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# PROTECTIVE EFFECT OF ALPHA-LIPOIC ACID ON HEPATO RENAL TOXICITY ON ACETAMINOPHEN INDUCED UREMIC MALE ALBINO RAT.

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## ABSTRACT
Hepatic toxicity can be produced by overdoses of acetaminophen, it-induced liver necrosis and renal insufficiency. This study was designed to investigate the protective effect of alpha-lipoic acid (ALA) on hepato-renal toxicity on rats induced by acetaminophen. There was a significant increase in plasma ALT, AST, ALP and malondialdehyde levels ($p< 0.05$) but a significant decrease in super oxide dismutase and catalase activity in hepatotoxic group (Group II) was compared with control group (Group I) and which are resettled to the ALA treated group (Group III and IV). In conclusion it established that ALA at high doses group (Group IV) has better hepatoprotective activities by different biochemical and histological observations.

**KEYWORDS:** Acetaminophen (APAP), Alpha Lipoic Acid (ALA), Hepato-renal toxicity.

## 1. INTRODUCTION
Acetaminophen (N-acetyl-p-aminophenol; APAP), also known as paracetamol, is the most widely used analgesic and antipyretic medication in the world, which has no adverse effect at therapeutic dosages.[1] But in high doses of APAP causes hepatic necrosis and renal failure in both humans and animals.[2, 3] The prime toxicity of APAP is the result of drug metabolism both in the liver and in extrahepatic tissues.[4] The initial step of its toxicity is formation of the reactive intermediate N-acetyl-p-benzoquinone imine (NAPQI) by cytochorom P450 which at therapeutic doses is removed by conjugation with glutathione solfidryle (GSH). At therapeutic doses, APAP is metabolized via glucuronidation and sulfuration reactions occurring primarily in the liver, and results in water-soluble metabolites that are excreted via the kidney. As a result of metabolic conversion of APAP by the microsomal P-450 enzyme system, a highly reactive intermediate, N-acetyl-p-benzoquinone imine (NAPQI) is produced. This electrophilic metabolite is then reduced by glutathione (GSH) and subsequently excreted as mercapturic acid, a relatively benign compound.[5] High doses of acetaminophen results in the depletion of stored sulfate and GSH. This shunts the excess amount of APAP to the CYP-450 mixed function oxidase system, generating more of the reactive intermediate NAPQI. When large doses of APAP are ingested, there is severe GSH depletion and massive production of metabolites; this compounds the toxicity, leaving large amounts of reactive species unbound. These electrophilic intermediates then form covalent bonds to cellular protein macromolecules leading to hepatocellular injury.[5] This process disrupts homeostasis and initiates apoptosis, or programmed cell death, leading to liver tissue necrosis and, ultimately, organ dysfunction.[6, 7] Alpha Lipoic Acid (ALA) is a naturally occurring antioxidant and plays a fundamental role in metabolism. ALA has been shown to affect cellular processes, alter redox status of cells, and interact with thiols and other antioxidants.[8] One experimental study reported that lipoic acid administration was effective in hepatic function restoration.[9] These studies showed that lipoate pretreatment lowered the levels of SGOT and SGPT significantly. This fact has to be connected with the finding that a-lipoic acid is able to modulate cytochrome P450 reductase. Alpha-lipoic acid is able to inhibit both purified and microsomal P450 reductase by inducing a chemical modification of the SH-groups via a thiol–disulfide exchange reaction.[10] The present study was designed to investigate the protective effect of alpha-lipoic acid on hepato renal toxicity of acetaminophen induced uremic rat models.

