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PROTECTIVE EFFECTS OF VITAMIN C ON ACETAMINOPHEN-INDUCED LIVER AND KIDNEY TOXICITY IN MALE RATS

***[1]Shirley O. Ebhohon, [2]Ekene V. Asoya and [3]Harrison. E. Iyare**

[1]Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Nigeria.
[2]Department of Medical Biochemistry, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Edo State, Nigeria.
[3]Department of Biochemistry, Faculty of Life Sciences, University of Benin, Edo State, Nigeria.

*Corresponding authors' email: so.ebhohon@mouau.edu.ng

ABSTRACT

Acetaminophen (paracetamol) is a widely used over-the-counter analgesic and antipyretic medication known for its efficacy and safety when administered within recommended doses. However, overdose or prolonged use of acetaminophen can lead to severe hepatotoxicity and nephrotoxicity, posing a significant public health concern. This study aims to investigate the protective effects of vitamin C on acetaminophen-induced hepatotoxicity and nephrotoxicity in male Wistar rats. Six rats were randomly assigned to seven groups, with Group 1 designated as the control. Groups 2, 4, and 6 were given daily single oral doses of 100, 200, and 400 mg/kg of acetaminophen, respectively. Meanwhile, Groups 3, 5, and 7 received daily single oral doses of 100, 200, and 400 mg/kg of acetaminophen, followed by intraperitoneal administration of 100 mg/kg of vitamin C daily, for 14 days. Liver function markers (AST, ALT, total bilirubin, and total protein) and renal function indicators (urea and creatinine levels) were assessed, alongside antioxidant status in liver and kidney tissues through antioxidant assays (SOD, CAT, GSH, and MDA). The results demonstrated the protective influence of vitamin C on liver and kidney tissues, as indicated by the modulation of biochemical markers. These findings suggest that vitamin C may play a pivotal role in alleviating acetaminophen-induced liver and kidney damage across different dosage regimens, potentially serving as a therapeutic intervention for preventing or treating drug-induced organ injuries. Further investigations are essential to elucidate the underlying mechanisms and validate the translational potential of vitamin C as a protective agent against acetaminophen toxicity.

Keywords: Acetaminophen, Hepatoxicity, Nephrotoxicity, Vitamin C, Wistar Rats

INTRODUCTION

Acetaminophen is primarily administered orally in the form of tablets or liquid solutions. After ingestion, it is rapidly absorbed from the gastrointestinal tract, reaching peak plasma concentrations within 30 minutes to 2 hours (Offor *et al.*, 2022). The drug is readily distributed throughout the body due to its water-solubility. It easily crosses cell membranes, including the blood-brain barrier, allowing it to exert its effects in the central nervous system, as well as in peripheral tissues (Ghanem *et al.*, 2016). Acetaminophen metabolism occurs primarily in the liver, where it undergoes two main pathways: glucuronidation and sulfation. In the glucuronidation pathway, acetaminophen is conjugated with glucuronic acid by the enzyme UDP-glucuronosyltransferase (UGT). This conjugation forms a water-soluble and inactive metabolite, acetaminophen glucuronide. The glucuronide metabolite is then excreted in the urine (Mazaleuskaya *et al*., 2015). Acetaminophen can also undergo sulfation, which involves the addition of a sulfate group to the drug. This process is mediated by sulfotransferase enzymes. The resulting sulfate metabolite, acetaminophen sulfate, is water-soluble and is also excreted in the urine (Yamamoto *et al.*, 2015). While glucuronidation and sulfation are the primary detoxification pathways for acetaminophen, a minor metabolic pathway, catalyzed by the cytochrome P_{450} enzyme system (specifically CYP2E1), can lead to the formation of a reactive intermediate called N-acetyl-p-benzoquinone imine (NAPQI). Under normal circumstances and therapeutic doses, NAPQI is rapidly detoxified by glutathione, a critical antioxidant present in the liver. Glutathione conjugates with NAPQI to form a non-toxic compound, which is then excreted (Pingili *et al.*, 2019). In cases of acetaminophen overdose or excessive intake, the glucuronidation and sulfation pathways can become saturated. As a result, a greater proportion of acetaminophen is metabolized through the cytochrome P_{450} pathway, leading to the excessive production of NAPQI. If the production of NAPQI overwhelms the available glutathione stores, it can cause oxidative stress and damage to liver cells, leading to hepatotoxicity. This is the basis of acetaminophen overdose and the reason why excessive use of acetaminophen can be dangerous (El-Maddawy *et al.*, 2018). The majority of acetaminophen and its metabolites are excreted through the kidneys into the urine. As water-soluble compounds, both the glucuronide and sulfate metabolites are readily eliminated from the body. A small portion of the drug is also excreted in the faeces (Mazaleuskaya *et al*., 2015). Acetaminophen is relatively safe when used at therapeutic doses. However, certain drugs can interact with acetaminophen metabolism, affecting its bioavailability or detoxification pathways. For example, drugs that induce or inhibit specific cytochrome P450 enzymes may alter the balance between detoxification and the formation of toxic metabolites, increasing the risk of liver damage (Mazaleuskaya *et al*., 2015).

