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EC PHARMACOLOGY AND TOXICOLOGY
Research Article

Effect of $_{L}$-Arginine on Some Bio-indicators of Hepatic Functions of Paracetamol-Intoxicated Rats

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Received: April 28, 2020; **Published:** August 28, 2021

Abstract

Paracetamol (Acetaminophen) in toxic doses caused hepatic centrilobular necrosis while L-arginine could offer wide range of bio-benefits. Thus, this study investigated the effect of L-arginine on some bio-indicators of hepatic functions of Paracetamol-intoxicated Wistar rats. Twenty five rats (average weight, 88.00 g) were randomly allocated to five groups (n = 5). Rats in Group A (Control) were fed with feed and water while those in Group B (Arginine) were administered 60 mg/ kg body weight of L-arginine. Rats in Group C (Paracetamol) were administered 1000 mg/kg body weight of Paracetamol whereas those in Group D (Arginine/Paracetamol) were administered L-arginine and paracetamol at 60 mg/kg and 1000 mg/kg body weight, respectively while rats in Group E (High dose Arginine/Paracetamol) were administered 120 mg/kg and 1000 mg/kg body weight of L-arginine and Paracetamol, respectively. Treatment was daily and per oral for 14 consecutive days. Results showed that, compared with the control, alanine aminotransferase (ALT) activity was significantly increased ($p < 0.05$) in paracetamol-intoxicated group of rats but reduced ($P < 0.05$) in rats exposed to L-arginine together with intoxicating dose of Paracetamol. A converse but similar trend was observed in the albumin concentration of the rats. Aspartate aminotransferase (AST) and total bilirubin activities were significantly increased ($p < 0.05$) in all treated groups, compared with the control. Alkaline phosphatase (ALP) and total serum protein decreased significantly when compared with the control. Thus, L-arginine may mitigate Paracetamol-induced adverse effect on some bio-indicators of hepatic functions of rats.

Keywords: *L-Arginine; Paracetamol; Hepatic Functions; Alanine Aminotransferase; Albumin; Total Serum Protein; Aspartate Aminotransferase; Alkaline Phosphatase*

Abbreviations

ALT: Alanine Amino Transferase; AST: Aspartate Amino Transferase; ALP: Alkaline Phosphatase; TP: Total Protein; SPSS: Statistical Package for Social Sciences; LSD: Least Significant Difference; CNS: Central Nervous System; NAPO1: N-Acetyl-p-Quinone Imine

Introduction

Paracetamol (acetaminophen), a popular and commonly used analgesic and antipyretic drugs around the world, was discovered 100years ago [1]. Due to its availability, incident of accidental and intentional abuse are numerous. As a result of the high rate of abuse, paracetamol has been described as one of the most common cause of liver failure [2,3]. Paracetamol is metabolized in the liver *via* three pathways-glucuronidation, sulfation and hepatic cytochrome P450enzyme system. At intoxicated dose, paracetamol causes hepatic centrilobular necrosis [3], which has been linked with excessive generation of the highly toxic metabolite N-acetyl-P-benzo-quinone imine

(NAPQI). Paracetamol is oxidatively transformed to N-acetyl-P-benzo-quinone imine (NAPQI) by the Cytochrome P450 enzyme system particularly the CYP450 2E1 [4,5]. Endogenous glutathione binds to NAPQ1 and detoxifies it to a non toxic metabolite (Mercapturic acid) which is excreted in urine. However, at toxic level, hepatic glutathione depletion occurs when NAPQ1 formation exceeds the available supply of glutathione [5]. The undetoxified NAPQ1 eventually binds to cellular macromolecules [3,6] like cellular proteins resulting in impairment in mitochondrial respiration [7], opening of the mitochondrial permeability transition pores [8], elevation of the oxidative stress [8] as well as hepatic necrosis [9]. The risk of paracetamol toxicity increases with malnutrition [10], application of paracetamol combined with drugs inducing cytochrome P450 [11,12].

Paracetamol mechanism of action is dependent on the inhibition of prostaglandin and other pro-inflammatory chemical synthesis that takes place in the central nervous system (CNS) which blocks pain impulse generation. It provides relief from mild to moderate pain and fever [13,14].