## 2. MATERIALS AND METHODS
### 2.1. Reagents
#### 2.1.1. Drugs and Chemicals
Reagents such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) were from Merck Specialties Private Limited, Worli, Mumbai. All other chemicals were purchased from SRL, India, Sigma Aldrich, India and HiMedia Laboratories Pvt. Ltd., Mumbai, India. Different

biochemical parameters like plasma antioxidant enzyme profiles like super oxide dismutase (SOD), catalase (CAT) and oxidative stress marker like malondialdehyde (MDA) were measured by the absorbance of UV- VIS Spectrophotometer (Systronics, India). Alpha lipoic acid was purchased from Sun Pharmaceuticals-Symbiosis. All other chemicals used for Bio chemical tests including Methanol, ethylene diamine tetra acetic acid (EDTA), Potassium phosphate dibasic (K2HPO4), potassium dihydrogen phosphate (KH2PO4), Pyragallol, Tris, Trichloroacetic acid (TCA), Thiobarbituric acid (TBA) were collected from Merck Specialties Private Limited, Worli, Mumbai, HiMedia Laboratories Pvt. Ltd., Mumbai, India and Crest Biosystems, Goa, India.

**2.1.2. Drug doses:** Acetaminophen was injected intraperitoneally at a dose of 500/ kg body wt/day for 10 days **[11].** Alpha lipoic acid tablets (Sun Pharmaceuticals-Symbiosis): Alpha lipoic acid was co administered orally at conc. of 50, 100 mg/kg body weight /day for 20 days of experiment.[12]

**2.2. Selection of animals and care**

**2.2.1. Experimental subjects**

The present experiment was conducted on 30 male *Wistar* strain adult pathogen free, healthy albino rats having weight of 100±15 g (Supplied from Ghosh animal, animal foods and animal cages Supplier, Kolkata 54). They were housed at laboratory condition for 2 weeks prior to experimentation. Animals were housed three rats/cage in a temperature-controlled room (22± 20C) with 12–12 h dark–light cycles (8.00–20.00 h light, 20.00– 8.00 h dark) at a humidity of 50 ±10%. They were provided with standard food and water *ad libitum*. Animal care was provided according to the Guiding Principle for the Care and Use of Animals.[13] Our Institutional Animal Ethical Committee (IAEC) approved this study.[14]

**2.2.2. Experimental design**

The rats were divided into four equal groups as follows:

Group I or control – Six animals were subjected to control group. They were housed at room temperature (25± 30C) and feed normal diet and water ad libitum.

Group II or acetaminophen induced hepatotoxic rats – Six animals were randomly placed in cage with normal diet and injected with acetaminophen at the conc. of 500 mg with de-ionized water 5 mL/kg of body weight/day for 10 days to achieve hepatic toxicity.

Group III and IV or acetaminophen with alpha lipoic acid treatment group–This group of animal treated as group II and co-administered with ALA at the dose of 50 and 100 mg/ /kg body weight/rat for 20 days.

**2.3. Preparations of sample for biochemical studies**

This experimental design was continued for 20th days. On 21st day of experiment, the animals were sacrificed and blood and tissues were collected from the aorta after which the kidneys were collected for different biochemical analysis.

**2.4. Parameters**

**2.4.1. Biochemical estimation of liver toxicity study:**

Activities of plasma liver enzymes aspartate and alanine aminotransferases (AST and ALT) were chemically determined according to the method of Goel.[15] For the assessment of toxicity in plasma and liver biochemical estimation of alkaline phosphatase (ALP) were done spectrophotometrically.[16]

**2.4.2. Biochemical estimation of plasma antioxidant enzyme profiles and oxidative stress marker**

The whole blood was centrifuged and plasma fraction was separated. The super oxide dismutase (SOD) activity of plasma will be estimated by measuring the percentage of inhibition of the pyragallol auto-oxidation by SOD using spectrophotometer at 420 nm and values were expressed as mmol of H2O2 consumption/dl of plasma/min.[17] For the estimation of catalase (CAT) activity, in a spectrophotometric cuvette, 0.5 ml of hydrogen peroxide (H2O2) and 2.5 ml of distilled water were mixed and reading of absorbance was noted at 240 nm and plasma was added at volume of 40µl separately, the subsequent six reading were noted at 30 sec. interval[18] and values were expressed as mmol of H2O2 consumption/dl of plasma/min. Plasma level of malondialdehyde (MDA) was measured by spectrophotometer at 535 nm and values were expressed as n mol/dl of plasma.[19]

**2.4.3. Biochemical estimation of antioxidant enzyme profiles and oxidative stress marker of kidney tissue:**

kidney tissues were homogenized separately in 0.05 M Tris Hydrochloric acid (HCl) buffer solution (pH-7.0) at the tissue concentration of 50 mg/ml. These homogenate was centrifuged separately at 10,000 g at 4oC for 10 min and tissue supernatant was collected. The activities of SOD[17], CAT[18] and MDA[19] in kidney tissue homogenate were measured spectrophotometrically.

