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Original Research Article

ANTIOXIDANT EFFECTS OF L-ARGININE ON REDOX PROFILE OF CARBON TETRACHLORIDE (CCl_4) - INTOXICATED ALBINO RATS

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ABSTRACT

Acute exposure to carbon tetrachloride causes anaemia, electrolyte imbalance (hyponatremia and hyperkalemia) and hepatic centrilobular necrosis. This study aimed to investigate some biochemical effects of L-arginine on carbon tetrachloride-intoxicated albino rats. Thirty-six mature male albino rats of average weight, 135g were randomly assigned to six groups of six rats each. Group A (control) fed with normal rat chows and water, group B (negative control) received 1ml/kg of CCl_4 dissolved in 0.2mls of distilled water, group C (L-arginine) administered 50mg/kg of L-arginine only, group D (L-arginine+ CCl_4) administered 25mg/kg of L-arginine and 0.5ml/kg of CCL_4, group E (L-arginine+ CCl_4) administered 50mg/kg of L-arginine and 0.5ml/kg of CCL_4 and group F (L-arginine + CCl_4) administered 100mg/kg of L-arginine and 0.5ml/kg of CCL_4. Treatment was daily, per os and lasted for 14 days. The result of reduced glutathione (GSH), glutathione perioxidase (GPx) and superoxide dismutase (SOD) activities showed significant reduction in all treated groups. Treatment with L-arginine recorded a significant increase ($p<0.05$) in glutathione perioxidase when compared with the control. Histological examination reveals that treatment of albino rats with L-arginine at 50mg/kg showed normal hepatic histo-architecture. However, treatment with the negative control, and other groups showed the characteristics histological presentation of CCl_4 induced liver damage. Kidney section of all treated groups showed normal renal histo-architecture, with normal renal tubule. This study therefore suggests that L-arginine could be an alternate therapeutic agent in restoring reduced glutathione and glutathione perioxidase.

Keywords: Anemia, Electrolyte imbalance, Hepatic centrilobular necrosis, Histo-architecture, Hyperkalemia, Hyponatremia

INTRODUCTION

L-arginine is an amino acid that is considered essential for proteogenesis. It occurs naturally in the proteins that make up our diets. It is abundant in seafoods, watermelon, nuts, seeds, seaweed, meat, fish, concentrated rice and soy proteins (Bescos*et al.,* 2012). L-arginine plays an important role in the metabolism of an organism. It is the precursor for the synthesis of proteins, nitric oxide (NO) and other molecules of great biological importance to the human body. NO is generated in the presence of oxygen and by the action of the enzyme nitric oxide synthase (NOS), of which three isoforms are known: neuronal (nNOS), inducible (iNOS) and endothelial (eNOS) (Centeno *et al.,* 2017). For young organisms, L-arginine is an essential amino acid for optimal growth and development. Therefore, it must be provided in the diet. For adults, L-arginine is a semi-essential or conditionally essential amino acid, especially in such conditions as trauma, burn injury, small bowel resection, and renal failure (Scibior & Czeczot, 2004). In adults, L-arginine is produced in the kidneys from circulating citrulline synthesized by enterocytes in the small intestine (Wouter *et al.*, 2002; Centeno *et al.,* 2017).

Oral L-arginine is emerging as a new effective approach of reducing blood pressure by improving endothelial function in hypertensive patients reported following a placebo-controlled study which included 54 individuals including 19 healthy individuals (Control) and 35 patients with hypertension (Ast *et al.,* 2010). The patients received either

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L-arginine (2 or 4 g three times daily) or received placebo. One-month L-arginine supplementation at a dose of 12 g daily was associated with considerable lowering of systolic and diastolic blood pressure (Ast *et al.*, 2010).

