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# Hepatoprotective activity of aqueous extract of roots of *Antegonone leptopus* against Paracetamol induced hepatotoxic albino rats

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**ABSTRCT**

This study has been planned to find out hepatoprotective activity of aqueous extract of roots of *A. leptopus* (AERAL) against paracetamol induced hepatotoxicity in albino rats. The extract was analyzed for presence of phytochemicals and Gallic acid is used as standard to estimate the total phenolic content. The three doses of (AERAL) i.e., low (125 mg/kg), medium (250 mg/kg) and high (500 mg/kg) were selected to test hepatoprotective activity in hepatotoxic rats. The paracetamol (2g/kg p.o.) and Silymarin (50 mg/kg) were used as toxicant and standards in this study. The hepatotoxicity was induced by administration of paracetamol in rats. The biochemical parameters (AST, ALT, ALP and total Bilirubin) and physical parameters (liver weight and volume) were estimated. In this study AERAL has exhibited a significant hepatoprotection activity by maintaining the biochemical and physical parameters.

**KEY WORDS**: Hepatoprotective, *Antigonone leptopus*, aqueous extract, roots, rats.

## 1. INTRODUCTION

The liver is the largest glandular organ regulating homeostasis in the body and responsible for biochemical pathways, immunity, nutrition supply and energy production. It is responsible for detoxification of a variety of drugs and xenobiotics metabolized in liver. Even though, the scientific advancement was achieved, liver disorders were not constrained. Number of deaths was reported due to two major hepatic diseases like Jaundice and hepatitis.

Liver is the most important organ to metabolize all foreign substances, due to which, a variety of disorders may occur predominantly to it such as hepatisis, cirrhosis, liver cancer and drug induced hepatic diseases. Exposure of different pollutants of environment and xenobiotics like paracetamol, $CCl_4$, thioacetamide and alcohol etc., to liver causes damage mainly by releasing reactive oxygen species (ROS). The continuous aerobic life is exposed to sustained release of free radicals particularly ROS which are very dangerous for living organisms. The reactive species damage biomolecules and induce dangerous alterations in DNA, proteins, lipids and imply age related diseases. Lipid peroxidation is produced by ROS through covalent bonding that causes tissue injury. Antioxidants scavenge released free radicals and prevent fibrosis process and protect living tissues or organs. Free radicals are gets involved in progressive decrease in potentiality of immune system.

The allopathic hepatoprotective drugs are inadequate and also produce serious adverse effects, this made the thrust to explore the traditional medicinal plants and found herbal based natural products are more useful to cure disorders of liver. The phenolic compounds that are present in plant products are known to be excellent antioxidants to scavenge the liberated free radicals and protect the living organisms from a variety of disorders.

*Antigonon leptopus* Hook. et Arn (syn: *Corculum leptopus* family: Polygonaceae) is grown in parks and gardens throughout India. It is most commonly grows in the upper Ganges plains and Himalayan regions and Mexico and commonly found in tropical Asia, Africa, the Caribbean and the Americas. It is a fast growing climber, heart shaped green leaves, flowers throughout the summer to autumn with coral pink to red flowers hanging in panicles up to 15 cm long and will climb up to 40 ft. *A.leptopus* plant reported with anti-thrombin, analgesic and anti-inflammatory and antilipid peroxidation activities. Traditionally, *A. leptopus* is used as hepatoprotective and also used in the treatment of diabetes, asthma, liver and spleen disorders, and conditions of cough and throat constriction.

In the present study an attempt was made to evaluate the hepatoprotective activity of AERAL against paracetamol induced hepatotoxicity in rats. AERAL (500 mg/kg) has shown a significant hepatoprotective activity by decreasing AST, ALT, ALP and total Bilirubin against paracetamol (2g/kg) induced hepatic injury. Paracetamol administration (2g/kg) for 03 days) resulted in increased ALT, AST, ALP, Bilirubin levels and liver weight and liver volumes.

## 2. EXPERIMENTAL SECTION

**Preparation of Aqueous Extract:** The roots of *A.leptopus* were collected from fields of Andhra University, Visakhapatnam in the month of June-July and dried under sunshade. The roots of *A.leptopus* were washed thoroughly with water to clean the adhering dirt and sandy material, made into small pieces, dried under shade and powdered. Coarse powder was extracted with chloroform water by maceration technique under reduced pressure and the concentrated extract was obtained by using rotary evaporator. AERAL was in dark brown coloured solid mass and yield was 11.13 % w/w.

According to the OECD 423 guidelines the acute toxicity studies were conducted in mice (15-20 g) with the limit test dose of 2000 mg/kg and no death was reported at this highest dose level.

