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HEPATO-CARDIO PROTECTIVE POTENTIAL OF ALPHA LIPOIC ACID IN 5-FLUOROURACIL-INDUCED TOXICITY OF WISTAR RATS

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ABSTRACT

The aim of this study was to assess the protective potential of Alpha Lipoic Acid in 5-Fluorouracil induced hepato-cardio toxicity in Wistar rats. Wistar rats were randomly divided into six different groups (*n*=6 each). The first group received normal saline (1ml/kg) for four days. The second group received a daily dose of (1ml/kg) normal saline and 5-fluorouracil (50 mg/kg), orally for four days. The third group received Silymarin 100 mg/kg orally for four days. The fourth group received a daily dose of alpha lipoic acid (ALA), 100 mg/kg orally, and the fifth group received a daily dose of ALA (200 mg/kg, orally) for four days. The Silymarin and alpha lipoic acid treatments were given an hour before 5-fluorouracil administration. The sixth group received 5-fluorouracil (50 mg/kg, orally) for four days and 400mg/kg alpha lipoic acid for 14 days. All the experimental animals were sacrificed at the end of the experimental procedures. Single intraperitoneal injection of 50 mgkg-1 of 5-FU was associated with significant ($p<0.05$) increases in the malondialdehyde (MDA), lactose dehydrogenase (LDH) and creatinine phosphokinase (CPK) levels in the toxicity model control when compared with untreated control rats. However, a single intraperitoneal injection of 50 mg/kg 5-FU was associated with significant ($p<0.05$) decrease in the Glutathione (GSH), Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx), levels in the toxicity model control when compared with untreated control rats. Pretreatment and post treatment with ALA attenuated these changes.

Keywords: Alpha Lipoic Acid, Hepatotoxicity, Cardiotoxicity, 5-Fluorouracil, Wistar rats

INTRODUCTION

Cardiotoxicity is a serious side effect of the treatment with 5-fluorouracil (5-FU), but the underlying mechanisms are not fully understood. The pathogenesis of 5-FU induced cardiotoxicity may involve oxidative stress, as increased levels of superoxide anion were demonstrated in H9c2 cells after 5-FU treatment (Polk *et al.*, 2014). Reactive oxygen species (ROS), such as superoxide anions, are under normal physiological conditions cleared by antioxidant defense systems, such as sodium

oxide dismutase (SOD) and glutathione peroxidase. Diseases mediated by oxidative stress are treated effectively with alpha lipoic according to several reports. (Castro *et al.,* 2013). Alpha lipoic acid (ALA) is a cofactor of α-ketoacid dehydrogenase complexes and plays a fundamental role in fuel metabolism (Siti *et al*., 2008).It reduces the oxidized forms of other antioxidants,,chelates metal ions.It is an amphiphilic antioxidant that quenches reactive oxygen species. It restored hepatic function in chloroquine intoxicated rats (Pari & Murugavel, 2004) and can inhibit xenobiotic-induced liver toxicity as reported in adriamycin-induced hepatotoxicity in rats (Anandakumar *et al*., 2007).The present study was designed to investigate the hepato-cardioprotective potential of ALA in 5-FU induced cardiotoxicity of Wistar rats.

MATERIALS AND METHODS

Animals

Wistar rats of either sexes weighing 225±5g were obtained from the Animal House facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University Zaria. They were given free access to standard feed and water *ad libitum*. All experimental protocols were as approved by the University Animal ethics committee with Ethical Approval number ABUCAUC/2020/013. All experiments performed on laboratory animals in this study were in accordance with Ahmadu Bello University Research policy and followed the "principles of laboratory animal care" (NIH Publication, 1985, revised 1996).

Chemicals

Alpha lipoic Acid (ALA) was obtained from Sigma Chemical Company, United States of America (USA). All other chemicals were of analytical grade and obtained from local commercial sources.

Experimental Design

This experimental design was as described by Al-Asmari *et al.* (2016) with some modifications. Wistar rats were randomly divided into six different groups (*n*=6 each).

Group 1: received normal saline (1ml/kg).
Group 2: received a daily dose of (1ml/kg) and 5-fluorouracil (50 mg/kg body weight, orally for four days.
Group 3: received Silymarin orally, 100mg/kg.
Group 4: received a daily dose of ALA (100 mg/kg orally)
Group 5: received a daily dose of ALA (200 mg/kg orally). The Silymarin and alpha lipoic acid treatments were given an hour before 5-fluorouracil administration. Groups 1-5 served as the pre-treatment groups.
Group 6: received 5-fluorouracil (50 mg/kg orally) for four days and 400mg/kg alpha lipoic acid for 14 days. This served as the post-treatment group. All the experimental animals were sacrificed at the end of the experimental procedures.

Determination of Antioxidant Indices

The dissected tissues were washed with 50 mM sodium phosphate-buffered saline (100 mM Na_2HPO_4/NaH_2PO_4, pH 7.4) in an ice-containing medium, with 0.1 mM EDTA to remove any RBCs and clots. Then tissues were homogenized in 5–10 ml cold buffer per g tissue and were centrifuged at 5000 rpm for 30 min. The resulting supernatant was transferred into an Eppendorf tube and was preserved by deep freezing into aliquots for the spectrophotometric estimation of tissue MDA (a biomarker of lipid peroxidation, LPO), SOD, catalase (CAT), GSH, glutathione peroxidase (GSH-Px).

Determination of Biochemical Parameter

Serum biochemical parameters of cardiac injury biomarkers, Lactase dehydrogenase (LDH) and Creatinine Phosphokinase (CPK) enzyme activities were measured

spectrophotometrically according to manufacturers' instructions.

Statistical Analysis

The differences between the obtained values (mean ± SEM, n = 6) were analyzed with One-Way Analysis of Variance followed by the Tukey–Kramer multiple comparison using Graph pad prism 5 software (GraphPad Software, Nc., La Jolla, CA, USA) The differences were considered statistically significant when $p \leq 0.05$.

RESULTS

Hepatic Antioxidant Indices

Single intraperitoneal injection of 50 mg/kg of 5-FU caused a significant ($p<0.05$) increase in the MDA level in the toxicity control when compared with untreated control rats. However, daily oral pre-treatment with 100 mg/kg (ALA and silymarin), 200 mg/kg (ALA) and 400 mg/kg ALA (post treatment) before and after 5-FU injection significantly ($p<0.05$) attenuated increase in the MDA level in a dose-dependent manner when compared to the toxicity model group. (Fig 1-8).

