 21 days during co-administration of the leaf extract along with lead acetate.

Hepatoprotective effect of *Leucas aspera* is further exemplified by the result of serum total protein estimated in the present study. While a duration dependent decrease in serum total protein was seen which is more significant after 14-21 days of exposure to lead acetate. Co-administration of *Leucas aspera* is found to suppress the influence of the metal as seen by the absence of any alterations in serum total protein after 7-21 days of exposure.

CONCLUSION

The Hydroalcoholic leaf extract of *Leucas aspera* has found to possess hepatoprotective property by suppressing the abnormal elevation of liver enzymes induced by lead acetate. The foregoing discussion indicates that the leaf extract has got suppressive effects on the influence of lead acetate and the parameter chosen for the present study namely biochemical parameters related to hepatic function. Thus, the plant seems to possess medicinal constituents that can overcome the toxic potential of lead which is a common environmental pollutant.

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