

## ORIGINAL RESEARCH ARTICLE

**Effect of Hydroalcoholic Leaf Extract of *Leucas aspera* on Lead Acetate Induced Alterations in Membrane Bound Enzymes****M.Thenmozhi<sup>1</sup>, M.Dhanalakshmi<sup>\*1</sup>, K.Susila<sup>2</sup>, S.Thenmozhi<sup>1</sup>, K.Manjula Devi<sup>1</sup>**<sup>1</sup>Department of Pharmacy, Swamy Vivekanandha College of Pharmacy, Tiruchengode, Namakkal, Tamil Nadu, India<sup>2</sup>Department of Pharmacy, Enoah I-solution, Taramani, Chennai, Tamil Nadu, India

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**ABSTRACT**

The objective of the present study is to evaluate the protective effect of hydroalcoholic leaf extract *Leucas aspera* on membrane bound enzyme (serum ATPase) levels on male albino wistar rats. The membrane bound enzymes (serum ATPase) levels 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 were undertaken for the study and evaluated by the Oxidative stress test. A dose of 400 mg/kg hydroalcoholic leaf extract of *Leucas aspera* showed a significant increase in the membrane bound enzymes ( $P < 0.05$ ) in the dose dependent manner. Hence the hydroalcoholic leaf extract of *Leucas aspera* have shown protective effect on the membrane bound enzyme levels by suppressing lead acetate induced toxic effects.

**Keywords:** *Leucas aspera*, Membrane bound enzymes, Oxidative stress test, Lead acetate.**INTRODUCTION**

It has been known since ancient times that heavy metals may cause poisoning in man. Heavy metals are trace metals that are taken into the body via inhalation, ingestion and skin absorption. Lead and other heavy metals create reactive radicals which damage cell structure including DNA and cell membrane [1]. Lead is a natural element and widespread in the environment. The two major routes of lead entry into the body are the alimentary and respiratory tracts [2]. Lead poisoning may affect numerous organ systems and is associated with a number of morphological, biochemical and physiological changes, including kidney dysfunction, abnormal glucose metabolism, nervous system disturbances, impairment of liver function and hematological disorders [3-5]. Excess lead is known to reduce the cognitive development and intellectual performance in children. Hyperglycemia is one of the signs associated with lead poisoning [6]. Lead is reported to have an inhibitory action on the membrane bound enzymes such as Sodium-Potassium, Calcium and Magnesium adenosine triphosphatase ( $\text{Na}^+ - \text{K}^+$ ,  $\text{Ca}^{2+} + \text{Mg}^{2+}$ )ATPase in various vital organs [7]. Any alteration in

membrane lipid leads to change in membrane fluidity, which in turn alters the ATPase activities and cellular functions [8]. The decrease in the levels of  $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ ,  $\text{Ca}^{++} - \text{ATPase}$ , and  $\text{Mg}^{++} - \text{ATPase}$  could be due to enhanced lipid peroxidation by free radicals in lead-treated animals [9]. Herbs are staging a comeback and herbal renaissance is happening all over the globe. The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment [10]. *Leucas aspera* is one such herb that is found to reduce the metabolic effects of sugar and hence it is being used in traditional medicine for treating diabetes [11]. Medicinally, it has been proven to possess various pharmacological activities like antifungal, antioxidant, antimicrobial, antinociceptive and cytotoxic activity and as an antidote to snake venom [12]. Since there is no previous study available to validate this research, the present study was undertaken to examine the influence of *Leucas aspera* in lead acetate induced alterations in serum ATPases ( $\text{Na}^{(+)} - \text{K}^{(+)}$ ,  $\text{Ca}^{(2+)}$  &  $\text{Mg}^{(2+)}$  ATPase).

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## MATERIALS AND METHODS

### Chemicals

Bovine serum albumin purchased from Sigma Chemical Co. USA. Tris-HCl Buffer Ethylene diamine tetra acetic acid, Adenosine Triphosphate substrate, Trichloroacetic acid, Ammonium molybdate, 1-amino-2-naphthol sulphonic acid (ANSA) & Folin's reagent were purchased from Sisco Research Laboratories Pvt. Ltd, Mumbai. Lead acetate purchased from Merck Laboratories Pvt.Ltd.

