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IN VIVO EVALUATION OF THE ROLE OF D-RIBOSE-L-CYSTEINE IN ACETAMINOPHEN- INDUCED LIVER DAMAGE USING WISTAR RAT

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ABSTRACT

Acetaminophen- induced hepatotoxicity is usually provoked by oxidative stress. D-ribose-L-cysteine (DRLC) is a precursor in the synthesis of glutathione (GSH). It minimizes oxidative stress. Experimental animals were grouped into 4(n=5). Group1received control (10mg/kg), group 2 received Acetaminophen 150mg/kg and DRLC 125mg/kg, group 3 received Acetaminophen 150mg/kg and DRLC 250 mg/kg while group 3 received Acetaminophen alone at 150mg/kg. The level of Glutathione level (GSH), Malondialdehyde (MDA), Catalase (CAT), Nitric oxide (NO), C-reactive protein (CRP) and Gamma glutamyl transferase (GGT) were analyzed. Administration of DRLC at both doses (125 and 250 mg/kg) post treatment with acetaminophen (150mg/kg), significantly elevated the hepatic level of GSH, GSH-Px and CAT in experimental rats compared to control. Lipid peroxidation was minimized seen with a decrease in the hepatic level of MDA. Liver GGT was also reduced whereas administration of Acetaminophen (150mk/kg) alone reduced the level of GSH, GSH-Px and CAT. However, there were no significant differences in the level of NO and C-reactive protein (CRP) of both the DRLC 125mg/kg and 250mg/kg compared to the control. Therefore, this investigation suggests that DRLC both at 125mg/kg and 250mg/kg mitigates acetaminophen-induced hepatotoxicity.

KEYWORDS: Acetaminophen, D-ribose-L-cysteine (DRLC), Glutathione, Oxidative stress.

1. INTRODUCTION

Drug induced liver injury (DILI) also known as drug-induced hepatotoxicity occurs as a result of some medications (prescription or OTC) especially acetaminophen (Garcia-Cortes et al., 2020). DILI often occurs via a structural disorder (mitochondrial dysfunction) and functional integrity defect of the liver, synthesis of metabolite that modifies hepatocellular structure and function, generation of a reactive drug metabolite that attaches to hepatic proteins to generate new antigenic drug-protein adducts which are targeted by host defenses and the trigger of a systemic hypersensitivity response (drug allergy) that destroys the liver (Garcia-Cortes et al., 2020).

Hepatotoxicity is the major undesirable effect produced by acetaminophen (n-acetyl-aminophenol, APAP) (Zhou et al., 2021).

Acetaminophen (paracetamol) is an effective analgesic and antipyretic agent, but has only weak anti-inflammatory properties. It has weak anti-inflammatory activities. Acetaminophen possibly produces an antipyretic action by a central effect on the hypothalamic heat-regulating centre to cause peripheral vasodilation resulting in increased blood flow through the skin, sweating and heat loss. The central action probably involves inhibition of prostaglandin synthesis in the hypothalamus. Concerns have been raised over the effects on the cardiovascular, respiratory, renal, and gastrointestinal and central nervous systems, as well as potential effects in the offspring of pregnant women ingesting acetaminophen (Przybyła et al., 2020). Acetaminophen causes analgesia by inhibiting the COX-1 and COX-2 enzymes, which prevent prostaglandin synthesis from arachidonic acid. Additionally, it has been noted that acetaminophen particularly affects the third COX isoenzyme, COX-3, an exon splice variation of COX-1. However, it soon became clear that COX-3 was absent in people, and other research points to paracetamol having no clinically meaningful effects on

the COX-1 exon splice variants that have so far been identified in humans. It is currently believed that paracetamol's primary analgesic mechanism is not the suppression of COX activity (Ohashi and Kohno, 2020).The blood-brain barrier is easily crossed by p-aminophenol, which is then transformed into AM404 by fatty acid amide hydrolase from paracetamol. Another mechanism for the metabolization of acetaminophen results in substances like N-acetyl-p-benzoquinoneimine (NAPQI), which likewise seems to generate analgesia by activating transient receptor potential ankyrin 1 receptors. The most significant mediator of paracetamol metabolite-induced analgesia, however, is universally acknowledged to be AM404. Although AM404 was previously assumed to only operate on CB1 receptors as an anandamide analog, it has now been demonstrated that AM404 also activates TRPV1 receptors. In particular, it is understood that TRPV1 receptors play a crucial role in the modulation of pain in the brain. The main non-addictive component of cannabis, cannabidiol, activates TRPV1 receptors in the dorsal raphe nucleus to cause analgesia.

