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Nephroprotective Potential of Alpha Lipoic Acid in 5-Fluorouracil-Induced Toxicity of Wistar Rats

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ABSTRACT

5-Fluorouracil is a widely used anticancer drug but its therapeutic potential is limited due to its nephrotoxic and hepatotoxic side effects. This study was therefore designed to study the protective effects of Alpha Lipoic Acid (ALA) in 5-Fluorouracil-induced nephrotic damage in Wistar rats. Intraperitoneal injection of 50 $mgkg^{-1}$ of 5-FU was associated with significant ($p \leq 0.05$) increases in the urea, uric acid creatinine, and potassium levels. However, intraperitoneal injection of 50 $mgkg^{-1}$ of 5-FU was associated with significant ($p \leq 0.05$) decrease in the sodium and magnesium levels in the toxicity model control when compared with untreated control rats. Pretreatment and posttreatment with ALA attenuated these changes. This is indicative of the nephroprotective potential of ALA in 5-FU-induced kidney injury.

Keywords: Alpha Lipoic Acid, 5-Fluorouracil, Kidney, Wistar rats.

Introduction

The kidney is a very vital organ needed by the body to carry out numerous essential functions including the preservation of homeostasis, control of the extracellular environment, like detoxification, and excretion of drugs and toxic metabolites. Therefore, the kidney can be regarded as a major target organ for the excretion of exogenous toxicants. Nephrotoxicity is a kidney-specific characteristic in which excretion does not go efficiently due to toxic chemicals or drugs.[1]

5-Fluorouracil (5-FU) is a fluoropyrimidine antimetabolite agent that plays a significant role in the treatment of cancers.[2] 5-Fluorouracil (5-FU) is a chemotherapeutic agent that functions throughout the S phase of the cell cycle.In the cancerous cell, it interferes with nucleoside metabolism and is incorporated into ribonucleic acid (RNA) and deoxyribonucleic acid (DNA), resulting in cytotoxicity and cell death.[2] 5-FU, thymidine phosphorylase activates thromidylate synthase inhibiting fluorodeoxyuridine, thus preventing DNA synthesis.This leads to cell growth and ultimately cell death. In addition, 5-FU is metabolized to 5-fluorouridine monophosphate (5-FUMP), which degrades its function by binding to RNA. 5-FU is metabolized by the liver and has a half-life of 10 min.5-FU is extensively metabolized in the liver and the production of toxic intermediate may trigger liver injury. 5-Fluorouracil (5-FU) is one of the most widely used antineoplastic drugs, mainly because of its efficacy against various malignancies.5-FU is a fluoropyrimidine antimetabolite agent, used to treat cancer of colon, breast, gastrointestinal, head, neck, and pancreas. However, serious toxicity and unwanted side-effects occur following its use and it is considered to be a nephrotoxic compound. A number of studies have been conducted on the use of natural therapies to avoid the side-effects of anticancer agents.[3]

Alpha-lipoic acid (ALA) is a naturally occurring dithiol compound synthesized from octanoic acid in the mitochondrion and acts as a coenzyme for the mitochondrial respiratory enzymes.[4] It is able to protect cells and tissues from ROS and free radicals due to its antioxidant properties. Diseases mediated by oxidative stress are treated effectively with alpha lipoic according to several reports.[5]

The objective of the current study was to determine the protective potential of ALA on 5-FU induced nephrotic injury in Wistar rats.

Materials and Methods

Animals

Wistar rats of either sexes (225 ± 5 g) were obtained from the Faculty Animal House Department of Pharmacology and Therapeutics, Ahmadu Bello University Zaria They were given access to pelletized growers marsh and water *ad libitum*. All experimental protocols were as approved by the University Animal ethics committee. The rats were acclimatized for two weeks in the home cages and environment before commencement of the experiment. All experimental protocols were in accordance with the Ahmadu Bello University research policy (NIH publication number 85-23, revised1996) and of regulations governing the care and use of experimental animals with ethical approval number ABUCAUC/2020/013.