Single intraperitoneal injection of 50 mg/kg of 5-FU was associated with significant ($p<0.05$) decrease Catalase (CAT), Superoxide dismutase (SOD), Glutathione peroxidase (GPx) and reduced glutathione (GSH) levels in the toxicity model control when compared with untreated control rats (Fig. 1-8). However, daily oral pre-treatment with 100 mg/kg (ALA and Silymarin), 200 mg/kg (ALA) and 400 mg/kg ALA (post treatment) before and after 5-FU injection significantly ($p<0.05$) attenuated decrease in the Catalase (CAT), Superoxide dismutase (SOD), Glutathione peroxidase (GPx) and reduced glutathione (GSH) levels in a dose-dependent manner when compared to the toxicity model group (Fig 1-8).

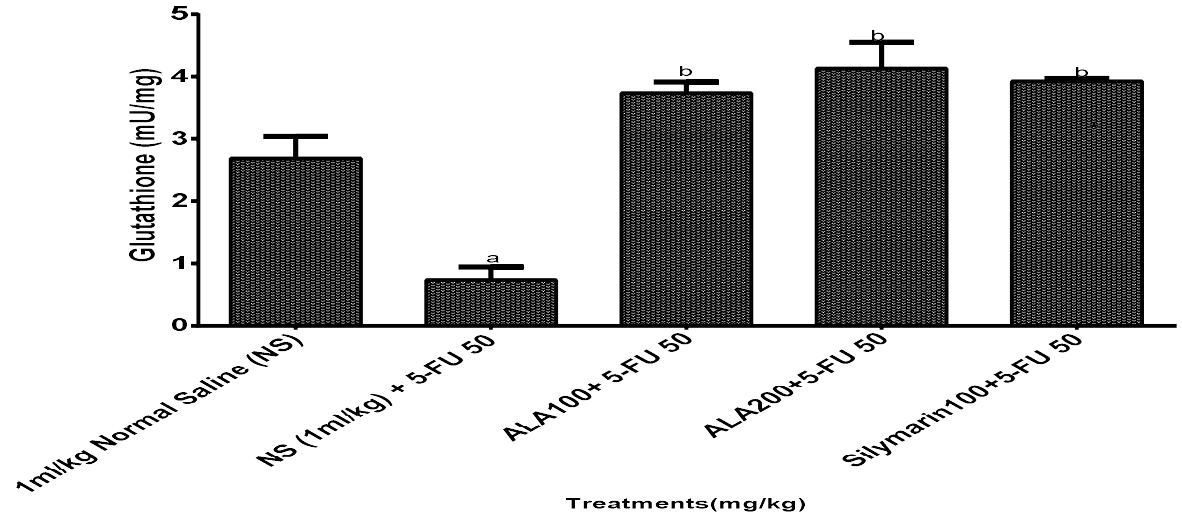

Fig. 1: Effect of Alpha Lipoic Acid on Glutathione (GSH) of 5-Fluorouracil Induced Toxicity in Wistar Rats (Pretreatment). Values are presented as mean ± SEM. Data was analyzed using One-way ANOVA followed by tukey post hoc test. [a]$p<0.05$ significant difference as compared to the negative control group (Normal Saline-1ml/kg). [b]$p<0.05$ significant difference as compared to the model or toxicity control group (Normal Saline-1ml/kg + 5-Fluorouracil-50mg/kg).

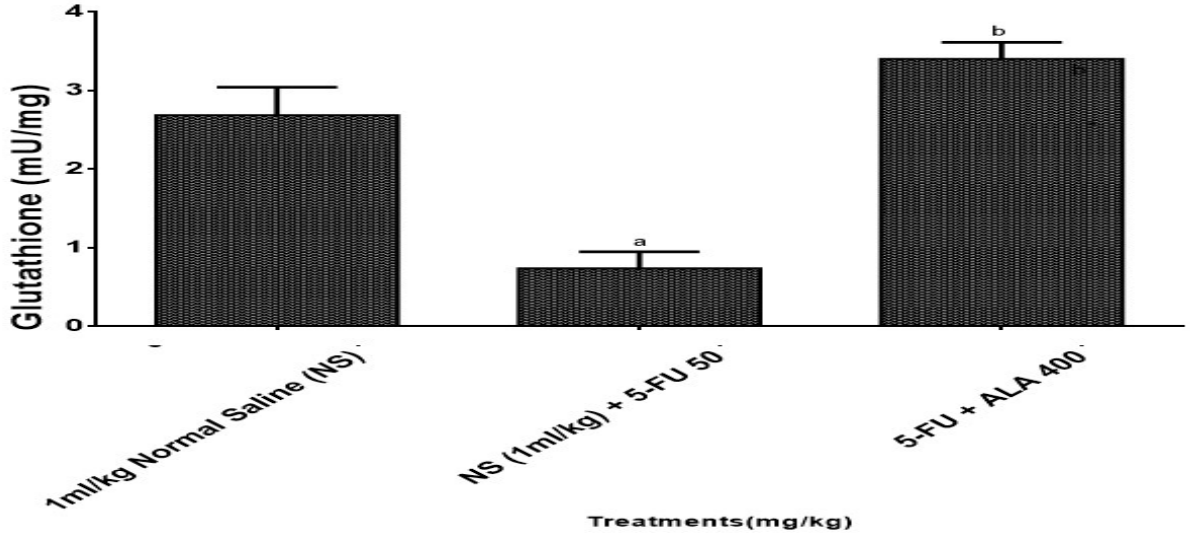

Fig. 2: Effect of Alpha Lipoic Acid on Glutathione (GSH) of 5-Fluorouracil Induced Toxicity in Wistar Rats (Post-treatment). Values are presented as mean ± SEM. Data was analyzed using One-way ANOVA followed by tukey post hoc test. [a]$p< 0.05$ significant difference as compared to the negative control group (Normal Saline-1ml/kg). [b]$p<0.05$ significant difference as compared to the model or toxicity control group (Normal Saline-1ml/kg + 5-Fluorouracil-50mg/kg).