D-ribose-L-cysteine (DRLC) is a precursor in the synthesis of glutathione (GSH). D-ribose-L-cysteine promotes glutathione (GSH) levels with consequent elevation of the antioxidant defence system of the body (Adelakun et al., 2021). Study has shown that DRLC has potent antioxidant potential even on testis to promote spermatogenesis by reducing poisonous activity of aluminium chloride on the testes (Olanrewaju et al., 2021). It facilitates cellular activities and reduces lipid peroxidation via over expression of GSH. DRLC is a source of antioxidants that inhibit the activities of reactive oxygen species (Akingbade et al., 2021). GSH deficiency has been linked to oxidative stress, hypertension, cardiovascular disease and hepatotoxicity. GSH deficiency is associated with inactivation and sequestration of nitric oxide (NO) (mediated by reactive oxygen species (ROS) which leads to diminished NO availability. D-ribose-L-cysteine causes elevation of GSH levels in organs such as heart, muscle tissue, liver, kidney and lungs without producing adverse effects in healthy mice. Previous investigation has shown that DRLC attenuate memory deficit induced by lipopolysaccharide in mice models (Emokpae et al., 2020).

This study seeks to investigate the role of D-ribose-L-cysteine (DRLC) in acetaminophen- induced liver damage using wistar rat.

2. MATERIALS AND METHODS

2.1 Preparation of D-ribose-L-Cysteine solution

D-ribose-L-Cysteine (GlaxoSmithKline®) was obtained from Max International, Salt Lake City, Utah, USA. 2g of DRLC was dissolved in 400ml physiological saline at 30mg/kg body weight.

2.2 Animal

Twenty experimental animals were procured from the animal house of the Department of Pharmacology and Toxicology of the Faculty of Pharmaceutical Sciences, University of Port Harcourt and caged appropriately with access to clean water and rat chow. Ethical approval (UPH/6788) was obtained from the Animal Care and Use Research Ethics Committee of the University of Port Harcourt, Nigeria.

2.3 Experimental design

Control group 1 (n=5) was orally administered with 10 mg/kg of distilled water daily for eight weeks. Group 2 (n=5) received Acetaminophen 150mg/kg + DRLC 125mg/kg daily for eight weeks while Group 3 (n= 5) received Acetaminophen 150mg/kg + DRLC 250mg/kg daily for eight weeks. Group 4 (n=5) were given Acetaminophen alone at 250mg/kg

Distilled water and DRLC were administered through an oral cannula. At the end of the study (week 8), the animals were anaesthetized by infusion of ketamine (60 mg/kg) and xylazine (10 mg/kg) and blood was collected.

The liver tissues of the rats were excised and a uniform portion (100 g) was homogenized in 10% w/v ice-cold 0.1 M phosphate buffer (pH 7.4) and centrifuged at 1372 x g for 15 min, at 4°C – using a cold centrifuge (Centurion Scientific Ltd., West Sussex, and United Kingdom). The resultant supernatant serum samples were pipette into separate plain bottles and used to assay for antioxidant parameters.

2.4 Chemicals

Lipid peroxidation (malondialdehyde assay kits) and Nitric oxide were obtained from Oxford Biomedical Research, Inc. (USA). Ellman reagent [5,5-dithiobis- (2-nitrobenzoate) DTNB] and thiobarbituric acid (TBA) were from (Sigma–Aldrich, St. Louis, USA). C-reactive protein was from (Elabscience Biotechnology Inc, USA) and D-Ribose-L-Cysteine was obtained from Max International, Salt Lake City, Utah, USA.

2.5 Determination of glutathione (GSH)

GSH was measured on the principle of oxidation of reduced GSH by 5,5,-dithiobis-2-nitrobenzoic acid (DTNB) (aromatic disulphide compound), to form glutathione oxidized form and 5,thio-2-nitrobenzoic acid (Beutler et al., 1963). At absorbance of 412nm, yellow colour formed as a result of DTNB reduction was measured.