Chemicals

Alpha lipoic acid (ALA) was obtained from Sigma Chemical Company, 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*.[6] Wistar rats were randomly divided into six different groups (n=6 each). The first group received normal saline (1 mL/kg) and used as a negative control. The second group received a daily dose of

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(1 mL/kg) and 5-fluorouracil (50 mg/kg body weight, orally for four days. This served as the positive control. The third group received silymarin orally, 100 mg/kg bwt. The fourth group received a daily dose of ALA (100 mg/kg body weight, orally),[7] and the fifth group received a daily dose of ALA (200 mg/kg body weight, orally). The Silymarin and alpha lipoic acid treatments were given an hour before 5-fluorouracil administration. They served as the prophylactic groups. The sixth group received 5-fluorouracil (50 mg/kg body weight, orally) for four days and 400 mg/kg alpha lipoic acid for 14 days. This served as the hepatocurative group. All the experimental animals were sacrificed at the end of the experimental procedures under chloroform anesthesia. At the end of the experimental period all rats were sacrificed and blood was collected, by carotid bleeding, in centrifuge tubes. Serum was separated and used freshly for the assessment of kidney function tests. Kidneys were quickly harvested and immediately stored at -20ºC till further biochemical estimations.

Biochemical analysis

Serum urea was assessed using the method described by Fawcett and Scout.[8] Creatinine was estimated according to the method of Bartels and Bohnzer.[9] Uric acid was estimated in the serum according to the method of Pileggi *et al.*[10] Potassium and Sodium were estimated using the method of Kokko and Tannen.[11] Serum magnesium was estimated using the method described by Ajith *et al.*[12]

Histopathology study

After the animals were euthanized, kidneys were removed from the rats and fixed in 10% formalin for at least 48 h. They were then processed routinely, and the tissues were embedded in paraffin wax. Histological sections were cut at 5 – 6 µm and stained with routine haematoxylin and eosin (H & E). Detailed microscopic examinations were carried out by a consultant histopathologist. Photomicrographs of the organs were taken at various magnifications (× 100, × 250, and × 400).

Statistical analyses

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, Inc., La Jolla, CA, USA) The differences were considered statistically significant when $p \leq 0.05$.

Results and Discussion

The Study investigated the potential protective effect of ALA against 5-fluorouracil-induced kidney damage. 5-FU is used in the treatment of cancers. Some of these cancers include that of the pancreas, neck. head, gastrointestinal tract, breast and colon.[3] It is however found to be hepatotoxic and nephrotoxic. The study assessed the ameliorative potential of alpha lipoic acid in 5-FU induced nephrotoxicity of Wistar rats.

Intraperitoneal injection of 50 $mgkg^{-1}$ of 5-FU was associated with significant ($p \leq 0.05$) increase in the urea, uric acid and creatinine levels in the toxicity model control when compared with untreated control rats (Table 1). However, daily oral pre-treatment with 100 $mgkg^{-1}$(ALA and Silymarin), 200 $mgkg^{-1}$(ALA) and 400 $mgkg^{-1}$ ALA (posttreatment) before and after 5-FU injection significantly ($p<0.05$) attenuated increase in the urea,uric acid and creatinine levels in a dose-dependent fashion when compared to the toxicity model group. 5-FU is a known nephrotoxicant[13,14] and this was confirmed in the present study by the elevated urea and creatinine levels in the toxicity control model as compared to the negative control. Co-treatment with alpha lipoic acid significantly reduced the creatinine and urea levels when compared to the positive control. Urea and creatinine taken together gives very accurate estimation of kidney function; however, creatinine is a more accurate predictor of kidney damage or injury than urea, though both the liver and kidney must be functioning properly for the body to maintain a normal level of urea in the blood.[15]