$_{L}$-Arginine, a semi-essential amino acid found in many dairy products. [15]. It plays a role in several important mechanisms in the body; 'in cell division [16], in healing of wounds [15,18], in the removal of ammonia from the body, immune function, and the secretion of important hormones [19,20,21]. $_{L}$-Arginine is required for synthesis of proteins and serves as a precursor for synthesis of creatine, agmatine, urea, polyamines, proline, glutamate [22]. The body also uses arginine to synthesize nitric oxide, which relaxes the blood vessels (vasodilation) [23].

Bio indicators of hepatic functions such as: Serum Bilirubin, Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST) and Alkaline Phosphatase (ALP) are used for the investigation of suspected liver diseases and injuries [24].

Elevated levels of these bio-indicators of hepatic functions reveals hepatocellular damage, toxic or ischemic liver injury, liver tissue degeneration and necrosis [25,26].

Aim of the Study

In view of these, the present work aimed to evaluate the effect of $_{L}$-arginine on some Bio indictors of paracetamol intoxicated wistar rats.

Materials and Methods

Chemicals and reagents

$_{L}$-Arginine (Anala R) manufactuered by BDH Chemical Ltd, Poole England and purchased from Fecotex Chemical Ltd, Abia state, Nigeria, Paracetamol (Acetaminophen) manufactured by Emzor, procured from Orchad Pharmacy, Nigeria. All other chemical reagents were of analytical grades.

Concentration determination/justification

$_{L}$-arginine concentration (60 mg/kg b.wt) used in this study was based on the recommendation from WHO as used in previous studies [23,24-30]. Paracetamol dose used in this study (1000 mg/kg b.wt) was based on intoxicating doses used in earlier studies [31].

Animals

Twenty-five male Wistar rats (Average weight, 88.4g) procured from the animal house of the College of Vertinery, Micheal Okpara University of Agriculture Umudike. The animals were housed in cages under standard hygienic condition in accordance with the guidelines of the National Institute of Health, USA, for ethical treatment of laboratory animals as adopted by Ethical Committee of Michael Okpara University of Umudike.

Experimental design

Animals were randomly assigned into five groups of (n = 5). Group 1 and group from 2 to 5 served as control and experimental groups respectively. Group 2 were administered 60 mgkg body weight of $_{L}$-arginine dissolved in 0.2 mls of distilled water. Group 3 were administered 1000 mgkg body weight of paracetamol dissolved in 1.0 mls of distilled water. Group 4 were administered 60 mgkg (low dose) of $_{L}$-arginine and 1000 mgkg of paracetamol dissolved in 1.2 mls of distilled water. Group 5 were administered 120 mg kg (high dose) of L arginine and 1000 mg /kg of paracetamol dissolved in 1.4 ml of distilled water. Treatment was daily, oral intubation and lasted for 14 days. The rats were sacrificed on the fifteenth day of experiment.

Blood sample collection and preparation

24 hrs following the last set of treatments, the animals were sacrificed via cervical dislocation, Blood samples were collected through cardiac puncture. Whole blood were allowed to coagulate in plain containers, from where serum was separated for other biochemical assay, while the liver of the rats were harvested after dissecting through a midline abdominal incision passing through the abdominal wall musculature into the peritoneal cavity. The organs tissues were viewed, washed in cold saline and fixed in 10% normal saline for histopathological examination.

Biochemical assays

Determination of alanine aminotransferase (ALT)

ALT activity was determined following the principles described by Reitman and Frankel (1957).

Determination of aspartate aminotransferase (AST)

AST activity was determined following the principles described by Reitman and Frankel (1957).

Determination of total serum proteins

The principle is based on the fact that at alkaline pH 7.0, proteins form a stable complex with Cu^{2+}, which is photometrically measured.

Determination of total bilirubin

This was done according to the method of Jendrassik and Grof (1938) as contained in Randox assay kits manual.

Determination of alkaline phosphatase (ALP)

ALP activity was determined following the principles described by Englehardt (1970).

Statistical analysis

SPSS version 20 was used for the statistical analysis. Results were analyzed using mean ± Sem (Standard error of the mean). The differences between the groups were tested using the least significant difference (LSD) p-values < 0.05 were considered statistically significant.