2.6 Glutathione peroxidase (GSH-Px) assessment

Measurement of GSH-Px was determined by a previously described method (Paglia and Valentine, 1967). GSH-Px in the presence of hydrogen peroxide (H_2O_2) facilitates the oxidation of reduced glutathione (GSH) to oxidized glutathione (GSSG). GSSG is reduced to GSH through nicotinamide adenine dinucleotide

phosphate (NADPH) and glutathione reductase. GSH-Px activity was then calculated by reading absorbance reduction at 340nm during NADPH to NADP oxidation.

2.7 Determination of catalase (CAT) activity

CAT was assessed through a previously described method (Aebi, 1974). H2O2, by catalase, is broken down to oxygen and water and it is presented in the ultraviolent spectrum as reduced absorbance. At 240 nm, H2O2 reaches maximum absorbance. This absorbance reduction is directly proportional to CAT enzymes activities.

2.8 Determination of malondialdehyde (MDA) level

Determination of Malondialdehyde (MDA) level principle was based on thiobarbituric acid (TBA) and MDA reaction at 95ºC (Draper and Hadly, 1990). TBA and MDA react at 532 nm absorbance to form pink pigment. For 15 min, the reaction was performed at pH 2–3 and sample mixed (2.5 vol of 10% (w/v) trichloroacetic acid) to precipitate protein. Through centrifugation, precipitate was pelleted and supernatant reacted with TBA (0.67%) for 15 min in boiling water-bath. Absorbance was read after 15 min at 532 nm. Values were compared with standard solutions (1,1,3,3-tetramethoxypropane) and results expressed in μM.

2.9 Determination of Nitric oxide (NO) and C-reactive protein (CRP)

Level of nitrite was calculated being the main product of NO oxidation in aqueous solution and this was used to determine plasma NO. Sulphanilic acid is converted by reaction with nitrite acid solution quantitatively to diazonium salt. This forms azo-dye through coupling of N-(1-naphthyl) ethylenediamine and can be quantified at 548nm absorbance spectrophotometrically. C-reactive protein (CRP) was determined using ELISA and the procedure followed was in accordance with the manufacturer's instructions.

2.10 Gamma glutamyl transferase (GGT)

The test principle is based on substrate L-γ-glutamyl-3-carboxy-4- nitroanide. In the presence of glycylglycine, it is converted to 5-amino-2- nitrobenzoate by γ-GT and absorbance is measured at 405nm. The increase in absorbance is directly proportional to GGT activity. The test procedure was followed according to manufacturer's instruction.

2.11 Statistical analysis

All data were presented as mean standard error of the mean (SEM). Statistically significant differences among groups were calculated using one-way analysis of variance (ANOVA) followed by Tukey post-hoc test for multiple comparisons. Statistical significance was defined as $p < 0.05$.

3. RESULTS

3.1. DRLC mediated changes in antioxidants status

Administration of DRLC at both doses (125 and 250 mg/kg) post treatment with acetaminophen (150mg/kg), significantly elevated the level of GSH 125mg/kg (3.8 ± 2.0) and 250mg/kg (4.2 ± 0.3) compared to control (2.6 ± 0.4). It also increased the level of GSH-Px 125mg/kg (3.9 ± 0.6) and 250mg/kg (3.3 ± 0.2) compared to control (2.2 ± 0.2), with consequent elevation in CAT 125mg/kg (3..9 ± 0.6) and 250mg/kg (3.3 ± 0.2) in experimental rats compared to control (2.3 ± 0.4) as shown in the Table 1 below. GSH was only significantly elevated in the DRLC 250 group ($P < 0.01$). Administration of Acetaminophen alone reduced the level of GSH, GSH-Px and CAT.

3.2 DRLC mediated changes in lipid peroxidation malondialdehyde (MDA) Nitric oxide (NO) and C-reactive protein (CRP)

Administration of DRLC 250mk/kg decreased MDA hepatic level (0.9 + 0.1) compared to control (4.5 + 0.2). Liver GGT was significantly decreased in DRLC 125mg/kg (3.2 ± 1.0) and DRLC 250mg/kg (2.1 ± 0.4) compared to control (8.5 ± 0.3). However, there were no significant differences in body NO and C-reactive protein (CRP) of both the DRLC 125mg/kg and 250mg/kg compared to the control from Table 2 and figure 1 shown below.