Carbon tetrachloride also called tetrachloromethane and perchloromethane is a colourless liquid with a sweetish odour (Peters, 2024). CCl_4 has been used as Industrial Solvent (to dissolve oils, fats, lacquers, and rubber), cleaning agent, refrigerant, and in fire extinguishers. Individuals may be exposed to CCl_4 through broken antique fire extinguishers, inhalation or skin contact (Malik, 2014). Carbon tetrachloride has been established as a hepatotoxin which induces toxicity in rats in a manner similar to human cirrhosis (Mrwad *et al.*, 2025). Experimentally, liver diseases have similarly been caused by the administration of (CCl_4), thioacetamide, paracetamol, etc (Singh *et al.*, 2024). The CCl_4-induced hepatotoxicity model is frequently used for the investigation of hepatoprotective effects of drugs and plant extracts in experimental animals. The CCl_4 hepatotoxicity arises from the CY450- catalyzed reductive dehalogenation of CCl_4 in the smooth endoplasmic reticulum of hepatocytes, leading to the generation of an unstable complex tricholoromethyl (CCl_3) radical. This trichloromethyl radical reacts rapidly with O_2 to yield tricholomethyl peroxy radical which has been reported to be a highly reactive species. These free radicals attack microsomal lipids leading to their peroxidation and also bind covalently to microsomal lipids and proteins to initiate a site of secondary biochemical processes that ultimately cause the pathological consequences of CCl_4 metabolism (De Groot & Haas, 1980; Evan *et al.*, 2011).

The administration of CCl_4 produces pathological changes not only in liver but also in different organs (Dashti *et al.*, 1995). In their studies, Patil *et al.*, (1989) and Enstar, (2012), reported that the exposure to CCl_4 caused significant alterations in the lipolytic activities of liver, adipose tissue and the kidney. The administration of CCl_4 induces oxidative stress via the production of free peroxy radicals and lipoperoxides thereby damaging proteis, DNA and lipids (Khan & Younus, 2011). Gluthathione (GSH) is a crucial determinant of tissue susceptibility to oxidative damage, and the depletion of hepatic Gluthathione content has been shown to be associated with the enhanced toxicity of many chemicals, including CCl_4 (Navarro *et al.*, 1999; Entsar, 2012). Liver is the main target for CCl_4 toxicity while the kidney is the main site of CCl_4 accumulation. Despite carbon tetrachloride is widely used in industries, it induces oxidative damage and liver injury due to its ability to generate free radicals via cytochrome P450 metabolism. L-Arginine is a semi-essential amino acid known for its roles in nitric oxide (NO) synthesis, immune function, vascular health, and antioxidant defence (Pendrazini *et al.*, 2024). However, this study examines the ability of L-arginine to ameliorate various effects propelled by intoxication of Albino rats with carbon tetrachloride.

MATERIALS AND METHOD

ANIMALS

Thirty-six (36) male Albino rats (Average weight, 135±2.34g) were obtained from the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike. The animals were housed in cages under hygienic conditions and were fed with rat chow and water *ad labium.*

EXPERIMENTAL DESIGN

Animals were randomly assigned into six groups of (n=6)

Group A- served as control group and fed with normal rat chow and water for 2 weeks

Group B- served as the negative control fed with 1mls/kg of CCL_4 dissolved in 0.2mls of distilled water.

Group C- were administered 50mg Kg of L-Arginine, dissolved in 0.2ml of distilled water.

Group D- were administered 25mg Kg of L-arginine and 0.5mls/kg of CCL_4 dissolved in 0.1mls of distilled water

Group E- were administered 50mg kg of L arginine and 2mls/kg of CCL_4 dissolvedin 0.4mls of distilled water.

Group F- were administered 100mg/kg L-Arginine and 2mls/kg of CCL_4 dissolved in 0.4mls of distilled water.

The rats were sacrificed on the fifteenth day of experiment.