**2.4.4. Histopathological studies**

Liver tissues from the experimental rats were fixed in 10% buffered formalin solution embedded in paraffin wax and 5µ sections were prepared with a rotary microtome. These thin sections were stained with hematoxylin and eosin (H and E), mounted on glass slides and observed for pathological changes under a binocular microscope according to Mani, 2010.[20]

**2.5. Statistical analysis**

Data were expressed as mean ± SE (n=6). ANOVA followed by Bonferroni multiple two-tail t-test to detect inter group differences and bars with different superscripts (*, #, Δ) differ from each other significantly ($p< 0.05$).[21]

## 3. RESULTS AND DISCUSSION

Paracetamol is a widely used antipyretic and analgesic drug which is safe in therapeutic doses but can cause fatal hepatic damage in human and animals at higher toxic doses. According to several experimental studies, the generation of reactive oxygen species has been established as a mechanism by which many chemicals can induce nephrotoxicity.[22] ALT and AST are enzymes normally present in the liver, heart, muscles and blood cells. They are basically located within hepatocytes. So when liver cells are damaged or die, transaminases are released into blood stream, to measure the index of liver injury. The elevated levels of ALT, AST and ALP are indicative of cellular leakage and loss of functional integrity of the hepatic cell membranes implying hepatocellular damage.[23] The levels of plasma AST, ALT, ALP, were markedly elevated (P<0.05) in acetaminophen treated rats of Group II animals in comparison with control (Group I). After treatment of ALA at higher doses (Group IV), there was a significantly (p<0.05) decreased levels of these marker enzymes. Administration of ALA at the dose of 50 and 100mg/kg body weight for 20 days to acetaminophen induced hepatotoxic rats restored significantly ($p < 0.05$) of the level of plasma ALT, AST and ALP offering the maximum hepatic protection with respect to different liver marker enzymes **(Table 2 and 3).** Previous studies have clearly demonstrated that acute APAP overdose increases the lipid per oxidation and suppresses the antioxidant defense mechanisms in renal tissue.[24] This is ascribed to a free radical-mediated chain reaction that damages cell membranes[25, 26] and MDA is a good indicator of the degree of lipid peroxidation. Alpha-lipoic acid is characterized by high reactivity towards reactive oxygen species and its capability of increasing tissue levels of anti-oxidant enzymes.[27] It has been demonstrated that lipoic acid reduces oxidative stress in healthy adults and diabetic patients by decreasing significantly lipid hydro peroxide formation.[28, 29] However in the acetaminophen treated animals (Group II), the MDA levels were increased significantly ($p < 0.05$), when compared to group I rats. Co-administration of ALA at two different doses (Group III and IV), the levels of MDA in plasma and kidney tissues were decreased significantly ($p < 0.05$) when compared to acetaminophen treated rats (Group II) and these values were resettled to the control group or group I rats (**Table 5)**. During kidney injury, superoxide radicals are generated at the site of damage and modulate SOD and CAT, resulting in the loss of activity and accumulation of superoxide radical, which damages kidney. SOD and CAT are the most important enzymes involved in ameliorating the effects of oxygen metabolism.[30] The present study also demonstrated that acetaminophen overdose resulted in a significant decrease ($p < 0.05$) in the SOD and CAT activities in plasma and kidney, when compared with normal control rats. It is due to enhanced lipid peroxidation or inactivation of the ant oxidative enzymes. When rat were co administered with the ALA at two different doses, the activity of SOD and CAT was increased significantly (p<0.05) when compared with acetaminophen treated group (P<0.01) (Group II) and higher doses of ALA (Group IV) showed highest activity than lower dose (Group III) **(Table 4)**. Depletion of renal GSH is one of the primary factors that permit lipid peroxidation; it is suggested to be closely related to APAP tissue damage. It has been reported that renal glutathione content, and glutathione peroxidase and reductase activity of kidney tissue, which are critical constituents of the GSH-redox cycle, were significantly reduced by treatment with acetaminophen.[31]