Diseases mediated by oxidative stress are treated effectively with alpha lipoic.[5] It also reduces apoptosis in the liver because of its ability to reduce oxidative stress.[16] It protects cells and tissues from ROS and free radicals due to its antioxidant properties .[17] 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.[7,18] ALA and DHLA derivatives also have anti-inflammatory activities.[19] Alpha lipoic acid interacts with other antioxidants as well as thiols and changes cellular metabolic processes. It also changes the redox status of cells.[20] It reduces the oxidized forms of other antioxidants, chelates metal ions. It is amphiphilic antioxidant that quenches reactive oxygen species.[21]

Intraperitoneal injection of 50 mgkg-1 of 5-FU was associated with significant ($p \leq 0.05$) increase in the potassium levels in the toxicity model control when compared with untreated control rats (Table 2). However, daily oral pre-treatment with 100 $mgkg^{-1}$(ALA and Silymarin), 200 $mgkg^{-1}$(ALA) and 400 mgkg-1 ALA (posttreatment) before and after 5-FU injection significantly ($p < 0.05$) attenuated increase in the potassium levels in a dose-dependent fashion when compared to the toxicity model group .Also daily oral pre-treatment with 100 mgkg-1(ALA and Silymarin), 200 $mgkg^{-1}$(ALA) and 400 $mgkg^{-1}$ ALA (posttreatment) before and after 5-FU injection non-significantly ($p > 0.05$) attenuated increase in the potassium level in a dose-dependent fashion when compared to the untreated control group. However, intraperitoneal injection of 50 $mgkg^{-1}$ of 5-FU was associated with significant ($p \leq 0.05$) decrease in the sodium and magnesium levels in the toxicity model control when compared with untreated control rats (Fig. 7-12). However, daily oral pre-treatment with 100 mgkg-1(ALA and Silymarin), 200 mgkg-1(ALA) and 400 mgkg-1 ALA (posttreatment) before and after 5-FU injection significantly ($p < 0.05$) attenuated decrease in the sodium and magnesium levels in a dose-dependent fashion when compared to the toxicity model group. Also daily oral pre-treatment with 100 mgkg-1(ALA and Silymarin), 200 $mgkg^{-1}$(ALA) and 400 $mgkg^{-1}$ ALA (posttreatment) before and after 5-FU injection non-significantly ($p>0.05$) attenuated decrease in the sodium and magnesium level in a dose-dependent fashion when compared to the untreated control group. In the same vein, the nephrotoxic effects of subacute 5-FU toxicity may also be due to oxidative stress caused by free radical production and the subsequent depletion of intracellular antioxidant enzymes, ultimately elevating serum levels of creatinine and urea.[22]

When 5-FU is given intravenously in the treatment of breast and gastrointestinal cancers, it is metabolized in tissues to its active form, 5-fluoro-deoxyuridinemonophosphate, which inhibits thymidylate synthase.5-FU is also catabolized primarily in the liver, as dihydrouracil, and the reduced compound is then cleaved to α-fluoro-β-alanine, ammonia, urea, and carbon dioxide which cause the hepatic and nephrotoxicity. The toxicity may be decreased if the catabolism is blocked by inhibiting dihydrouracil dehydrogenase. Protein depletion results in increased toxicity to 5-FU, which is associated with a significantly decreased rate of hepatic metabolism and clearance of 5-FU and a significant decrease in hepatic DPD activity. In this study 5-FU was found to cause significant kidney injury as evidenced in the histopathology and biochemical parameters. Plate I depicts architecture of normal rat liver showing intact glomerulus and tubules.. However, intraperitoneal injection of 50 $mgkg^{-1}$ of 5-FU was associated with remarkable tubular distortion.(B) when compared to normal liver architecture of the kidney (A).Rat kidneys pretreated with 100 $mgkg^{-1}$ (ALA and Silymarin) and 400 $mgkg^{-1}$ (ALA) Showed moderate tubular necrosis (C) while rat kidneys pretreated with pretreated 200 $mgkg^{-1}$ ALA and 100 $mgkg^{-1}$ silymarin showed normal kidney features (D and E,