Vitamin C, scientifically known as ascorbic acid, is a water-soluble vitamin that plays a crucial role in various bodily functions. It is renowned for its antioxidant properties, which help protect cells from damage caused by free radicals (Gęgotek and Skrzydlewska, 2022). Despite its primary association with enhancing the immune system, vitamin C offers a range of health benefits (Carr and Maggini, 2017). As an antioxidant, vitamin C helps neutralize free radicals—unstable molecules produced as a byproduct of normal body processes and through exposure to environmental factors such as pollution and ultraviolet radiation (Agwu *et al.*, 2023). By neutralizing these free radicals, vitamin C may play a role in reducing the risk of chronic diseases and slowing down the

ageing process (Monacelli *et al.,* 2017; Chambial *et al.,* 2013). This study aims to contribute valuable insights into the therapeutic potential of vitamin C as a protective agent against APAP-induced liver and kidney damage, ultimately paving the way for the development of preventive strategies and interventions in clinical settings. Overall, this research contributes to the broader understanding of the interplay between antioxidant supplementation and drug-induced organ toxicity, offering potential avenues for clinical applications in mitigating drug-associated adverse effects.

MATERIALS AND METHODS

Chemicals and Reagents

All chemicals used in this study, including acetaminophen and vitamin C, were of analytical grade and procured from Sigma Chemical Company (St. Louis, MO, USA). Assay kits for alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and bilirubin, were obtained from Randox (Antrim, U.K). Teco Diagnostics (California, USA) assay kits were used for urea and creatinine measurements.

Preparation of Acetaminophen

Acetaminophen (Sigma-USA) was dissolved in a few drops of tween 80, then adjusted to a known volume by adding saline solution. Three different doses were prepared (100, 200 and 400 mg/kg body weight). The prepared solution was administered orally using a gavage.

Preparation of Vitamin C

Vitamin C (Sigma-USA) was reconstituted in saline solution and a dosage of 100 mg/kg body weight was prepared. The prepared solution was administered intraperitoneally (i.p.).

Experimental animals

The experimental animals used in this study were male Wistar rats. During the study, adult male Wistar rats with an average weight of 180 ± 20 g were housed in a controlled environment under standard laboratory conditions. The animal facility provided a regulated temperature (20-25°C) and humidity, as well as a 12-hour light-dark cycle to simulate the natural diurnal rhythm. Adequate ventilation and sanitation were ensured to maintain a hygienic living environment for the animals. The rats had unrestricted access to standard laboratory chow, formulated to meet their nutritional requirements. Fresh water was also available *ad libitum* to ensure proper hydration throughout the study period. Before the commencement of the experimental procedures, the rats underwent an acclimatization period of at least one week which allowed the animals to adjust to the laboratory conditions and minimized stress associated with transportation and handling. The study was conducted in accordance with ethical guidelines and regulations for the care and use of laboratory animals. The research protocol was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC). All efforts were made to minimize the number of animals used and to reduce any potential discomfort or suffering during the study.