Liver is the main organ that is capable of detoxification**.** Probably, treatment with ALA could have enhanced detoxification of acetaminophen thereby maintaining the liver architecture. Morphological observations showing normal histology of liver of control group rats (Group I) with radiating chords of hepatocytes around central vein indicate well organized histoarchitecture **(Fig 5 Section A).** Figure four showed severe disorganization of liver cells after acetaminophen injection of 500 mg/kg body weight for 10 days in Group II rats. Damaged hepatocytes and blood vessels were seen prominently **(Figure 4 Section B).** But after treatment of ALA at the dose of 50 and 100mg/kg body wt/day for 20 days, there was the normal hepatocytes are seen as well as slightly affected ones with a small disorganization in Group IV than Group III rats **(Figure 4 Section D).**

**Table No: 1 Effect of two different doses of Alpha-lipoic acid on body weight of Acetaminophen induced hepato toxicity in male rats. Data are expressed as Mean ± SE (n=6). ANOVA followed by multiple two-tail t-test and data with different superscripts ((*, # Δ) in a specific vertical column differ from each other significantly (*P*< 0.05).**

| Groups | Initial body weight (g) | Final body weight (g) | Elevation/diminution in body growth (g%) |
|---|---|---|---|
| I | 169.65±2.82 | 252.45±2.55 | 82.8 |
| II | 174.25±0.67 | 188.62±3.15 | 14.63 |
| III | 173.20±.53 | 190.6±4.1 | 17.39 |
| IV | 176.48±1.6 | 195.43±6.12 | 18.95 |

**Table No: 2 Effect of two different doses of Alpha-lipoic acid on plasma aspartate aminotransferase and alkaline transaminase level on acetaminophen induced hepato toxicity on male rats. Data are expressed as Mean ± SE (n=6). ANOVA followed by multiple two-tail t-test and data with different superscripts ((*, # Δ) in a specific vertical column differ from each other significantly (*P*< 0.05).**

| Groups | ALT (U/L of plasma) | AST(U/L of plasma) |
|---|---|---|
| I | 20.72±0.9 | 24.05±1.75 |
| II | 44.53±1.9* | 49.5±7.38* |
| III | 22.41±6.93# | 23.91±7.18# |
| IV | 20.61±1.7# | 22.70±3.88# |

**Table No: 3 Effect of two different dose of Alpha-lipoic acid on plasma alkaline phosphatase on acetaminophen induced hepato toxicity on male rats. Data are expressed as Mean ± SE (n=6). ANOVA followed by multiple two-tail t-test and data with different superscripts (*, # Δ) in a specific vertical column differ from each other significantly (*P*< 0.05).**

| Groups | ALP(U/L of plasma) |
|---|---|
| I | 108.26±2.94 |
| II | 374.83±13.64* |
| III | 103.20±16.46# |
| IV | 96.49±10.30Δ |