### 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 OXIDATIVE STRESS PARAMETERS:

### a) Estimation of Na<sup>+</sup>-K<sup>+</sup>-ATPase<sup>[13,14]</sup>

Serum sample of volume 0.1 ml taken in centrifuge tubes was incubated in a medium containing 1.5 ml Tris-HCl buffer, 0.1 ml each NaCl, KCl, MgSO<sub>4</sub>, EDTA and 0.1 ml ATP for 30 min at 37°C. The reaction was arrested by the addition of 1.0 ml of 10% TCA. The test blank taken separately contained the buffer, NaCl, KCl, MgSO<sub>4</sub>, EDTA and ATP was incubated on par with the test. In this tube 0.1 ml of serum was added after addition of 1.0 ml of TCA. The precipitate formed on addition of TCA in both the test and test blank tubes was removed by centrifugation and the supernatant was transferred to fresh tubes. The reagent blank contained 3.1 ml of Tris-HCl buffer. The standard (KH<sub>2</sub>PO<sub>4</sub>) taken at a concentration range of 4 to 12 µg were placed in distilled water and were made upto 3.1ml with Tris-HCl buffer. To all the above tubes, 1 ml of ammonium molybdate and 0.4 ml of ANSA was added and left for 20 min for the development of

blue colour, which was read at 620 nm against the reagent blank using spectrophotometer. The activity of Na<sup>+</sup>-K<sup>+</sup>-ATPase in the serum was expressed as µmoles of phosphorous liberated/min/mg protein.

### Estimation of Mg<sup>2+</sup>-ATPase<sup>[14, 15]</sup>

Serum sample of volume 0.1 ml taken in a centrifuge tubes was incubated in a medium containing 1.5 ml of Tris-HCl buffer, 0.1ml of MgCl<sub>2</sub> and 0.1 ml of ATP for 30 min at 37°C. The reaction was arrested by the addition of 1.0 ml of 30% TCA. The serum (test) blank taken separately contained the buffer, MgCl<sub>2</sub> and ATP was incubated on par with the test. In this tube 0.1 ml of serum was added after addition of 1.0 ml of TCA. The precipitate formed on addition of TCA in both the test and test blank tubes was removed by centrifugation and the supernatant was transferred to fresh tubes. The reagent blank contained 2.8 ml of Tris-HCl buffer. The standard (KH<sub>2</sub>PO<sub>4</sub>) taken at a concentration range of 4 to 12 µg were placed in distilled water and were made upto 2.8ml with Tris-HCl buffer. To all the above tubes, 1 ml of ammonium molybdate and 0.4 ml of ANSA was added and left for 20 min for the development of blue colour, which was read at 620 nm against the reagent blank using spectrophotometer. The activity of Mg<sup>2+</sup>-ATPase in the serum was expressed as µmoles of phosphorous liberated/min/mg protein.

### Estimation of Ca<sup>2+</sup>-ATPase<sup>[14, 16]</sup>

Serum sample of volume 0.1 ml taken in centrifuge tubes was incubated in a medium containing 1.5 ml Tris-HCl buffer, 0.1 ml CaCl<sub>2</sub> and 0.1 ml ATP for 30 min at 37°C. The reaction was arrested by the addition of 1.0 ml of 20% TCA. The test blank taken separately contained the buffer, 0.1 ml CaCl<sub>2</sub> and ATP was incubated on par with the test. In this tube 0.1 ml of serum was added after addition of 1.0 ml of TCA. The precipitate formed on addition of TCA in both the test and test blank tubes was removed by centrifugation and the supernatant was transferred to fresh tubes. The reagent blank contained 2.8 ml of Tris-HCl buffer. The standard (KH<sub>2</sub>PO<sub>4</sub>) taken at a concentration range of 4 to 12 µg were placed in distilled water and were made upto 2.8ml with Tris-HCl buffer. To all the above tubes, 1 ml of ammonium molybdate and 0.4 ml of ANSA was added and left for 20 min for the development of blue colour, which was read at 620 nm against the reagent blank using spectrophotometer. The activity of Ca<sup>2+</sup>-ATPase in the serum was

expressed as  $\mu\text{moles}$  of phosphorous liberated/min/mg protein.

## RESULTS

### a) Estimation of $\text{Na}^+\text{-K}^+\text{-ATPase}$

There was an increase in the mean  $\text{Na}^+\text{-K}^+\text{-ATPase}$  level in G-I on day 14 & 21, but statistical

analysis did not show any significant decrease. G-II & G-III showed a statistically significant decrease in the mean  $\text{Na}^+\text{-K}^+\text{-ATPase}$  level on day 14 & 21 when compared with baseline (day 0) (Table 1).

**Table 1: Mean Serum Na-K ATPase ( $\mu\text{m}$  of inorganic phosphate liberated/mg of protein/min)**

Groups	Day 0	Day 7	Day 14	Day 21
Group-I	0.052 $\pm$ 0.009	0.052 $\pm$ 0.009	0.058 $\pm$ 0.008	0.058 $\pm$ 0.009
Group-II	0.061 $\pm$ 0.008	0.047 $\pm$ 0.009	0.035 $\pm$ 0.009*	0.024 $\pm$ 0.009*
Group-III	0.065 $\pm$ 0.009	0.041 $\pm$ 0.008*	0.042 $\pm$ 0.008*	0.045 $\pm$ 0.002*

\* Within groups ( $p > 0.05$ )  $n = 6$ ; Values are expressed as mean  $\pm$ SD

### b) Estimation of $\text{Mg}^{2+}\text{-ATPase}$

G-II showed a statistically significant decrease in  $\text{Mg}^{2+}\text{-ATPase}$  level on day 21 when compared with baseline (day 0). In G-I & G-III, there was an

increase in mean  $\text{Mg}^{2+}\text{-ATPase}$  level on 21<sup>st</sup> day but statistical analysis did not show any significant decrease (Table 2).