BLOOD COLLECTION AND SEPARATION

After two weeks, the animals were sacrificed by cervical dislocation, 24hours after the completion of the final round of treatments. Blood samples were obtained via heart puncture. The whole blood samples, were allowed to cloth in plain sterile test tubes, and the resulting sera separated for other biochemical assays. The liver and kidneys of the rats were obtained by a midline abdominal incision that passed through the abdominal wall musculature into the peritoneal cavity. The tissues were exacted, rinsed with cold saline solution, and preserved in a 10% normal saline solution for further histological fixation and analyses.

ANTIOXIDANT ENZYMES DETERMINATION

SUPEROXIDE DISMUTASE

The activity of the superoxide dismutase was evaluated as described by Sun and Zigman (1978).

REDUCED GLUTATHIONE (GSH) ESTIMATION

The Ellman (1959) method as described by Alam *et al.* (2013) and Sapakal *et al*. (2008) was used in the estimation of reduced glutathione level.

CATALASE ACTIVITY

The catalase activity in each serum sample was determined as described by Goth (1991).

GLUTATHIONE PEROXIDASE DETERMINATION

The activity of glutathione peroxidase was determined by a popular method described by Rotruck *et al.*, 1973 and modified by Ayeleso *et al.* (2018).

HISTOLOGICAL STUDY

After 2 weeks of experimental period, the excised organs (Liver, Kidneys and Testes) were transferred into 10% saline solution, subsequently preserved in 10% formaldehyde solution, and then embedded in paraffin wax and sectioned into 4-6 microns. The sections were stained with haematoxylin and eosin and photographed.

RESULTS

ANTIOXIDANT PARAMETERS

The result in table 1 below showed that GSH level in the normal control group (Group A) was 12.76 ± 0.44 u/L. However, exposure to carbon tetrachloride (CCl_4) in the negative control group (Group B) significantly depleted GSH levels to 8.97 ± 0.05 u/L. L-Arginine alone at 50 mg/kg (Group C) markedly increased GSH levels to 15.44 ± 0.38 u/L. In groups co-treated with CCl_4 and L-Arginine at 25 mg/kg (Group D) and 50 mg/kg (Group E), GSH levels were moderately restored to 10.19 ± 0.18 u/L and 10.20 ± 0.28 u/L, respectively.

In Group A (normal control), GPx activity was 38.33 ± 0.72 u/L. CCl_4 treatment alone (Group B) led to a significant reduction in GPx activity to 27.51 ± 0.69 u/L. In contrast, animals treated solely with L-Arginine (Group C) exhibited elevated GPx levels of 39.66 ± 0.96 u/L. Group E (CCl_4 + 50 mg/kg L-Arginine) also showed an appreciable improvement in GPx activity (34.26 ± 0.92 u/L), while GPx was not reported for Groups D and B.

In the normal control group (Group A), SOD activity was 31.46 ± 0.71 u/L. Exposure to CCl_4 (Group B) drastically reduced SOD levels to 17.10 ± 0.68 u/L. While SOD activity was not reported for Group C (L-Arginine only), the group co-treated with CCl_4 and 50 mg/kg of L-Arginine (Group E) recorded a significant increase to 30.03 ± 0.69 u/L nearly equivalent to the normal value.

In the normal control group (Group A), CAT activity was measured at 25.16 ± 0.80 u/L. CAT was only measured in Group E among the treated groups, and the activity was significantly elevated to 30.03 ± 0.69 u/L.

HISTOLOGY RESULT

The result in Figure I showed normal hepatocytes arranged in interconnecting cords around the central veins (v). Normal components of the portal triads (hepatic artery, hepatic vein and bile ducts) were also observed at the portal area (P).

Figure I: Photomicrograph of the liver section of rats in group A (control) Magx100. Central veins = V; Portal area = P.

The result in Figure II showed normal glomeruli (G) in their respective Bowman's capsules, surrounded by a sea of normal renal tubules (arrow) and suspended in a highly vascularized connective tissue matrix.