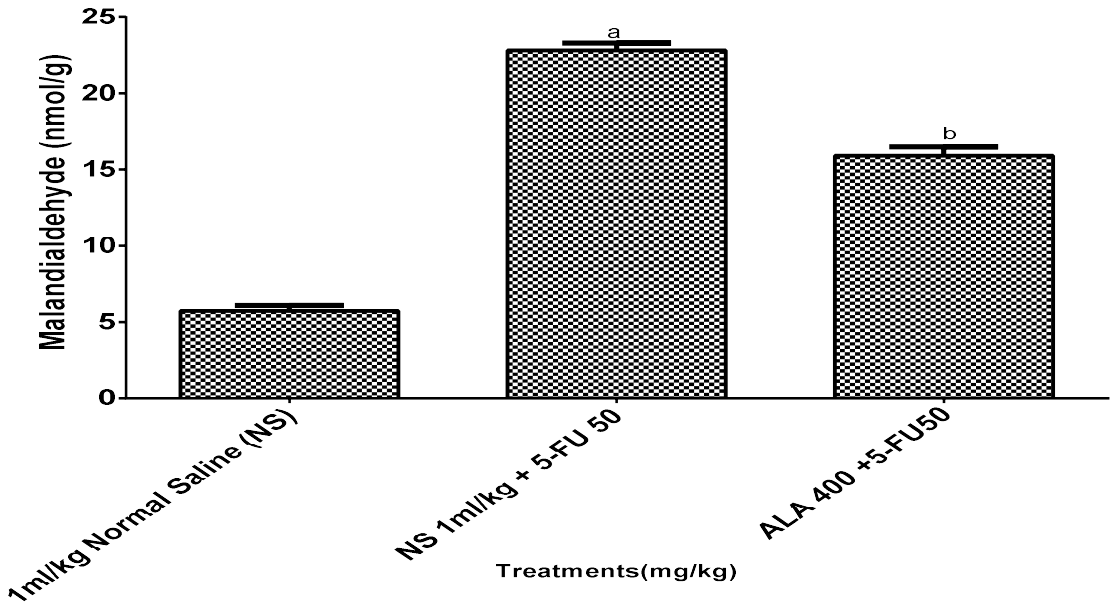

Fig. 3: Effect of Alpha Lipoic Acid on Malondialdehyde (MDA) of 5-Fluorouracil Induced Toxicity in Wistar Rats (Post-treatment). Values are presented as mean ± SEM. Data was analyzed using One-way ANOVA followed by tukey post hoc test. [a]$p<0.05$ significant difference as compared to the negative control group (Normal Saline-1ml/kg). [b]$p<0.05$ significant difference as compared to the model or toxicity control group (Normal Saline-1ml/kg + 5-Fluorouracil-50 mg/kg).

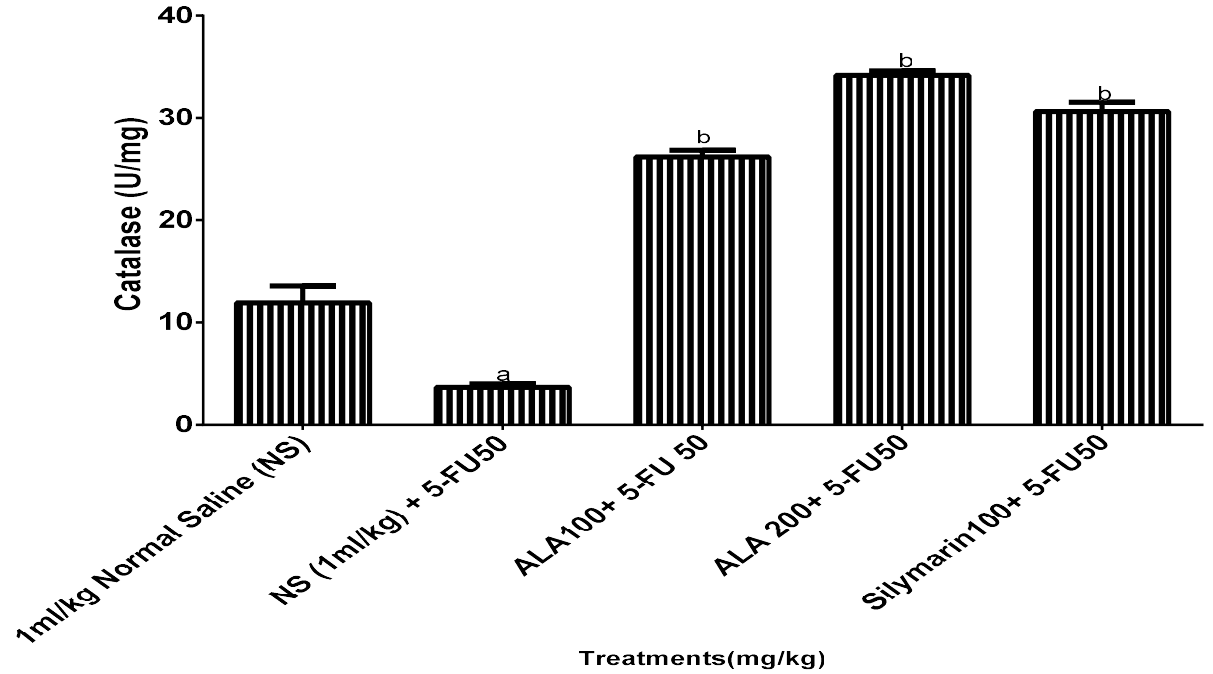

Fig. 4: Effect of Alpha Lipoic Acid on Catalase of 5-Fluorouracil Induced Toxicity in Wistar Rats (Pre-treatment). Values are presented as mean ± SEM. Data was analyzed using One-way ANOVA followed by tukey post hoc test. [a]$p<0.05$ significant difference as compared to the negative control group (Normal Saline-1ml/kg). [b]$p<0.05$ significant difference as compared to the model or toxicity control group (Normal Saline-1ml/kg + 5-Fluorouracil-50mg/kg).