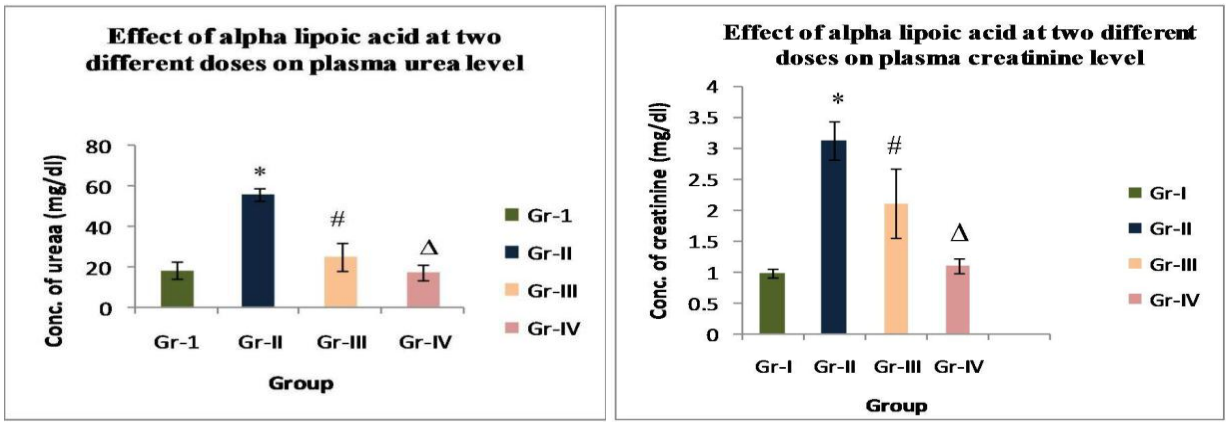



**Figure 1: Effect of two different doses of Alpha-lipoic acid on plasma urea and creatinine level on acetaminophen induced hepato toxicity on male rats. Data are expressed as Mean ± SE (n=6). ANOVA followed by multiple two-tail t-test and data with different superscripts (*, #, Δ) in a specific vertical column differ from each other significantly (p <0 .05)**

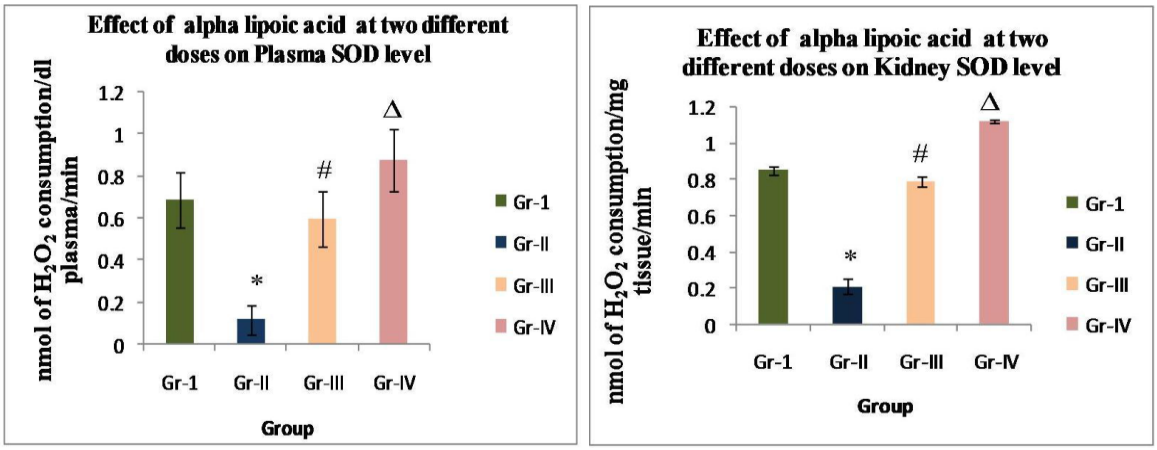



**Figure 2: Effect of two different doses of Alpha-lipoic acid on plasma and kidney SOD level on acetaminophen induced uremic male rats. Data are expressed as Mean ± SE (n=6). ANOVA followed by multiple two-tail t-test and data with different superscripts (*, #, Δ) in a specific vertical column differ from each other significantly (p <0 .05)**