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respectively). Histological examination of the kidney showed that 5-FU treatment caused abnormal ultrastructural alterations in these organs. This is consistent with the Afolabi *et al*.[2] In ALA treated animals, the changes were less compared to the 5-FU group, showing that ALA administration could improve the changes brought about by the drug on the kidney. Abidem *et al*.[23] also previously reported that Photomicrographs from the kidney samples showed cortical necrosis and thin glomeruli basement membrane caused by 5-FU and signs of local hemorrhage due to 5-FU. This confirms the nephrotoxic effects of 5-FU observed from the results of the biochemical analysis in which case deterioration of renal function, indicated by increase in creatinine and urea, was observed. Gelen *et al*.[3] also reported that histopathological examination of renal tissues in the 5-FU- treated group suggested cell injuries and necrosis in the liver and renal tissues.[24,25] Kidney tissue assessment, degeneration, and/or necrosis of the renal tubular epithelial cells were identified due to 5-FU administration.[3]

Table 1: Effect of alpha lipoic acid on Renal Function Parameters (Urea, Creatinine and Uric Acid) of 5-fluorouracil induced Toxicity in Wistar Rats

Groups	Urea (mmol/L)	Creatinine(mg/dL)	Uric acid (mg/dL)
Normal Saline (NS-1 mL/kg)	10.55 ± 1.223	1.815 ± 0.2978	32.87 ± 4.084
NS (1 mL/kg) + 5-FU (50 mg/kg)	32.47 ± 2.238[a]	11.32 ± 0.6058[a]	127.7 ± 5.244[a]
ALA (100 mg/kg) + 5FU (50 mg/kg)	18.57 ± 0.5506[b]	2.883 ± 0.1851[b]	45.07 ± 2.480[b]
ALA(200mg/kg) + 5FU(50mg/kg)	13.63 ± 0.8728[b]	3.233 ± 0.1476[b]	52.12 ± 1.579[b]
5FU (50 mg/kg) + ALA (400 mg/kg)	19.98 ± 1.530[b]	3.583 ± 0.2386[b]	61.37 ± 2.113[b]
Silymarin (100 mg/kg) + 5FU (50 mg/kg)	16.40 ± 0.5228[b]	2.083 ± 0.1851[b]	36.03 ± 1.870[b]

Values are presented as mean ± SEM. Data was analyesd using Oneway ANOVA followed by tukey post hoc test. [a]$p \leq 0.05$ significant difference as compared to the negative control group (Normal Saline-1ml/kg). . [b]$p \leq 0.05$ significant difference as compared to the model or toxicity control group (Normal Saline-1mL/kg + 5-Fluorouracil-50mg/kg).

Table 2: Effect of alpha lipoic acid on Electrolytes (Sodium, Potassium and Magnesium) of 5-fluorouracil induced Toxicity in Wistar Rats

Group	Sodium (mmol/L)	Potassium (mmol/L)	Magnesium (mg/dL)
Normal Saline (NS-1 mL/kg	54.83 ± 3.422	5.567 ± 0.3904	28.83 ± 2.455
NS (1 mL/kg) + 5-FU (50 mg/kg)	172.5 ± 3.544[a]	44.27 ± 1.916[a]	8.133 ± 1.203[a]
ALA (100 mg/kg) + 5FU (50 mg/kg)	202.5 ± 3.462[b]	13.22 ± 0.3842[b]	44.50 ± 1.945[b]
ALA (200 mg/kg) + 5FU (50 mg/kg)	182.2 ± 3.452[b]	8.500 ± 0.5502[b]	74.17 ± 2.056[ab]
5FU (50 mg/kg) + ALA (400 mg/kg)	154.2 ± 7.245[b]	16.63 ± 0.4161[b]	32.93 ± 1.752[b]
Silymarin (100 mg/kg) + 5FU (50 mg/kg)	132.7 ± 4.061[b]	11.42 ± 0.4556[b]	55.80 ± 3.228[b]

Values are presented as mean ± SEM. Data was analyesd using Oneway ANOVA followed by tukey post hoc test. [a]$p \leq 0.05$ significant difference as compared to the negative control group (Normal Saline-1 mL/kg). . [b]$p \leq 0.05$ significant difference as compared to the model or toxicity control group (Normal Saline-1 mL/kg + 5-Fluorouracil-50 mg/kg).