Experimental Groups/Design

The animals were randomly distributed into seven (7) groups, each comprising six (6) animals. Group 1 (Control) was administered the vehicle solution (tween 80 in normal saline) without acetaminophen or vitamin C. Group 2 received a daily single oral dose of 100 mg/kg of acetaminophen dissolved in the vehicle solution. Rats in Group 3 were given a daily single dose of 100 mg/kg of acetaminophen orally and 100 mg/kg of vitamin C intraperitoneally (i.p.), both dissolved in the vehicle solution. Group 4 received a daily single dose of 200 mg/kg of acetaminophen orally dissolved in the vehicle solution. In contrast, Group 5 received a daily single dose of 200 mg/kg of acetaminophen and 100 mg/kg of vitamin C intraperitoneally, both dissolved in the vehicle solution. Group 6 was administered a daily single oral dose of 400 mg/kg of acetaminophen dissolved in the vehicle solution. Conversely, Group 7 received a daily single oral dose of 400 mg/kg of acetaminophen and 100 mg/kg of vitamin C intraperitoneally, both dissolved in the vehicle solution. The duration of this experiment was 14 days.

Blood Sample and Tissue Collection

Before blood collection, the animals were fasted overnight to ensure consistent baseline values for biochemical parameters. To facilitate blood collection and reduce potential distress, the rats were anaesthetized with an intraperitoneal injection of urethane and sacrificed immediately. Liver and kidney were excised while blood samples were drawn using a 5 ml syringe via cardiac puncture (Arunachalam and Sasidharan, 2021) into sample tubes, and centrifuged at 4000 rpm for 10 minutes to obtain serum which was used for biochemical assays in this study. The blood sample and tissue collection procedures adhered to ethical guidelines and were approved by the Institutional Animal Care and Use Committee (IACUC).

Preparation of Liver and Kidney Homogenates

The tissues (liver and kidney) were homogenized in cold phosphate buffer (0.05M, pH 7.0) with a Teflon homogenizer. 1g of the tissue was homogenized in 9 ml of phosphate buffer to give 10% homogenate. The homogenate was centrifuged at 4000rpm for 10 minutes. The supernatant obtained was stored frozen at -20^{0}C until required for the analyses of CAT and SOD activities as well as GSH and MDA levels.

Biochemical Assays

Liver Function Tests

The activities of AST, ALT, and ALP, as well as the concentration of total bilirubin were determined in serum using their respective Randox (Antrim, U.K) assay kits.

Renal Function Tests

Serum urea nitrogen and creatinine were determined using (Fawcett and Scott, 1960) and (Bartels and Bohmer, 1972) methods respectively as outlined in the Teco diagnostic kit leaflet.

Antioxidant Assays

Estimation of Catalase Activity

Catalase activity in serum was determined using the modified method described by (Atawodi, 2011).

Estimation of SOD Activity

Superoxide dismutase (SOD) activity in serum was determined using the method described by (Sun et al., 1988).

Estimation of GSH Levels

GSH levels in serum were determined using the method described by (Tietze,1969).

Estimation of Glutathione Peroxidase (GPx) Activity

Glutathione Peroxidase (GPx) activity in serum was determined using the method described by (Flohe and Gunzler,1984).

Assessment of Lipid Peroxidation

The concentration of MDA in serum was determined using the method described by (Draper and Hadley 1960).

Ultrastructural Investigations of Rat Liver and Kidney

At the end of the experimental period, the rats were euthanized, and liver and kidney tissues were carefully excised. The collected tissues were immediately fixed in 10% neutral buffered formalin to preserve the cellular architecture and prevent autolysis. The liver and kidney tissues were dehydrated in a graded series of ethanol solutions and then cleared using xylene. Subsequently, the tissues were embedded in paraffin wax to facilitate sectioning. The paraffin-embedded tissues were sectioned at a thickness of approximately 4-5 µm using a microtome. The obtained tissue sections were mounted onto glass slides and subjected to routine histological staining procedures. Hematoxylin and eosin (H&E) staining was employed to visualize the general tissue morphology and assess cellular integrity. The stained tissue sections were examined under a light microscope (Leica DM 500, Leica Biosystems, Germany) and multiple microscopic fields were assessed to obtain representative images of each tissue sample. Histopathological changes, such as alterations in cell structure, tissue architecture, and the presence of inflammation or lesions, were carefully documented.