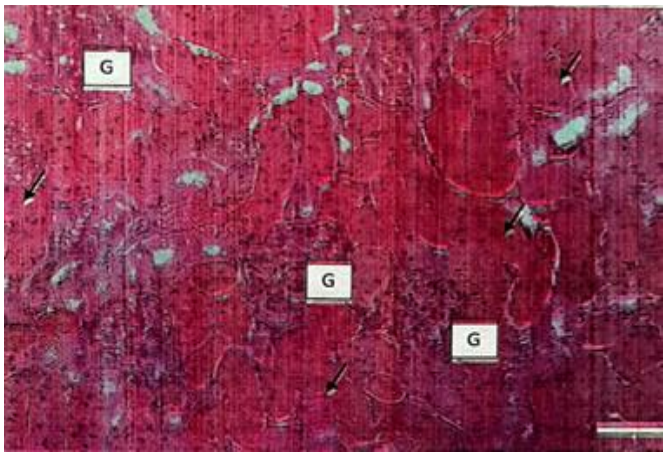

Figure II: Photomicrograph of the kidney section of rats in group A Magx100. G = Glomeruli

The result in Figure III showed the hepatocytes in the centrilobular and mid-zonal areas of the hepatocytes showed marked vacuolar degeneration and necrosis (arrow). The hepatocytes in the peripheral areas of the hepatic lobules showed a relatively normal hepatocyte population at the central vein (V) and portal area (P).

TABLE I: ANTIOXIDANT PARAMETERS OF DIFFERENT EXPERIMENTAL GROUPS

Treatment groups	GSH (u/l)	GPx(u/l)	SOD(u/l)	CAT(u/l)
Group A: Normal control	12.76±0.44[a]	38.33±0.72	31.46±0.706[a]	25.16±0.80[a]
Group B: Negative control (CCl4 only)	8.97±0.05[d]	27.51±0.69[a]		17.10±0.68[b]
Group C: L-Arginine only (50mg/kg)	15.44±0.38[c]	39.66±0.96[c]		
Group D: CCl_4+L-Arginine (25mg/kg)	10.19±0.18[b]			
Group E: CCl4+L- Arginine (50 mg/kg)	10.20±0.28[b]	34.26±0.92[b]		
Group E: CCl4+L-Arginine (100mg/kg)			30.03±0.69[b]	

Results are presented as Mean ± SEM. Means within a column with same alphabets superscript (such as a, a and b, b) are not significantly different, while, means within a column with different alphabets superscripts (such as a, b and b, c) are significantly different P<0.05.

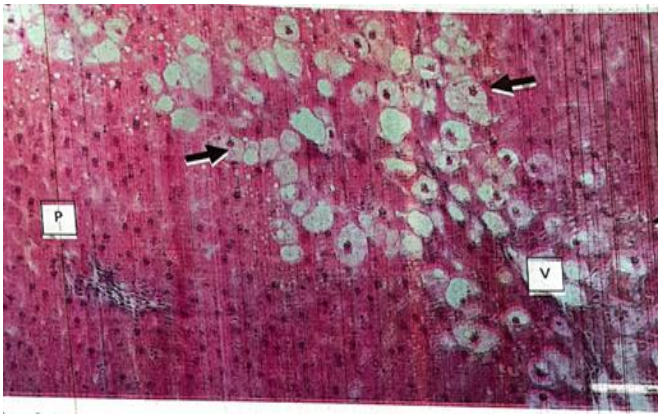

Figure III: Photomicrograph of the liver section of rats in group B Magx100. Central veins = V; Portal area = P.

The result in Figure IV showed normal glomeruli (G) in their respective Bowman's capsules, surrounded by a sea of normal renal tubules (arrow) and suspended in a highly vascularized connective tissue matrix.