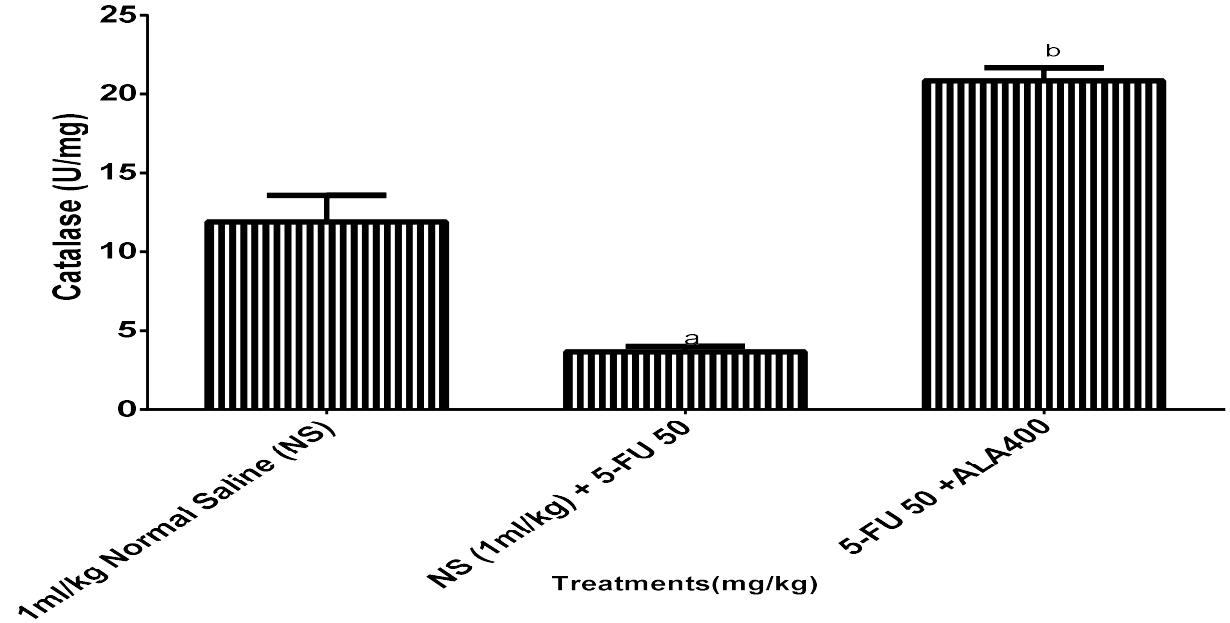

Fig. 5: Effect of Alpha Lipoic Acid on Catalase of 5-Fluorouracil Induced Toxicity in Wistar Rats (Post-treatment). Values are presented as mean ± SEM. Data was analyzed using One-way ANOVA followed by tukey post hoc test. [a]$p<0.05$ significant difference as compared to the negative control group (Normal Saline-1ml/kg). . [b]$p<0.05$ significant difference as compared to the model or toxicity control group (Normal Saline-1ml/kg + 5-Fluorouracil-50 mg/kg).

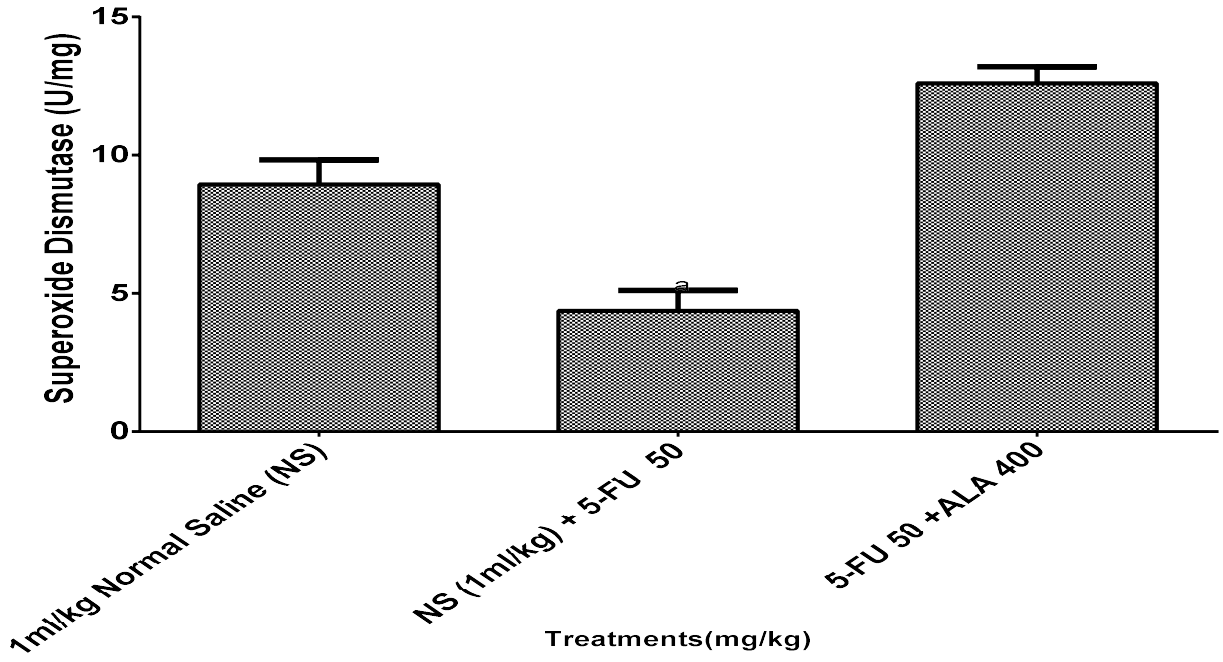

Fig. 6: Effect of Alpha Lipoic Acid on Superoxide Dismutase (SOD) of 5-Fluorouracil Induced Toxicity in Wistar Rats (Post-treatment). Values are presented as mean ± SEM. Data was analyzed using One-way ANOVA followed by tukey post hoc test. [a]$p<0.05$ significant difference as compared to the negative control group (Normal Saline-1ml/kg). [b]$p<0.05$ significant difference as compared to the model or toxicity control group (Normal Saline-1ml/kg + 5-Fluorouracil-50mg/kg).