Statistical Analysis

The experimental results were presented as mean ± standard deviation and analyzed using SPSS 20. Differences between groups were statistically analyzed using analysis of variance (ANOVA), and mean values that were significantly different from each other were identified by Duncan's multiple range test. $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Table 1: Effect of Acetaminophen-induced toxicity and Vitamin C (Ascorbic acid) on serum ALT, AST, ALP, Total Bilirubin and Total Protein

GROUP	TREATMENT	ALT(IU/L)	AST(IU/L)	TOTAL BILIRUBIN (mg/dL)	Total Protein (g/dL)
1	Control	0.104 ± 0.02^{a}	0.076 ± 0.02^{a}	0.10 ± 0.12^{a}	6.23 ± 0.23^{a}
2	100 mg/kg b.w.t of Acetaminophen	0.111 ± 0.01^{b}	0.081 ± 0.04^{b}	0.22 ± 0.17^{b}	4.22 ± 0.20^{b}
3	100 mg/kg of acetaminophen and 100 mg/kg b.w.t of Vitamin C	0.108 ± 0.01^{c}	0.077 ± 0.01^{a}	0.20 ± 0.13^{c}	4.60 ± 0.32^{c}
4	200 mg/kg b.w.t of Acetaminophen	0.123 ± 0.02^{d}	0.088 ± 0.01^{c}	0.38 ± 0.20^{d}	5.20 ± 0.20^{d}
5	200 mg/kg of acetaminophen and 100 mg/kg b.w.t of Vitamin C	0.118 ± 0.01^{e}	0.081 ± 0.02^{b}	0.35 ± 0.12^{e}	5.40 ± 0.25^{e}
6	400 mg/kg b.w.t of Acetaminophen	0.136 ± 0.01^{f}	0.096 ± 0.01^{d}	0.52 ± 0.19^{f}	5.38 ± 0.32^{f}
7	400 mg/kg of acetaminophen and 100 mg/kg b.w.t of Vitamin C	0.125 ± 0.01^{g}	$0. 084 \pm 0.01^{e}$	0.44 ± 0.10^{g}	5.51 ± 0.46^{g}

Values are expressed as mean ± standard deviation. Values on the same column with different superscripts are statistically significant at $p < 0.05$.

Elevated ($p<0.05$) levels of ALT, AST, and total bilirubin were observed in the serum of rats receiving the varying doses of acetaminophen alone in a dose-dependent manner (Table 1) when compared to both the control and treated groups. This was accompanied by a significant ($p < 0.05$) decrease in total protein concentration in the rat serum, as compared to both the control and treated groups (Table 1). Notably, the administration of vitamin C to the acetaminophen-exposed rats resulted in a marked ($p<0.05$) reduction in serum ALT and AST activities, along with a significant ($p<0.05$) decrease in total bilirubin concentration, when compared to both control and treated animals (Table 1). A significant ($p < 0.05$) increase in total protein concentration was also observed in the serum of both the control and treated animals after the administration of vitamin C.

Table 2: Effect of Acetaminophen-induced toxicity and Vitamin C (Ascorbic acid) on serum Urea and Creatinine

GROUP	TREATMENT	Urea (mg/dL)	Creatinine (mg/dL)
1	Control	11.53 ± 0.04^{a}	0.42 ± 0.01^{a}
2	100 mg/kg b.w.t of Acetaminophen	17.58 ± 0.21^{b}	0.60 ± 0.01^{b}
3	100 mg/kg of acetaminophen and 100 mg/kg b.w.t of Vitamin C	16.20 ± 0.17^{c}	0.51 ± 0.02^{c}
4	200 mg/kg b.w.t of Acetaminophen	22.02 ± 0.40^{d}	1.52 ± 0.12^{d}
5	200 mg/kg of acetaminophen and 100 mg/kg b.w.t of Vitamin C	19.02 ± 0.29^{e}	1.32 ± 0.02^{e}
6	400 mg/kg b.w.t of Acetaminophen	29.39 ± 0.37^{f}	2.25 ± 0.02^{f}
7	400 mg/kg of acetaminophen and 100 mg/kg b.w.t of Vitamin C	23.32 ± 0.20^{g}	2.10 ± 0.12^{g}

Values are expressed as mean ± standard deviation. Values on the same column with different superscripts are statistically significant at $p < 0.05$.