**Table 2: Mean Serum Mg ( $\mu\text{m}$  of inorganic phosphate liberated/mg of protein/min)**

Groups	Day 0	Day 7	Day 14	Day 21
Group-I	0.045 $\pm$ 0.009	0.052 $\pm$ 0.012	0.060 $\pm$ 0.013	0.062 $\pm$ 0.013
Group-II	0.042 $\pm$ 0.008	0.041 $\pm$ 0.007	0.041 $\pm$ 0.003	0.030 $\pm$ 0.006*
Group-III	0.045 $\pm$ 0.010	0.045 $\pm$ 0.013	0.058 $\pm$ 0.014	0.058 $\pm$ 0.011

\* Within groups ( $p > 0.05$ )  $n = 6$ ; Values are expressed as mean  $\pm$ SD

### c) Estimation of $\text{Ca}^{2+}\text{-ATPase}$ ( $\mu\text{m}$ of inorganic phosphate liberated/mg of protein/min)

G-II showed a statistically significant decrease in  $\text{Ca}^{2+}\text{-ATPase}$  level on day 21 when compared

with baseline (day 0). In G-I & G-III, there was an increase in mean  $\text{Ca}^{2+}\text{-ATPase}$  level on 21<sup>st</sup> day, but statistical analysis did not show any significant decrease (Table 3).

**Table 3: Mean serum  $\text{Ca}^{2+}\text{-ATPase}$  ( $\mu\text{m}$  of inorganic phosphate liberated/mg of protein/min)**

Groups	Day 0	Day 7	Day 14	Day 21
Group-I	0.025 $\pm$ 0.009	0.025 $\pm$ 0.001	0.026 $\pm$ 0.006	0.029 $\pm$ 0.003
Group-II	0.026 $\pm$ 0.007	0.024 $\pm$ 0.004	0.019 $\pm$ 0.006	0.015 $\pm$ 0.003*
Group-III	0.020 $\pm$ 0.006	0.016 $\pm$ 0.008	0.028 $\pm$ 0.005	0.032 $\pm$ 0.007

\* Within groups ( $p > 0.05$ )  $n = 6$ ; Values are expressed as mean  $\pm$ SD

## DISCUSSION

Lead is one of the most ubiquitous toxic materials encountered in everyday life. At present lead is commonly used for commercial purposes and many household products. Lead poisoning is one of the oldest occupational and environmental diseases in the world. Despite its recognized hazards, lead continues to have widespread commercial application. The highest level of exposures of lead occurs principally among people working in lead smelters.

Herbal plants have been used to overcome toxic manifestations of metals in Indian Traditional System of Medicine. In the present study *Leucas aspera* has been screened for its influence on lead induced biological changes with respect to selected parameters like serum ATPases.

ATPases are membrane bound enzymes which play a vital role in cellular level. These are the most sensitive enzymes for any toxic effect. The decrement in the enzyme activity is indication of

cellular dysfunction. In the present study,  $\text{Na}^+\text{-K}^+\text{-ATPase}$  had been found to be suppressed during daily exposure to lead acetate. The decrement in the enzyme activity is found to be statistically significant after 14 & 21 days of exposure. This effect of lead acetate has been found to be suppressed on co-administration of hydroalcoholic extract of *Leucas aspera*. However the effect of lead acetate has not been totally overcome by administration of *Leucas aspera* since, the significant reduction in enzyme activity is seen after 14 to 21 days of exposure.

Influence of lead acetate was reflected on serum  $\text{Mg}^{2+}\text{-ATPase}$  also which was shown by gradual reduction which is significant after 21 days of exposure. However this impact of lead acetate has been found to be annealed by the extract since co-administration of extract along with lead acetate has not shown any reduction in  $\text{Mg}^{2+}\text{-ATPase}$  level. Similar to  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}\text{-ATPase}$  was also been found to be suppressed by lead acetate. Significant

reduction has been seen after 21 days of exposure. This inhibitory effect of lead acetate on Serum ATPases, was found to be overcome by hydroalcoholic extract of *Leucas aspera*. The membrane bound enzymes important for conduction of nerve impulses and oxidative phosphorylation. Thus *Leucas aspera* seems to have suppressive effect on lead acetate induced alterations of serum ATPase activity, the usefulness of *Leucas aspera* in combating these effects observed in the present study can have therapeutic implications.

## CONCLUSION

*Leucas aspera* increases serum ATPase level and also seems to possess protective effect on the membrane bound enzymes by suppressing lead acetate induced toxic effects. 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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