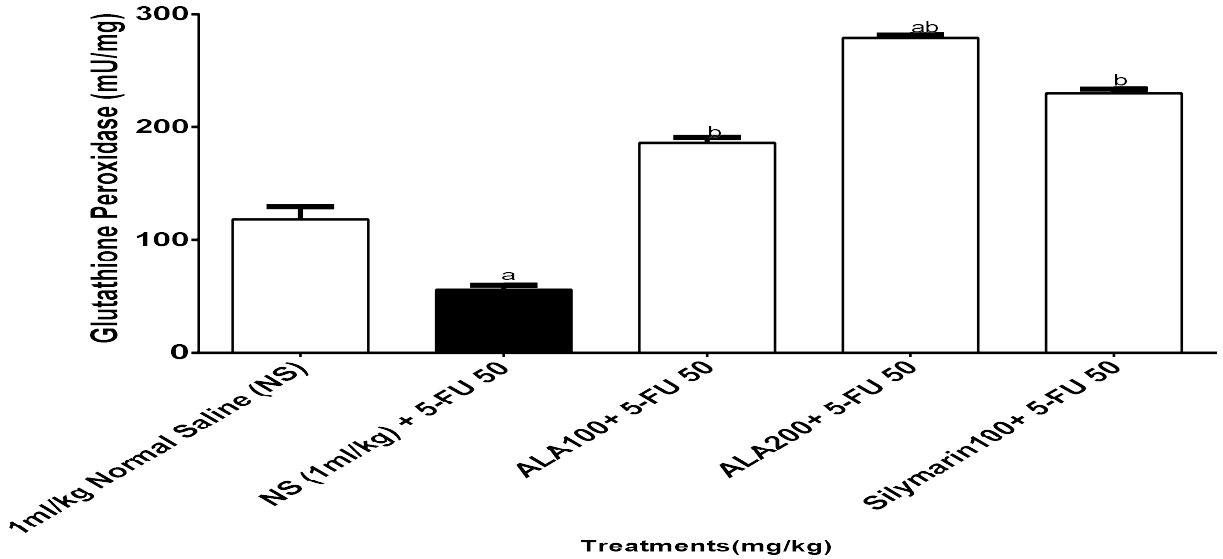

Fig. 7: Effect of Alpha Lipoic Acid on Glutathione Peroxidase (GPx) of 5-Fluorouracil Induced Toxicity in Wistar Rats (Pre-treatment). Values are presented as mean ± SEM. Data was analyesd using One-way ANOVA followed by tukey post hoc test. [a]$p<0.05$ significant difference as compared to the negative control group (Normal Saline-1ml/kg). [b]$p<0.05$ significant difference as compared to the model or toxicity control group (Normal Saline-1ml/kg + 5-Fluorouracil-50 mg/kg).

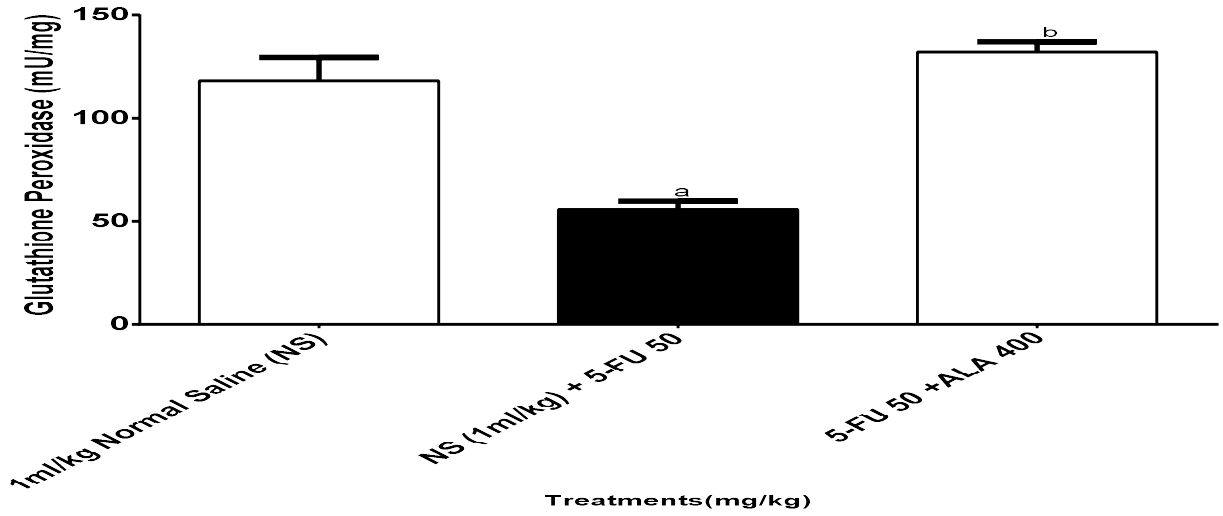

Fig. 8: Effect of Alpha Lipoic Acid on Glutathione Peroxidase (GPx) of 5-Fluorouracil Induced Toxicity in Wistar Rats (Post-treatment). Values are presented as mean ± SEM. Data was analyesd using Oneway ANOVA followed by tukey post hoc test. [a]$p<0.05$ significant difference as compared to the negative control group (Normal Saline-1ml/kg). [b]$p<0.05$ significant difference as compared to the model or toxicity control group (Normal Saline-1ml/kg + 5-Fluorouracil-50 mg/kg).

Effect of Oral Pretreatments with 100 mg/kg (ALA & Silymarin), 200 mg/kg (ALA) and Post-treatment with 400 mg/kg on Cardiac Injury Markers (Lactose Dehydrogenase and Creatinine Phosphokinase) in 5-Fluorouracil-induced Toxicity in Wistar Rats

Single intraperitoneal injection of 50 mg/kg of 5-FU was associated with significant (*p<0.05*) increase in the Lactose dehydrogenase and Creatinine Phosphokinase levels in the toxicity model control when compared with untreated control rats (Fig. 9-12). However, daily oral pre-treatment with 100 mg/kg (ALA and Silymarin), 200 mg/kg (ALA) and 400 mg/kg ALA (post treatment) before and after 5-FU injection significantly ($p<0.05$) attenuated increase in the Lactose dehydrogenase and Creatinine Phosphokinase levels in a dose-dependent manner when compared to the toxicity model group (Fig 9-12).Also daily oral pre-treatment with 100 mg/kg (ALA and Silymarin), 200 mg/kg (ALA) and 400 mg/kg ALA (post treatment) before and after 5-FU injection non-significantly ($p>0.05$) attenuated increase in the Lactose dehydrogenase and Creatinine Phosphokinase level in a dose-dependent manner when compared to the untreated control group (Fig 9-12).