In Table 2, the administration of the varying doses of acetaminophen alone led to a substantial ($p < 0.05$) increase in urea and creatinine concentrations. Conversely, treatment with vitamin C resulted in a marked ($p < 0.05$) reduction in serum urea and creatinine concentrations in both the control and treated animals.

Table 3: Effect of Acetaminophen-induced toxicity and Vitamin C (Ascorbic acid) on Superoxide dismutase (SOD), Catalase (CAT), reduced glutathione (GSH) and Malondialdehyde (MDA) of liver homogenate in experimental animals

GROUP	TREATMENT	SOD (U/mL)	CAT (U/mL)	GSH (U/mL)	MDA x 10^{-3}(mmole/mL)
1	Control	25.60 ± 9.50^{a}	83.93 ± 5.42^{a}	34.15 ± 3.71^{a}	1.51 ± 0.08^{a}
2	100 mg/kg b.w.t of Acetaminophen	21.80 ± 22.59^{b}	79.09 ± 4.76^{b}	29.87 ± 2.70^{b}	1.58 ± 0.25^{b}
3	100 mg/kg of acetaminophen and 100 mg/kg b.w.t of Vitamin C	23.00 ± 16.18^{c}	81.95 ± 2.26^{c}	32.94 ± 3.11^{c}	1.53 ± 0.74^{a}
4	200 mg/kg b.w.t of Acetaminophen	18.85 ± 5.45^{d}	75.58 ± 5.54^{d}	27.91 ± 3.89^{d}	1.63 ± 0.05^{c}
5	200 mg/kg of acetaminophen and 100 mg/kg b.w.t of Vitamin C	20.83 ± 22.08^{b}	79.98 ± 2.41^{b}	29.64 ± 2.09^{b}	1.60 ± 1.65^{d}
6	400 mg/kg b.w.t of Acetaminophen	14.35 ± 11.02^{e}	78.20 ± 14.51^{e}	22.00 ± 0.63^{e}	1.72 ± 0.04^{e}
7	400 mg/kg of acetaminophen and 100 mg/kg b.w.t of Vitamin C	15.52 ± 37.52^{f}	81.15 ± 5.09^{c}	26.31 ± 2.38^{f}	1.69 ± 0.03^{f}

Values are expressed as mean ± standard deviation. Values on the same column with different superscripts are statistically significant at $p < 0.05$.

The results shown in Table 3 indicate significant ($p < 0.05$) reductions in superoxide dismutase (SOD) and catalase (CAT) activities, as well as reduced ($p < 0.05$) glutathione (GSH) levels, and increased ($p < 0.05$) malondialdehyde (MDA) levels in the liver homogenates of rats exposed to the varying doses of acetaminophen when compared to the control and the vitamin C treated groups. However, treatment with 100 mg/kg body weight of vitamin C resulted in a significant ($p < 0.05$) increase in SOD and CAT activities, while GSH levels increased ($p < 0.05$) and MDA levels decreased significantly ($p < 0.05$) when compared to the groups treated with acetaminophen alone.