Results

Groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Albumin (g/dl)	Protein (g/dl)	T-Bil (mg/dl)
Group 1 Control	61.60 ± 1.03	33.80 ± 0.37	11.00 ± 1.48	3.20 ± 0.12	7.94 ± 0.08	1.20 ± 0.12
Group 2 Arginine	65.00 ± 5.47	35.00 ± 0.84	5.25 ± 0.37*	3.05 ± 0.16	7.35 ± 0.50	1.43 ± 0.03*
Group 3 Paracetamol	81.50 ± 2.69*	37.25 ± 1.65*	6.25 ± 0.19*	2.43 ± 0.08*	5.60 ± 0.18*	1.45 ± 0.04*
Group 4 ARG & PARA	78.20 ± 1.32*	26.60 ± 1.03*	6.60 ± 0.24*	3.64 ± 0.19*	5.42 ± 0.12*	2.53 ± 0.05*
Group 5 High Dose ARG and PARA	72.25 ± 1.53*	43.25 ± 0.97*	8.75 ± 0.37*	2.18 ± 0.07*	5.38 ± 0.14*	1.46 ± 0.03*

Table 1: *Effect of $_{L}$-arginine on Bio-markers of Hepatic functions of paracetamol intoxicated wistar rat. Results = Mean ± SEM. Means marked * is significantly different from normal control (P < 0.05).*

Discussion

Following acetaminophen toxicity, activities of ALT and AST increased significantly in the positive control group. This is in line with the findings of Ghaffar and Jadvi [7] which showed dramatic increase in AST and ALT activities following paracetamol intoxication. Damage to hepatic cells, can cause hepatocellular enzyme leakage into the serum from the cytosol, which shows peak activities between 24 to 48 hour after a toxic insult [23,32]. However, treatment with L- arginine decreased the activity of ALT and AST. Results of ALP activity showed significant reduction in the positive control group compared to the normal. This observation was different from that reported by Oyedepo [16] who observed an increase in ALP activity after intoxication with paracetamol at 2 g/kg body weight. This could mean that at 1 g/kg, paracetamol do not cause alterations in the excretion of bile by hepatocytes. Liver injury as a result of toxicants can lead to defects in the excretion of bile by hepatocytes which are manifested as their activity increases in the serum [19].

The results of this study indicated decrease in the activity of AST and ALT in the groups administered $_{L}$- arginine after intoxication with paracetamol. AST activity was reduced dose dependently. The therapeutic effect of $_{L}$-arginine may be the result of stabilization of plasma membrane, in other words preserving structural integrity of the cell as well as the repair of damages induced on the hepatic tissues by paracetamol [16]. The significant increase in the activity of serum AST in paracetamol intoxicated rats was also accompanied by significant increase in total bilirubin. In addition, the hepatotoxicity of acetaminophen was confirmed by the results of total protein (TP) and albumin concentrations. As both were significantly decreased. Most plasma proteins are synthesized by the hepatocytes, abnormality of which indicates a lower serum concentration. Decreases in total protein and albumin concentration was also observed at high dose paracetamol intoxication [9,16]. A reduction in TP and albumin concentration reflects synthetic incapability of the liver of rats intoxicated with paracetamol. This may interfere with active participation in fluid exchange binding and transport function, buffering action and enzyme activities of plasma protein [1].

The increase in total bilirubin in rats intoxicated with paracetamol, compared to the control reflects an increased breakdown of hemoglobin or other heme containing proteins. In addition, failure of a damaged liver to conjugate and excrete bilirubin results in the increased concentration of bilirubin in the serum [3,14]. Treatment of paracetamol intoxication with $_{L}$-arginine did not reduce serum total bilirubin concentration. Also observed, was the increase in serum total bilirubin in $_{L}$-arginine treated group. This indicates that administration of $_{L}$-arginine at 60 mg/kg body weight could result in moderate increase in serum total bilirubin concentration. This explains the non reduction of serum total bilirubin concentration in the groups treated with $_{L}$-arginine at different doses.

Conclusion

Intoxication of experimental species with paracetamol at 1000 mg/kg body weight caused liver damage with the extent of damage depending on the dose and duration of exposure. $_{L}$-arginine at 60 mg/kg body weight ameliorated the damaging effects of paracetamol overdose therefore; caution must be taken with the prescription and rampant use of paracetamol.

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Volume 9 Issue 9 September 2021