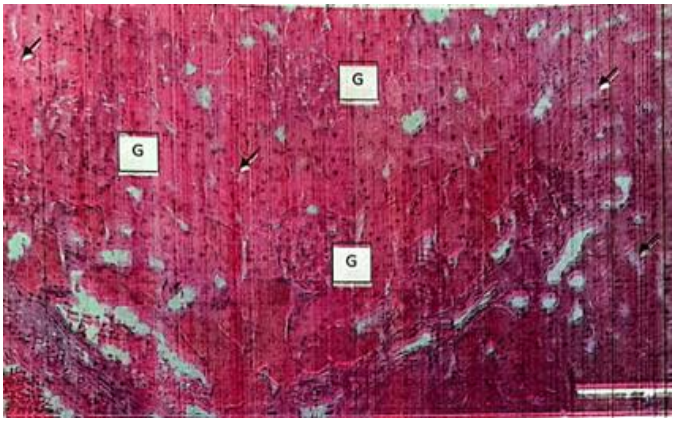

Figure IV: photomicrograph of the kidney section of rats in group B Magx100. G = Glomeruli

The result in Figure V showed that normal hepatocytes arranged in interconnecting cords around the central vein (V). Normal components of the portal triads (hepatic artery, hepatic vein, and bile duct) were also observed around the portal area (P).

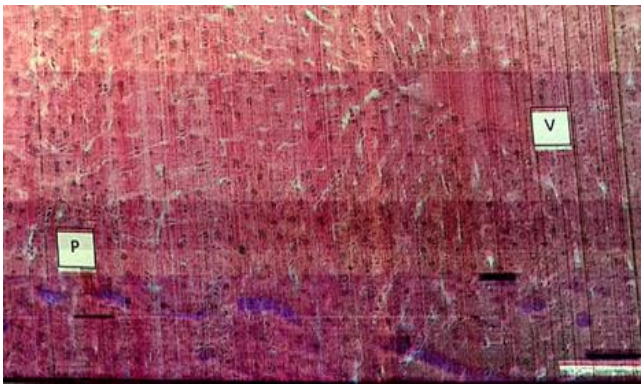

Figure V: Photomicrograph of the liver section of rats in group C Magx100. Central veins = V; Portal area = P.

The result in Figure VI showed normal glomeruli (G) in their respective Bowman's capsules, surrounded by a sea of normal renal tubules (arrow) and suspended in a highly vascularized connective tissue matrix.

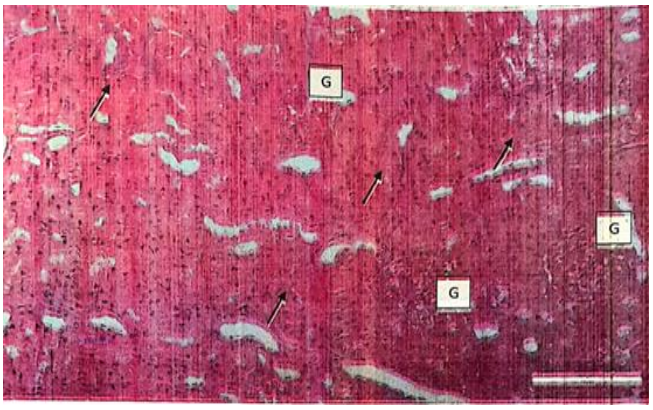

Figure VI: Photomicrograph of the kidney section of rats in group C Magx100. G = Glomeruli

The result in Figure VII showed the hepatocytes showed marked vacuolar degeneration and necrosis (arrow). The hepatocytes in the peripheral area of the hepatic lobules

showed a relatively normal hepatocyte population around the portal area (P).