**Determination of Total Phenolic Compounds:** The determination of total phenolic compounds in AERAL is estimated by spectrophotometric method. Extract prepared in the form of methanolic solution with the concentration of 1 mg/ml was used in this experiment. 0.5ml of methanolic solution and 2.5ml of 10% Folin-Ciocalteu's reagent were mixed initially and then diluted with water. Finally 7.5% $NaHCO_3$ was added to the mixture. The samples were carried for incubation at 45ºC for 45 minutes. Spectrophotometer was used to find out absorbance of the samples. Absorbance was carried out at a wavelength of 765 nm. The analysis was carried out in triplicate. Calibration curve for Gallic acid was drawn following the above said procedure.

**DPPH scavenging method**: Free radical scavenging activity of DPPH was measured by following the procedure given by Braca (2003). The solutions were prepared by mixing 3 ml of 0.0004% DPPH in ethanol and 0.1ml of AERAL in different concentrations. The mixture was allowed to stand for 30 minutes at room temperature with intermittent vigorous shaking. Decolourization of DPPH was observed at the absorbance of 517 nm. A control was prepared in the place of AERAL /ascorbic acid using 0.1 ml of respective vehicle. The percentage inhibition activity was calculated as $A_0$-$A_1/A_0 \times 100$

Where $A_0$ is the absorbance of control; $A_1$ is the absorbance of the AERAL /ascorbic acid.

Inhibition % = $A_0$-$A_1/A_0 \times 100$

$A_0$ is the absorbance control and $A_1$ is the absorbance with AERAL/ Ascorbic acid.

**The superoxide free radical scavenging activity:** The superoxide scavenging activity of the AERAL was determined by Mc Cord and Fridovich method, 1969, which depends on light induced superoxide generation by riboflavin and the corresponding reduction of nitroblue tetrazolium. 0.1 ml of different concentrations of AERAL (50, 100, 250, 500, 750 & 1000 µg) and 0.1 ml of 6 µM ethylenediamine tetraacetic acid containing NaCN, 0.1 ml of nitroblue tetrazolium, 0.05 ml of 2 µM riboflavin were transferred to a test tube, and final volume made up to 3 ml using phosphate buffer. Then the assay tubes were uniformly illuminated with an incandescent light (40 Watts) for 15 min and thereafter the optical densities were measured at 560 nm. A control was prepared in the place of AERAL /Ascorbic acid using 0.1 ml of respective vehicle. The percentage inhibition of superoxide production was evaluated by comparing the absorbance values of control and experimental tubes. Inhibition % = $A_0$-$A_1/A_0 \times 100$

$A_0$ is the absorbance control and $A_1$ is the absorbance with AERAL / Ascorbic acid.

**Acute Toxicity Studies:** The extract was subjected for acute toxicity studies at limit test dose of 2000 mg/kg according to OECD 423 guidelines. The mice were fasted and administered the plant extract as per the procedure and observed for mortality for 24 hrs.

**Experiment:** The albino rats (150-250g) were randomly selected and divided into 6 groups, each consists of six animals. They were maintained under standard conditions (Room temperature at 25±2$^0$C, 12 h light/dark and free access to food along with water up to 2 weeks before the experiment to adapt to laboratory conditions. The animals were deprived of food for 18 h and water allowed *ad libitum* prior to experiment.

Group A: Received vehicle for 10 days.

Group B: Received paracetamol (2 mg/kg p.o.) for first 3 days and vehicle for from 4$^{th}$ day to 10$^{th}$ day.

Group C: Received paracetamol (2 mg/kg p.o.) for first 3 days and Silymarin (100 mg/kg) from day 4 to 10$^{th}$ day.

Group D: Received paracetamol (2 mg/kg p.o.) for first 3 days and AERAL 125 mg/kg from day 4 to 10$^{th}$ day.

Group E: Received paracetamol (2 mg/kg p.o.) for first 3 days and AERAL 250 mg/kg from day 4 to 10$^{th}$ day.

Group F: Received paracetamol (2 mg/kg p.o.) for first 3 days and AERAL 500 mg/kg from day 4 to 10$^{th}$ day.

**Estimation of Biochemical Parameters**: The blood samples collected by rupturing retro-orbital plexus and centrifuged at 10000 rpm for 15 min. The serum was analyzed for biochemical parameters such as AST, ALT, ALP and total bilirubin. After collection of blood samples thiopental sodium (40 mg/kg) was administered and recorded for its sleeping time. Then the animals were sacrificed and wet, dry liver weights were recorded.