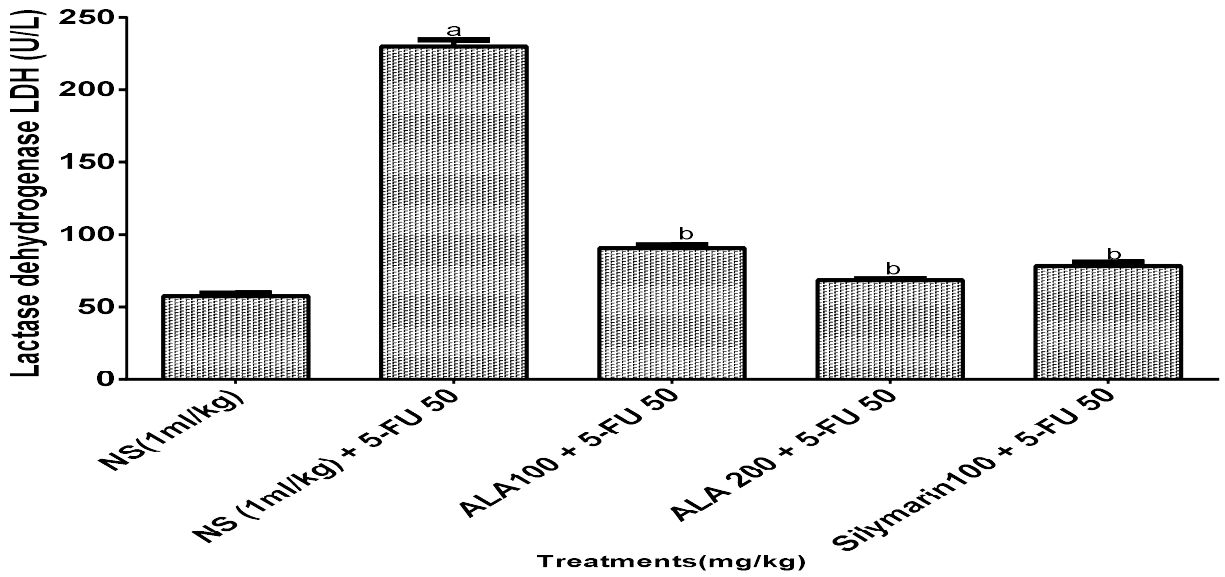

Fig. 9: Effect of Alpha Lipoic Acid on LDH levels of 5-Fluorouracil Induced Toxicity in Wistar Rats (Pre-treatment).
Values are presented as mean ± SEM. Data was analyzed using One-way ANOVA followed by tukey post hoc test. [a]$p<0.05$ significant difference as compared to the negative control group (Normal Saline-1ml/kg). [b]$p<0.05$ significant difference as compared to the model or toxicity control group (Normal Saline-1 ml/kg + 5-Fluorouracil-50 mg/kg).

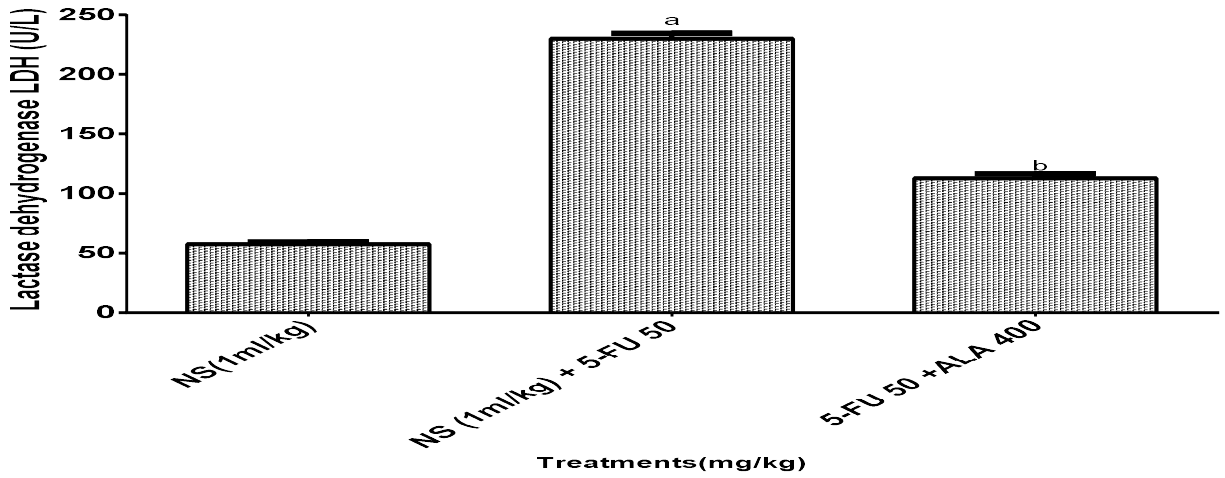

Fig. 10: Effect of Alpha Lipoic Acid on LDH Levels of 5-Fluorouracil Induced Toxicity in Wistar Rats (Post-treatment).
Values are presented as mean ± SEM. Data was analyzed using One-way ANOVA followed by tukey post hoc test. [a]$p<0.05$ significant difference as compared to the negative control group (Normal Saline-1ml/kg). . [b]$p<0.05$ significant difference as compared to the model or toxicity control group (Normal Saline-1 ml/kg + 5-Fluorouracil-50 mg/kg).