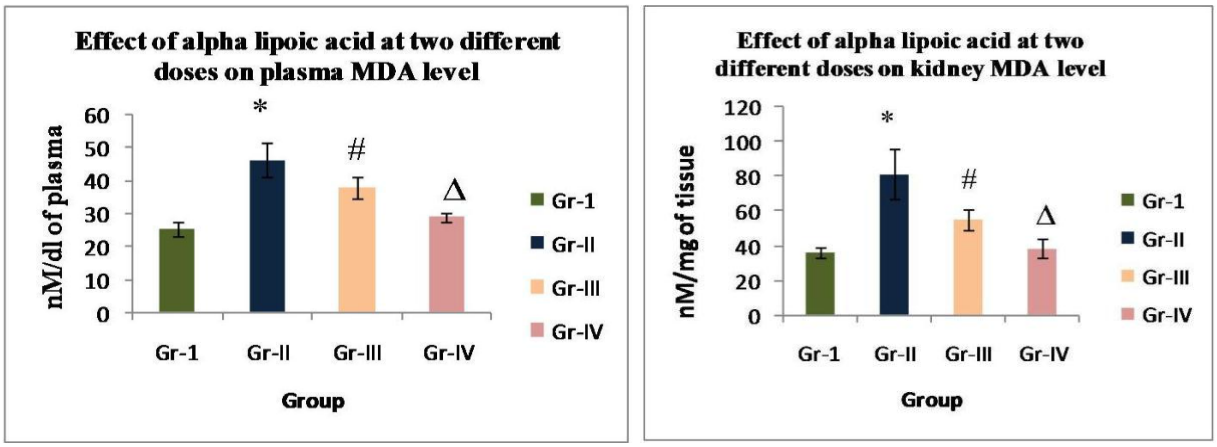



**Figure 3: Effect of different doses of Alpha-lipoic acid on plasma and kidney MDA level on acetaminophen induced hepato toxicity on male rats. Data are expressed as Mean ± SE (n=6). ANOVA followed by multiple two-tail t-test and data with different superscripts (*, #, Δ) in a specific vertical column differ from each other significantly (p <0 .05)**

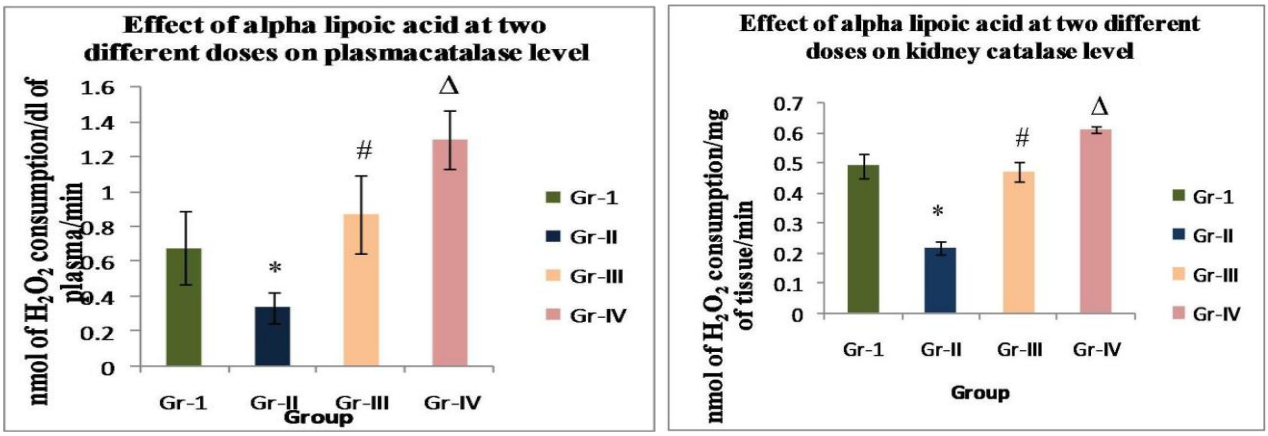
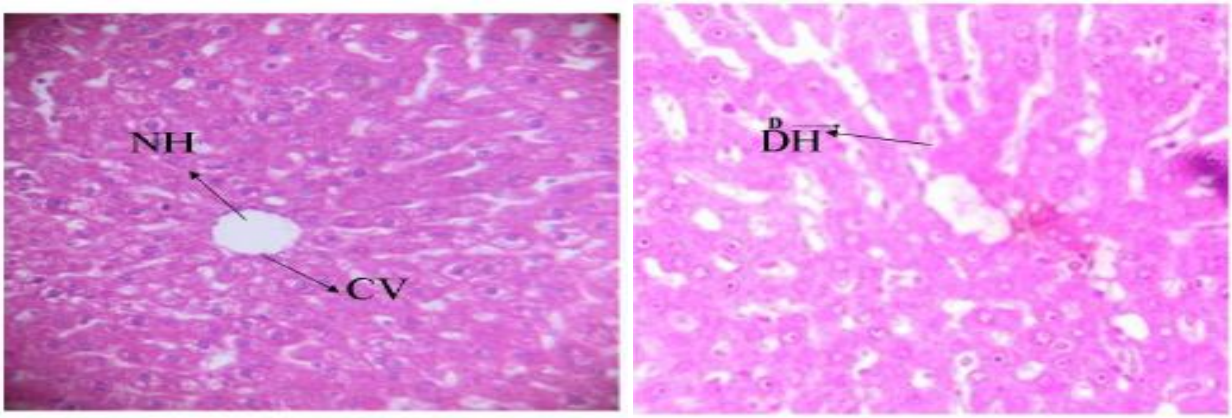
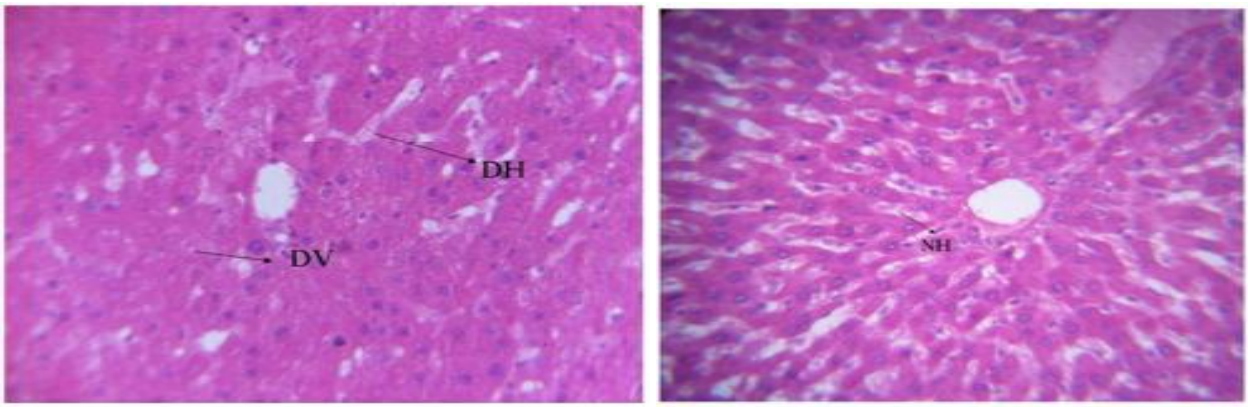