**Statistical Analysis**: All the experimental data was subjected to one-way analysis of variance (ANOVA) followed by Dunnett's't' test and P < 0.05*, P < 0.01**, P < 0.001***, were considered as statistically significant.

**Table1. Effect of AERAL on Biochemical & Physical Parameters in albino rats**

| Treatment | Biochemical parameters | | | | Physical parameters | |
|---|---|---|---|---|---|---|
| | AST (U/L) | ALT (U/L) | ALP (U/L) | T.BIL (mg/dl) | Liv. Wt. (g/100g) | Liv. Vol. (g/100g) |
| Group A | 84.69±0.73 | 44.72±0.89 | 125.80±0.93 | 0.42±0.01 | 3.29±0.02 | 3.44±0.04 |
| Group B | 369.10±1.52** | 322.50±1.51** | 408.67±0.96** | 3.17±0.03** | 5.93±0.06** | 6.76±0.12** |
| Group C | 84.85±1.62** | 43.82±1.53** | 127.45±1.60** | 0.44±0.0**1 | 3.26±0.03** | 3.73±0.05** |
| Group D | 216.98±1.01 | 140.19±1.08 | 281.31±0.98 | 2.89±0.01 | 5.28±0.03 | 6.17±0.04 |
| Group E | 170.55±4.08 | 110.16±1.70 | 204.89±1.62 | 2.09±0.03 | 4.65±0.02 | 5.71±0.02 |
| Group G | 97.90±1.16** | 62.20±0.61** | 138.47±1.07** | 0.62±0.01** | 4.06±0.06** | 4.48±0.04** |

n=6, Values are expressed as mean± SEM. Significant at P < 0.05*, P < 0.01**, P < 0.001***

**Table.2. Effect of AERAL on sleeping time in rats**

| Group | Time in min |
|---|---|
| Noraml | 52.83±1.20 |
| Toxicant | 256.66±1.24** |
| Sylimarin | 55.66±9.95** |
| AERAL 125 mg/kg | 222.36±2.63 |
| AERAL 250 mg/kg | 103.65±1.64** |
| AERAL 500 mg/kg | 67.35±1.54** |

n=6 Values are expressed as mean± SEM. Significant at P < 0.05*, P < 0.01**, P < 0.001***

## 3. RESULTS

The phytochemical instigation revealed that the AERAL contain phenolic compounds, flavonoids, tannins, glycosides, carbohydrates and saponins.

The Folin-Ciocalteu's reagent is used to determine total phenolic content present in the extract and expressed in terms of gallic acid equivalent (Y=0.097x+0.042 , $R^2$=0.997). The phenolic content of extract was 5.66 mg of GA/g of extract.

The antioxidant property of AERAL against DPPH was given in Fig.1. The dose dependent antioxidant activity of AERAL and Ascorbic acid was observed in the dose range of 50 µg to 1000 µg and $IC_{50}$ values were 130.25 and 185.65 µg/ml respectively.