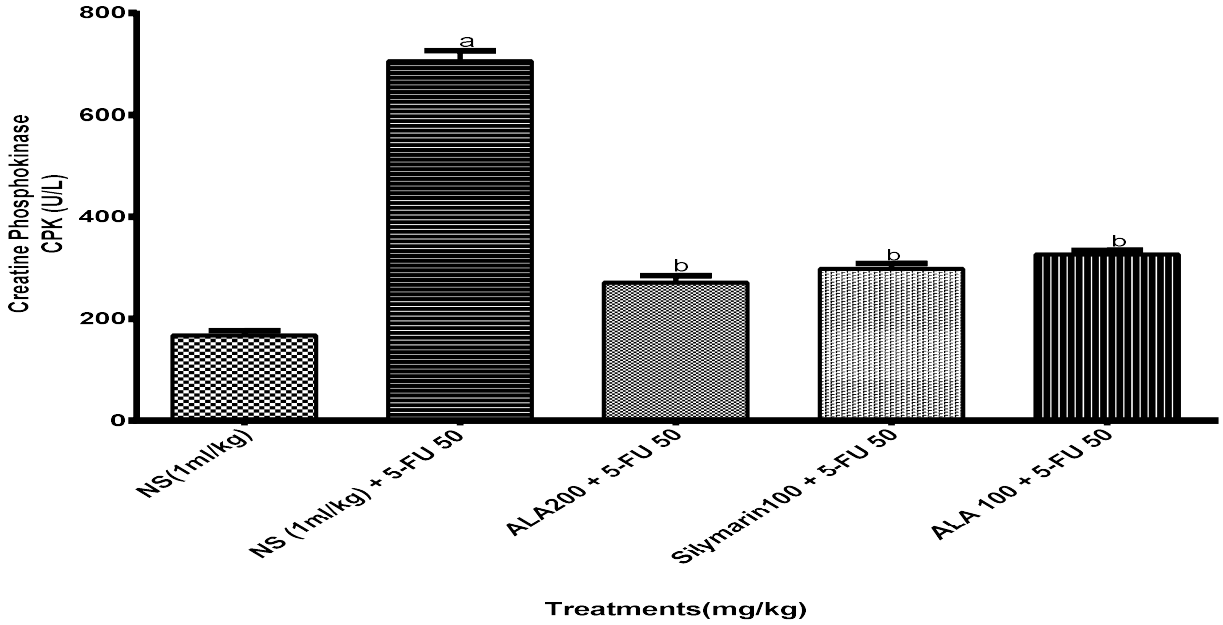

Fig. 11: Effect of Alpha Lipoic Acid on CPK Acid Levels of 5-Fluorouracil Induced Toxicity in Wistar Rats (Pretreatment).
Values are presented as mean ± SEM. Data was analyzed using One-way ANOVA followed by tukey post hoc test. [a]$p<0.05$ significant difference as compared to the negative control group (Normal Saline-1 ml/kg). . [b]$p<0.05$ significant difference as compared to the model or toxicity control group (Normal Saline-1 ml/kg + 5-Fluorouracil-50 mg/kg).

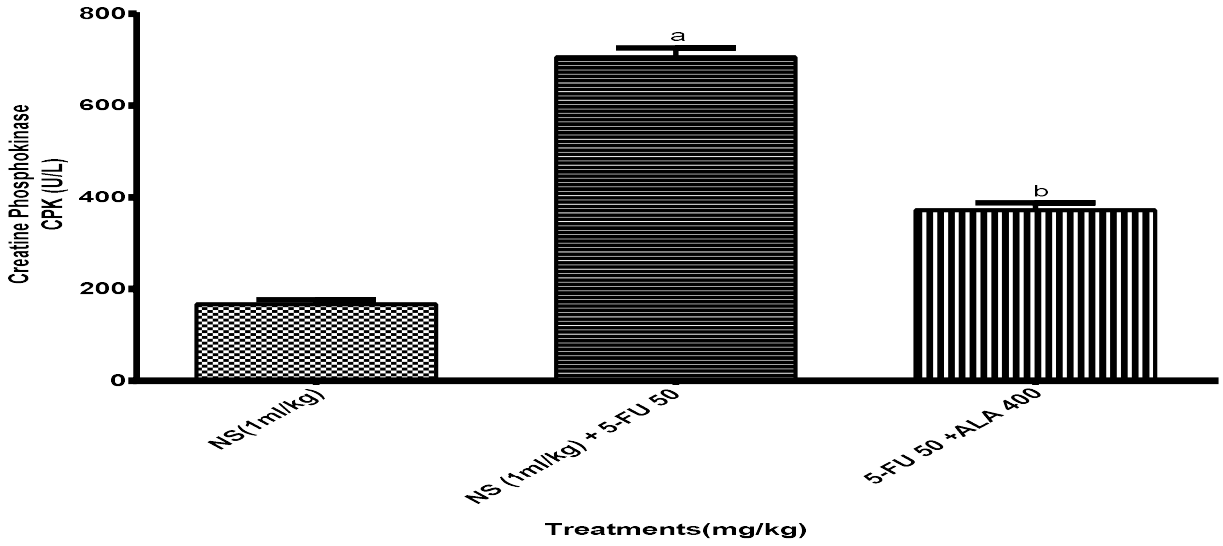

Fig. 12: Effect of Alpha Lipoic Acid on CPK Levels of 5-Fluorouracil Induced Toxicity in Wistar Rats (Post-treatment).
Values are presented as mean ± SEM. Data was analyzed using One-way ANOVA followed by tukey post hoc test. [a]$p<0.05$ significant difference as compared to the negative control group (Normal Saline-1 ml/kg). . [b]$p<0.05$ significant difference as compared to the model or toxicity control group (Normal Saline-1 ml/kg + 5-Fluorouracil-50 mg/kg).

DISCUSSION

Although a number of studies have been carried out on the protective effects of natural agents against chemotherapeutic drugs, this present study, to the best of our knowledge is the first to investigate the hepato-cardio protective potential of ALA against 5-fluorouracil induced hepato-cardio damage. 5-FU is used in the treatment of a wide range of cancers. Some of these cancers include that of the pancreas, neck. head, gastrointestinal tract, breast and that of the colon (Gelen *et al.*, 2018).It is however found to hepatotoxic and nephrotoxic. The present study was to assess the ameliorative potential of alpha lipoic acid in 5-FU induced toxicity of wistar rats.

Diseases mediated by oxidative stress are treated effectively with alpha lipoic according to several reports (Castro *et al.,* 2013). ALA protects cells and tissues from ROS and free radicals due to its antioxidant properties (Moini *et al*. 2002). Alpha lipoic acid helps in the regeneration of endogenous antioxidants such as intracellular reduced glutathione (GSH), vitamin E and vitamin C in order to scavenge free radicals (Wollin and Jones 2003; Abdou and Abdel- Daim 2014). ALA and derivatives also have anti-inflammatory activities (Kwiecień *et al.,* 2013). Alpha lipoic acid interacts with other antioxidants as well as thiols and changes cellular metabolic processes. It also changes the redox status of cells (Packer *et al*., 2001). It reduces the oxidized forms of other antioxidants,,chelates metal ions.It is amphiphilic antioxidant that quenches reactive oxygen species.It can inhibit xenobiotic-induced liver toxicity as reported in adriamycin-induced hepatotoxicity in rats (Anandakumar *et al*., 2007). The liver is constantly involved in biotransformation and this could lead to hepatotoxicity. Production of free radicals leads to oxidative stress and this is the basis of drug- induced hepatotoxicity (Anandakumar *et al*., 2007).