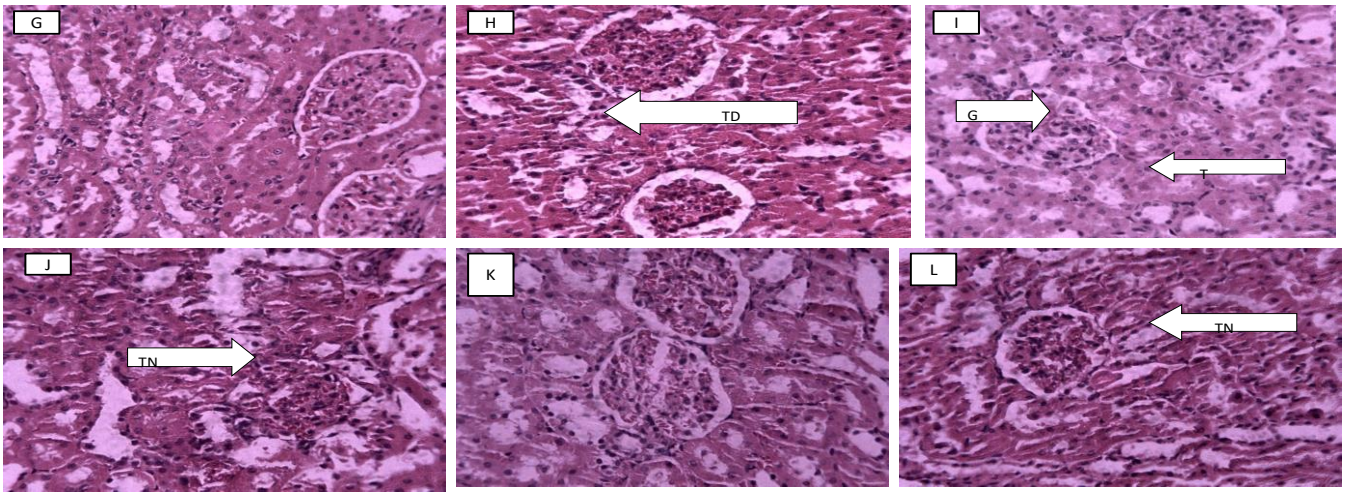

Plate I: Photomicrograph of Kidney from: (G) Normal Saline, NS (1 mL/kg) treatment. Kidney shows normal features. Magnification x10 (H and E stain) **(H)** NS (1 mL/kg) + 5-Fluorouracil (50 mg/kg) treatment. Kidney shows tubular distortion (TD). Magnification x 10 (H and E stain). **(I)** Silymarin (100 mg/kg) Treatment. kidney shows normal glomerulus **(G)** and tubule (T). Magnification x10 (H and E stain). **(J)** Alpha lipoic acid (100 mg/kg) treatment. Kidney shows Moderate Tubular Necrosis (TN). Magnification x 10 (H and E stain). **(K)** Alpha lipoic acid (200 mg/kg) treatment. Kidney shows normal features. Magnification x 10 (H and E stain). **(L)** Alpha lipoic acid (400 mg/kg) treatment. Kidney shows moderate tubular necrosis (TN). Magnification x 10 (H and E stain).

Conclusion

Alpha Lipoic acid has demonstrated an ample potential of mitigating the nephrotoxic effects of 5-FU-induced kidney damage. More research needs to be carried out on its clinical usefulness in humans with nephrotoxicity.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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