**Figure 4: Effect of different doses of Alpha-lipoic acid on plasma and kidney Catalase level on acetaminophen induced hepato toxicity on male rats. Data are expressed as Mean ± SE (n=6). ANOVA followed by multiple two-tail t-test and data with different superscripts (*, #, Δ) in a specific vertical column differ from each other significantly (p <0 .05)**




**A** **B**




C D

**Figure 5: Section A Showing normal histology of liver of control group (Gr-I) rats. Radiating chords of normal hepatocytes (NH) around central vein (CV) indicate well organized histoarchitecture. Section B Showing severe disorganization of rat liver cells after acetaminophen injection doses of 500mg/kg body weight for 15 days to Group II rats. Damaged hepatocytes (DH) and damaged blood vessels are seen (DV). Section C showing acetaminophen induced hepatotoxic rat liver cells (500mg/kg body weight) with co-administered of ALA (50 mg/kg) for 20 days to Gr-III rats showing both less damaged hepatocytes (DH). Section D showing acetaminophen induced hepatotoxic rat liver cells (500mg/kg body weight/day for 15 days) with co-administered of ALA (100mg/kg body wt/day) for 20 days to group IV rats showing both normal hepatocytes (NH) with a minimum disorganization (D).**

## CONCLUSION

In conclusion, the findings suggests that the potential use of alpha lipoic acid at two different doses is a novel therapeutically useful Hepato-Reno protective agent which improves hepatological derangements associated with repeated dose acetaminophen induced nephrotoxicity. It also observed that the higher dose of Alpha lipoic acid (100mg/kg/day) is the most effective dose for reducing hepato-renal toxicity due to its anti-oxidative properties on acetaminophen induced hepato-toxic male rats. It has great societal impact on community people as it is an alternative, cost effective easily affordable therapy for the management of hepato-renal toxicity.

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