The exhaustion of antioxidant defense enzymes ultimately leads to hepatocellular injury and necrosis and the release of intracellular enzymes such as LDH (Stevens et al., 2000). Increases in these biomarkers are evidence of active liver dysfunction. These elevations could be attributed to direct damage to hepatocytes caused by the 5-FU, which is confirmed by measuring antioxidant levels in liver tissue. Enzyme levels such as LDH are often used to assess hepatic damage. Liver injury causes membrane damage or necrosis, which allows intracellular enzymes to circulate and be detected in serum. In this study 5-FU administration caused a significant increase in enzyme levels of CPK and LDH when compared to the negative control. These were significantly restored by pre-and post-treatments with ALA. This reversal in enzyme levels after ALA treatment is probably due to membrane-stabilizing activities that inhibit intracellular enzyme leakage. This is indicative of the cardio-protective potential of ALA. Liver is the main detoxifying organ for many toxic agents and drugs that contribute to oxidative stress (Stevens et al., 2000). The increased ROS production due to depletion of GSH led to oxidative damage and a critical role in the development of hepatic damage (Gorąca *et al*., 2011). ALA is effective in preventing the development of hepatic damage (Min et al., 2010; Park et al., 2008) which is consistent with the findings of the present study. ALA is found to be a potential therapeutic agent in the treatment and prevention of different pathologies that are related to an imbalance of the oxidoreductive cellular status, which occurs in the case of hepatic disorder status. In addition, several researchers have recently reported the protective effects of ALA on the liver which is induced by oxidative

agents (Eswaran et al., 2015), which is also consistent with the findings of this current study.

Oxidative stress results when generation of excess reactive oxygen species exceeds the capacity of the antioxidant system. These reactive species mediate cell damage in a variety of pathophysiological conditions and are responsible for severe damage to macromolecules, tissues and organs through lipid peroxidation (LPO), protein modification and DNA strand breaks (Zaidi & Banu,2004). The increased hepatic lipid peroxidation evidenced by the elevated MDA concentration is an indication that 5-FU-induced hepatic damage is mediated in part, by the generation of free radicals. Increased production of ROS is known to be accompanied with the generation of oxidized proteins (Penna *et al*., 2009). Where there are more pro-oxidants than antioxidants, oxidative stress occurs and this is injurious to cells (Zaidi *et al*., 2014). Free radicals that are produced due to oxidative stress engage and overwhelm antioxidant enzymes, resulting in the depletion of the antioxidant defenses and induction of lipid peroxidation evident in elevation of MDA level. (Akindele *et al*., 2010; Awodele *et al*., 2015). This marks the beginning and progress of liver damage in a variety of hepatic disorders (Girish *et al*., 2009). **(**Blokhina *et al*.,2003**).** An elevation in MDA level usually occurs with a decrease in endogenous antioxidants (SOD, CAT, GPx, and GSH) in the presence of oxidative stress (Kaplowitz, 2000) which agrees with the findings of the current study. 5-FU significantly increased MDA levels and significantly decreased GSH, CAT, GPx and SOD levels in the positive control when compared to the negative control. This is an ample evidence of the ability of 5-FU to generate free radicals and cause oxidative stress culminating in liver damage. Co-administration with alpha lipoic acid significantly reduced MDA levels and increased hepatic GSH, CAT and SOD levels when compared to the toxicity control groups. This once again is indicative of the protective role of alpha lipoic acid in the presence of 5-FU. This was due to the inherent antioxidant activity of alpha lipoic acid. (Abidemi *et al*., 2017). ALA also protects the integrity of cell membranes by interacting with other antioxidants, namely glutathione and vitamins E and C (Nordberg and Arner, 2001).

It was previously reported that pre administration of ALA and vitamin E for 4 weeks in the LPS-challenged rats significantly decreased the formation of MDA in liver (Sena *et al*., 2007). Similarly, previous studies showed that treatment with ALA significantly decreased the MDA level, which may be partly due to the ability of ALA to scavenge free radicals. This effect can be explained on the basis that, ALA or its reduced form dihydrolipoic acid can prevent lipid peroxidation and protein damage via interaction with vitamin C and glutathione. It has been shown that ALA reduces the increased ROS generation and protein oxidation in the liver as a result of the potent antioxidant capacity of ALA (Tian *et al*., 2012). El-Feki *et al*. (2016) also reported that ALA-treated rats demonstrated enhanced activity of catalase. Similarly, Akpinar *et al.* (2009) found that, ALA contributes to antioxidant defense by increasing catalase activity. Moreover, administration of ALA contributed to an increase in the level of GSH, thus improving the hepatic redox status (Akpinar *et al*., 2009). Both ALA and its derivate DHLA may act as extra-and intracellular redox couples and potent free radical scavengers. This may imply that ALA prevents the oxidation of free or protein-bound thiols (Mihai *et al*., 2010). ALA improves a deficient thiol status by increasing the levels of hepatic GSH (Bertok, 2005). The

decreasing GSH activity might be related to the inhibitor 5-FU effect on Glutamyl-cysteine-synthetase activity, the enzyme that controls the biosynthesis of glutathione in liver, thus resulting in a reduction in GSH synthesis. This might be coupled to the 5-FU inhibiting ability of NADPH generating enzymes such as glucose 6-phosphate dehydrogenase and NADP-isocitrate dehydrogenase, resulting in a slowing down in the GSH regeneration (Newairy *et al*., 2009). We propose that ALA's antioxidants capacity may be due to its ability to chelate 5-FU to scavenge reactive oxygen species by the sulphydryl group to regenerate endogenous antioxidants (such as vitamin C, vitamin E,and GSH) and to repair oxidative damage of cellular macromolecules which prevented the increase in lipid peroxidation level and increased the antioxidants enzymes activities. Several reports (Winiarska *et al*., 2008; Abdel-Zaher *et al*., 2008) reported that ALA could prevent GSH depletion by scavenging reactive oxygen species and or increasing cysteine uptake, which is a rate limiting step for GSH biosynthesis (Ihcène *et al*., 2014).

Conclusion

Alpha Lipoic acid demonstrated a potential of mitigating the hepato-cardio protective effects of 5-FU induced toxicity. More research needs to be carried out on its clinical usefulness in humans with hepato-cardio injuries.

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