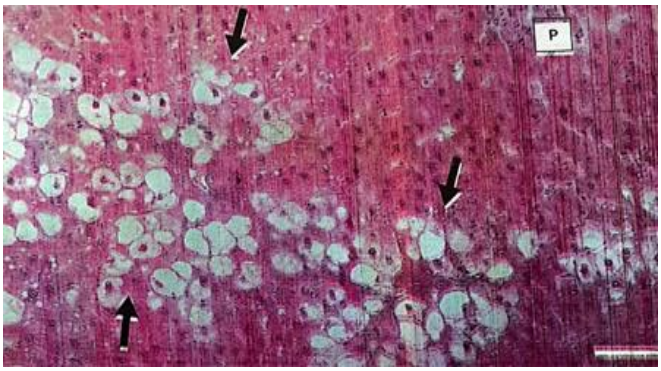

Figure VII: Photomicrograph of the liver section of rats in group D Magx100. Central veins = V; Portal area = P.

The result in Figure VIII showed normal glomeruli (G) in their respective Bowman's capsules, surrounded by a sea of normal renal tubules (arrow) and suspended in a highly vascularized connective tissue matrix.

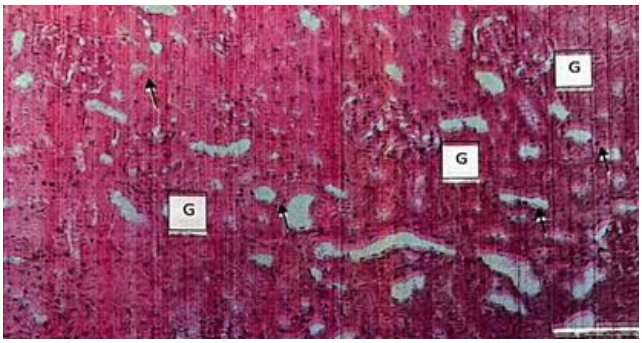

Figure VIII: Photomicrograph of the kidney section of rats in group D Magx1. G = Glomeruli

DISCUSSION

The results of the present investigation indicate that exposure to CCI_4 resulted in a considerable reduction in the activity of antioxidant enzymes (SOD, catalase, GR, GST, and GPx) in the liver, when compared to the control group. This finding is consistent with the findings of Dalaen *et al*. (2016) and indicated that the administration of L-arginine before and after CCI_4-induced intoxication in rats significantly increased the activity of hepatic antioxidant enzymes. According to a study conducted by Adawi *et al.* (1996), the administration of oral arginine supplementation demonstrated a significant reduction in the severity of liver injury in an acute liver injury model.

Organ histological-architecture is an important aspect of toxicological studies. Intoxication of rats with CCL_4, induced hepatotoxicity. This became evident in the negative control group, and other CCl_4-treated groups which showed the characteristic histological presentation of CCl_4-induced liver damage. The hepatocytes in the centrilobular and mid-zonal areas of the hepatocytes showed marked vacuolar degeneration and necrosis. Treatment of CCl_4 intoxicated group with L-arginine at 50 mg/kg showed the normal hepatic histo-architecture, indicating normal hepatocytes arranged interconnecting cords around the central veins. This reflects hepatic protection of L-arginine at 50 mg/kg. Kidney section of all treated groups including the negative control group showed the normal renal histo-architectures. These sections showed normal glomeruli in their respective Bowmans capsules, surrounded by a sea of normal renal tubules and suspended in a highly vascularized connective tissue matrix. This is in agreement with Li *et al.,* (2024); which stated that the kidney is the main site of CCl_4 accumulation. Hence, pathogenesis of CCl_4-induced renal dysfunction is not completely understood.

CONCLUSION

This study demonstrated that L-arginine possessed significant antioxidant, hepat-oprotective and nephron-protective effects against carbon tetrachloride-induced oxidative stress. The administration of CCl_4 led to reduction in the activities of endogenous antioxidant enzymes. The co-treatment with L-arginine mitigated the oxidative damage and enhanced reduced glutathione and glutathione perioxidase activity. The histology analysis showed the ability of L-arginine to preserve liver and kidney architecture with minimal necrosis.

CONFLICT OF INTEREST

No relevant conflict of interest relevant to this article was reported.

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