Table 1: Showing the effect of administration of DRLC on Antioxidant parameters after Acetaminophen-induced hepatotoxicity.

Parameters	Control/distilled water (10mg/kg)	ACE 150mg/kg + DRLC 125mg/kg	ACE 150mg/kg + DRLC 250mg/kg	ACE 150mg/kg
GSH (m M)	2.6 ± 0.4	3.8 ± 2.0	*4.2 ± 0.3	2.5± 0.1
CAT (μ mol/min/m L)	2.12 ± 0.4	*3.9 ± 0.6	3.3 ± 0.2	1.8 ± 0.2
GSH-P x (u/L)	2.2 ± 0.2	3.2 ± 0.8	*4.3 ± 0.8	1.6 ± 0.2

Values are expressed as Mean + SEM, * significantly different from control ($p < 0.05$)

Table 2: Showing the effect of administration of DRLC on Lipid peroxidation (MDA), Nitric oxide and C-reactive protein (CRP).

Parameters	Control/distilled water (10mg/kg)	ACE 150mg/kg + DRLC 125mg/kg	ACE 150mg/kg + DRLC 250mg/kg	ACE 150mg/kg
NO (μ M)	1.5 ± 0.2	1.4 ± 0.3	1.2 ± 0.3	2.7 ± 0.3
MDA (μ M)	4.5 ± 0.2	3.4 ± 0.2	*0.9 ± 0.1	
GGT (u/L)	8.5 ± 0.3	3.2 ± 1.0	*2.1 ± 0.4	5.5 ± 0.3

Values are expressed as Mean ± SEM, * significantly different from control ($p < 0.05$)

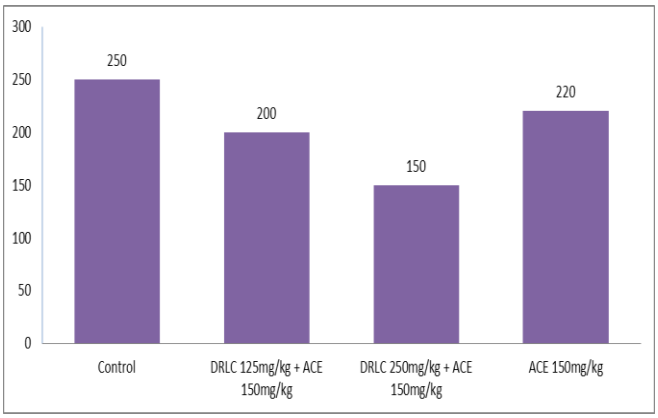

Figure 1: C-reactive protein (CRP) in control, DRLC (D-ribose-L-cysteine) 125mg/kg, DRLC (D-ribose-L-cysteine) 250mg/kg and Acetaminophen 150mg/kg alone of wistar rats after 8 weeks of DRLC treatment. n ¼ 5. Values are expressed as Mean SEM, * significantly different from control ($p < 0.05$).

4. DISCUSSION

Oxidative stress is a pathogenic factor that participates in acetaminophen (APAP)-induced acute liver failure (Xiao et al., 2022). Reactive oxygen species (ROS), a bye-product of oxidative stress participates in the initiation and progression of different pathological dysfunction. Thus, antioxidants are vital in ameliorating APAP-induced liver breakdown. But supplementation with antioxidant is widely employed in management of oxidative stress.

Glutathione (GSH) contributes immensely in recreating other antioxidants (such as ascorbate, tocopherols, alpha lipoic acid and co-enzymes), consequently enhancing the body's protective and defensive role against reactive oxygen species (ROS) (Kwon et al., 2019). Glutathione is naturally synthesized by the liver and also a major component of fruits, vegetables, meat and synthesized from cells by cysteine, glutamic and glycine. A number of factors can deplete glutathione concentration including poor nutrition, environmental toxins, xenobiotics (drugs), stress and some pathological conditions. The antioxidant defense property of glutathione seems to contribute in cell proliferation (Kwon et al., 2019).