Table 4: Effect of Acetaminophen-induced toxicity and Vitamin C (Ascorbic acid) on Superoxide dismutase (SOD), Catalase (CAT), reduced glutathione (GSH) and Malondialdehyde (MDA) of Kidney homogenate in experimental animals

GROUP	TREATMENT	SOD (U/mL)	CAT (U/mL)	GSH (U/mL)	MDA x 10^{-3}(mmole/mL)
1	Control	79.88 ± 7.34^{a}	81.29 ± 25.59^{a}	51.47 ± 0.70^{a}	3.16 ± 3.93^{a}
2	100 mg/kg b.w.t of Acetaminophen	71.75 ± 13.69^{b}	72.63 ± 0.43^{b}	44.42 ± 2.64^{b}	6.24 ± 1.35^{b}
3	100 mg/kg of acetaminophen and 100 mg/kg b.w.t of Vitamin C	73.14 ± 0.01^{c}	74.09 ± 0.01^{c}	47.77 ± 3.51^{c}	5.57 ± 3.40^{c}
4	200 mg/kg b.w.t of Acetaminophen	66.95 ± 6.60^{d}	67.09 ± 5.41^{d}	37.65 ± 0.84^{d}	6.64 ± 1.72^{d}
5	200 mg/kg of acetaminophen and 100 mg/kg b.w.t of Vitamin C	68.13 ± 0.05^{e}	70.04 ± 0.02^{e}	42.73 ± 2.96^{e}	5.39 ± 3.59^{e}
6	400 mg/kg b.w.t of Acetaminophen	63.65 ± 3.77^{f}	64.98 ± 28.52^{f}	30.19 ± 2.39^{f}	6.98 ± 1.49^{f}
7	400 mg/kg of acetaminophen and 100 mg/kg b.w.t of Vitamin C	65.11 ± 0.04^{g}	66.03 ± 0.01^{g}	34.10 ± 4.58^{g}	5.33 ± 1.82^{e}

Values are expressed as mean ± standard deviation. Values on the same column with different superscripts are statistically significant at $p < 0.05$.

The findings presented in Table 4 demonstrate significant ($p < 0.05$) decreases in superoxide dismutase (SOD) and catalase (CAT) activities, alongside reduced ($p < 0.05$) levels of glutathione (GSH), and elevated ($p < 0.05$) levels of malondialdehyde (MDA) in the kidney homogenates of rats exposed to varying doses of acetaminophen compared to both the control group and the groups treated with vitamin C. However, administration of 100 mg/kg body weight of vitamin C resulted in a significant ($p < 0.05$) increase in SOD and CAT activities, accompanied by increased ($p < 0.05$) GSH levels and decreased MDA levels, when compared to the acetaminophen-treated groups.

Effect of Acetaminophen-Induced Toxicity and Vitamin C on Liver Ultrastructure Following 14 Days of Administration in Rats

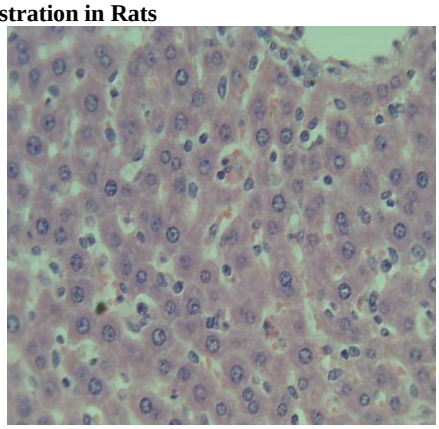

Plate 1: photomicrograph of liver from the control group (H&E; x 400)

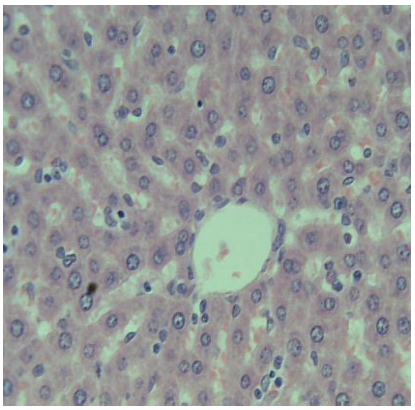

Plate 2: photomicrograph of liver from acetaminophen (100 mg/kg b.w.t) treated group (H&E; x 400)