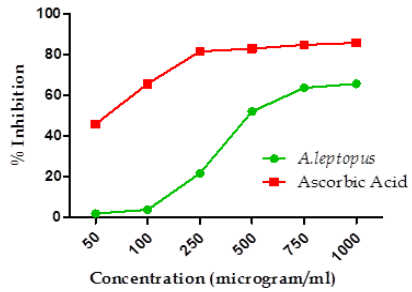


**Figure.1. DPPH free Radical Scavanging activity**

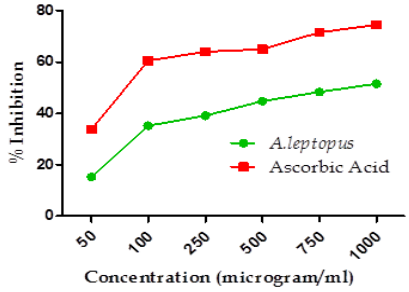


**Figure.2. Superoxide free Radical Scavanging activity**

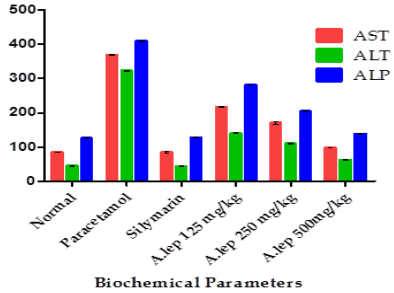


**Figure.3. Effect of AERAL on Biochemical parameters**

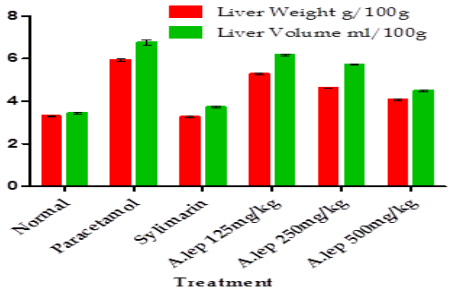


**Figure.4. Effect of AERAL on Physical parameters**

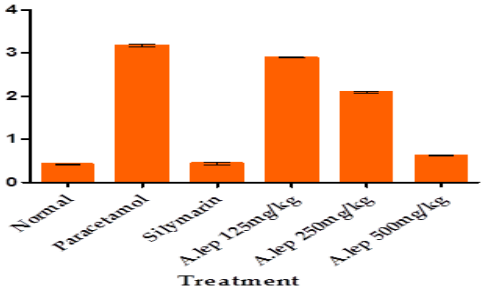


**Figure.5. Effect of AERAL on Bilirubin**

The antioxidant property of AERAL against superoxide radical was given in Fig.2. A dose dependent antioxidant activity of AERAL and Ascorbic acid was observed in the dose range of 50 µg to 1000 µg and $IC_{50}$ values were 135.65 and 204.98 µg/ml respectively.

The results of hepatoprotective activity of AERAL against paracetamol intoxicated rats are shown in Table.1. The biochemical parameters ALT (322.50±1.51 U/L), AST (369.10±1.52 U/L), ALP (408.67±0.96 U/L) and bilirubin (3.17±0.03 mg/dl) and physical parameters such as liver weight (5.93±0.06 g/100g) and liver volume (6.76±0.12 g/100g) were significantly increased in paracetamol treated rats when compared to normal group.

The increased biochemical parameters such as ALT (62.20±0.61 U/L), AST (97.90±1.16 U/L), ALP (138.47±1.07U/L), bilirubin (0.62±0.01 mg/dl) and physical parameters such as liver weight (4.06±0.06 g/100g) and liver volume (4.48±0.04 g/100g) were significantly decreased against paracetamol treated rats after treatment with AERAL and a significant hepatoprotective activity was observed with AERAL.

Paracetamol received group has shown a prolonged thiopental sodium induced sleeping time (256.66±1.24 min) when compared to normal (52.83±1.20 min) and it has been reversed with silymarin (55.66±9.95 min) and AERAL treated groups (67.35±1.54 min)

**DISCUSSION**

The preliminary phytochemical analysis revealed that the AERAL contains glycosides, carbohydrates and phenolic compounds such as flavonoids, Tannins. Paracetamol metabolized in the hepatocytes by cytochrome P-450 into hepatotoxin, N-acetyl-P-benzoquinoneimine (NAPQI), which causes oxidative stress and excessive release of NAPQI produces depletion of glutathione. Further NAPQI covalently binds to cellular macromolecules and initiate cell damage. Paracetamol is widely used as an analgesic & antipyretic drug which causes liver necrosis at toxic doses. Induction of cytochrome or depletion of glutathione is an indication for paracetamol induced hepatotoxicity.

The acute paracetamol poisoning is one of the leading causes of liver failures in developed countries. The toxic levels of paracetamol induce hepatic necrosis which can be fatal. Hepatocytes damage due to hepatotoxicants leads to leakage of enzymes such as AST, ALT and ALP. ALT is the specific enzyme for liver and important enzyme for detection of liver damage. The increased serum enzyme and bilirubin levels as these biochemicals are released into blood circulation due to damaged structural integrity of hepatocytes, an indication of liver toxicity.

Bilirubin is the important clinical parameter of fatal necrosis and its accumulation is a measure of binding, conjugation and excretory capacity of hepatocytes. Reduced serum bilirubin after treatment with AERAL against hepatotoxicity induced by paracetamol, indicated the effectiveness of the AERAL as a hepatoprotectant in restoring the normal function of the liver.

The measurement of thiopental sodium induced sleeping time is the indication of activity of hepatic enzymes (CYP's). The free radicals released from metabolites of xenobiotics cause damage to components of cell including CYP's. During destruction of hepatic CYP's thiopental sodium induced sleeping time is prolonged which is the fair indication of hepatic destruction. The hepatoprotective drugs prevent damage of CYP's and decreases prolongation sleeping time of thiopental sodium. The AERAL has reversed thiopental sodium induced sleeping time by protecting hepatic CYP 420 family.

**4. CONCLUSION**

The AERAL has showed a dose dependent hepatoprotective activity and more significant effect was observed at a dose of 500 mg/kg. The hepatoprotective activity may be due to the presence of phytoconstituents like phenolic and flavonoids that are present in the AERAL and further these chemical constituents are already reported for their hepatoprotective activities.

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