From this research study, supplementation of GSH through administration of D-ribose-L-cysteine (DRLC) both at the doses of 125mg/kg and 250mg/kg produced a remarkable increase in GSH level. Literature investigation has shown that 16 h after single DRLC administration at 2 g/kg in mice, increased and maintained elevation in heart GSH with increases equally found in other tissues as well (Ojetola et al., 2021). N-acetylcysteine (NAC) is also a precursor/pro drug of GSH that increases GSH level with associated side effects (Sawyer, 2020). NAC is antioxidants that stems from its potential to elevate the intracellular level of glutathione (GSH). Previous research has demonstrated that higher dose of NAC (1.0 g/kg b.w) was reported to be effective in increasing GSH levels but with adverse effects resulting from impulsive release of NAC in a toxic manner (Tenorio et al., 2021). D-ribose-L-cysteine helps generate glutathione to neutralize ROS. (Akingbade et al., 2021).

This research study has demonstrated that co-administration of acetaminophen and DRLC decreased the level of lipid peroxidation seen with reduction in MDA with associated increase in other antioxidant parameters including catalase and glutathione peroxidase. Intervening activity of DRLC resulted in suppression of acetaminophen induced hepatotoxicity via inhibition of oxidative stress (Adelakun et al., 2021). Again, Emokpae et al (2020) reported that D-ribose-L-cysteine enhances catalase activity in the prefrontal cortex of mice treated with lipopolysacharides. Assessment of the level of gamma glutamyl transferase (GGT) enzyme has been used to investigate liver functions and also evaluate the integrity of the hepatic tissue (Adeyemi et al., 2020) as damaged hepatic cells are known to discharge GGT. Normally, GGT is present in low levels but when the liver is injured, the level rises. Elevated level of the enzymes GGT indicates liver disease (Seppelt et al., 2022). A research study has also disclosed that GGT also serves the important role of metabolizing GSH extracellularly thereby resisting GSH intracellular degradation (Pitisuttithum, et al., 2020).

This implies that decrease in hepatic GGT indicates that GSH degradation is tightly regulated and well controlled by DRLC supplementation. Additionally, GGT activities from the result obtained above, indicates there was no damage to liver cells after prolonged exposure (8 weeks) which depicts a safety profile of DRLC. Furthermore, GSH degradation requires GGT as it is the only enzyme that initiates catabolism of GSH and GSH adducts (such as GSSG, glutathione S-conjugates, and glutathione complexes) in the extracellular space (Neuman et al., 2020). This is in consonance with evidences from previous studies that GSH maintains antioxidants homeostasis by regulating the release of DRLC in a pulsatile fashion for GSH synthesis.

C-reactive protein is synthesized in response to inflammation and it serves as a predictor of liver diseases. The liver discharges CRP in response to inflammation. Elevation in CRP level means serious health condition due to inflammation. High CRP level signifies liver diseases with reduce cognitive decline and brain GMV loss (Jiang et al., 2023). The outcome of this current research revealed DRLC to reduce CRP at 125mg/kg and 250 mg/kg in this study which indicates that it could reduce the risk of hepatotoxicity/damage. Previous investigation has disclosed that CRP decrease was dose-response dependent as humans with higher intake of fruits and vegetables were 65% less likely to have higher plasma CRP and homocysteine concentrations (Ojetola et al., 2021).

There were no changes in nitric oxide (NO) concentration after eight weeks of DRLC supplementation in normal wistar rats at both doses (125mg/kg and 250mg/kg). Literature has shown this effect could be due to decreases observed in CRP. CRP plays pivotal roles in atherogenesis and it inhibits endothelial NO synthase expression (which is responsible for NO production) in endothelial cells (Cheng et al., 2020).

5. CONCLUSION

This research investigation revealed that supplementation with D-ribose-L-cysteine (DRLC) at 125mg/kg and 250 mg/kg mitigate acetaminophen-induced hepatotoxicity via elevation of glutathione (GSH), catalase (CAT) and glutathione peroxidase enzymes and at the same time minimize lipid peroxidation via reduction in malondialdehyde (MDA) level. It also enhances decrease in C-reactive protein.

Declarations

Author contribution statement

Doris Ajibo: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper. Ajirioghene Akpotu, Emem John Offong, Akpan Williams and Silver Isosiya : Analyzed and interpreted the data; Wrote the paper. : Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

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Declaration of interests' statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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