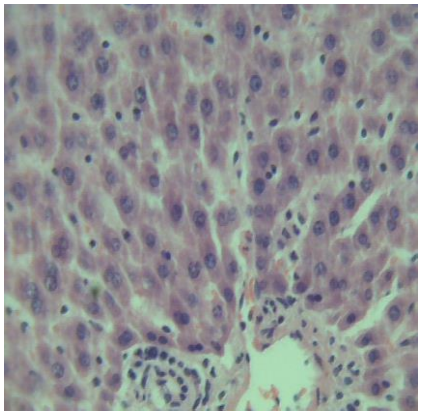

Plate 3: photomicrograph of liver from acetaminophen (100 mg/kg b.w.t) and Vitamin C (100 mg/kg b.w.t) treated group (H&E; x 400)

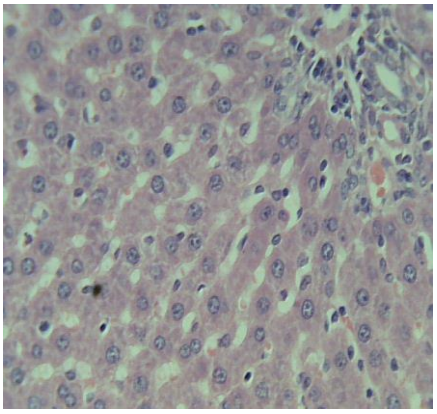

Plate 4: photomicrograph of liver from acetaminophen (200 mg/kg b.w.t) treated group (H&E; x 400)

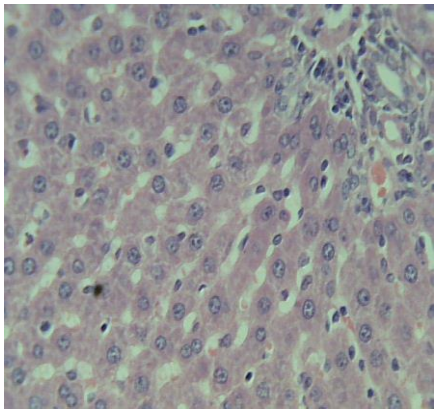

Plate 5: photomicrograph of liver from acetaminophen (200 mg/kg b.w.t) and Vitamin C (100 mg/kg b.w.t) treated group (H&E; x 400)

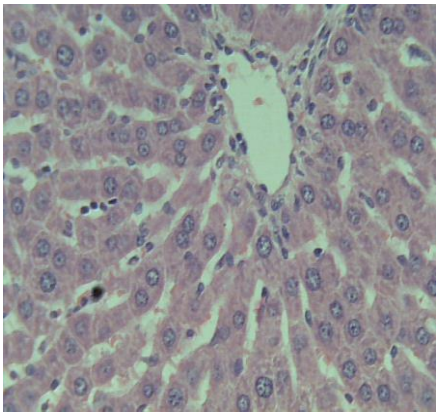

Plate 6: photomicrograph of liver from acetaminophen (400 mg/kg b.w.t) treated group (H&E; x 400)

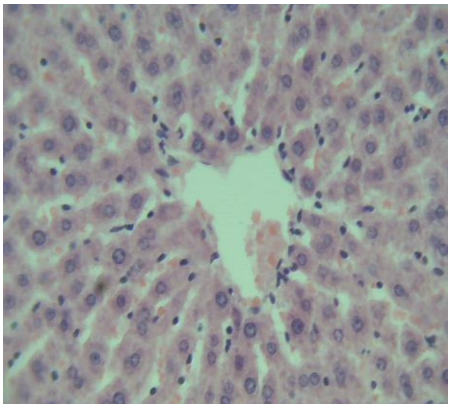

Plate 7: photomicrograph of liver from acetaminophen (400 mg/kg b.w.t) and Vitamin C (100 mg/kg b.w.t) treated group (H&E; x 400)

Effect of Acetaminophen-Induced Toxicity and Vitamin C on Kidney Ultrastructure Following 14 Days of Administration in Rats

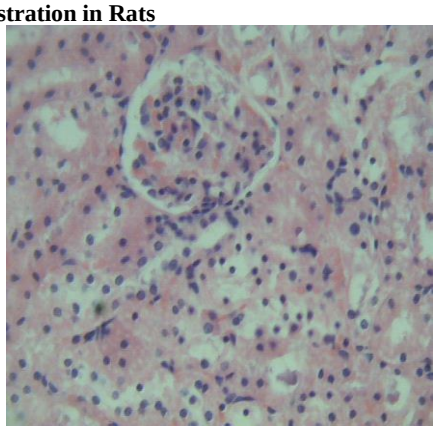

Plate 8: Photomicrograph of kidney from the control group (H&E; x 400)

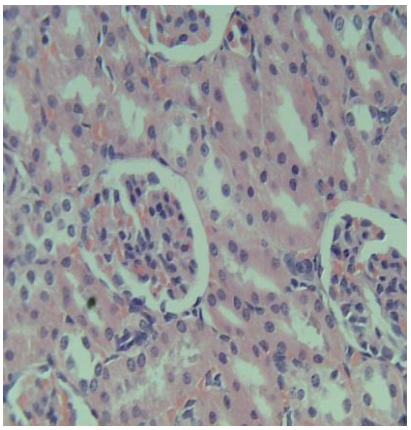

Plate 9: Photomicrograph of kidney from acetaminophen (100 mg/kg b.w.t) treated group (H&E; x 400)

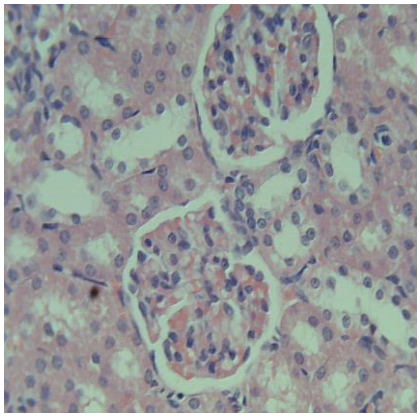

Plate 10: Photomicrograph of kidney from acetaminophen (100 mg/kg b.w.t) and Vitamin C (100 mg/kg b.w.t) treated group (H&E; x 400)

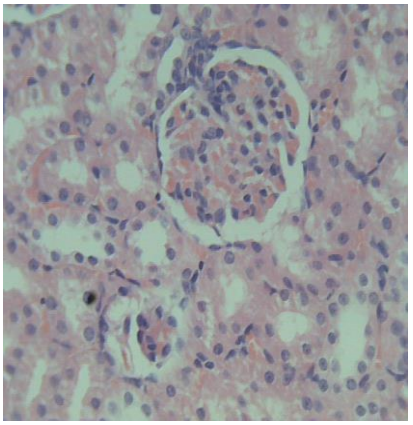

Plate 11: Photomicrograph of kidney from acetaminophen (200 mg/kg b.w.t) treated group (H&E; x 400)

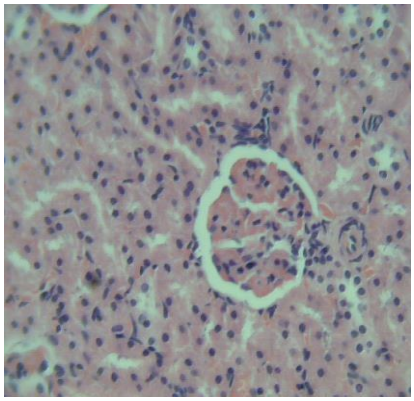

Plate 12: Photomicrograph of kidney from acetaminophen (200 mg/kg b.w.t) and Vitamin C (100 mg/kg b.w.t) treated group (H&E; x 400)

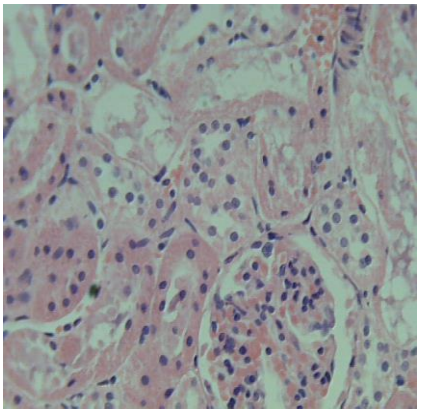

Plate 13: Photomicrograph of kidney from acetaminophen (400 mg/kg b.w.t) treated group (H&E; x 400)

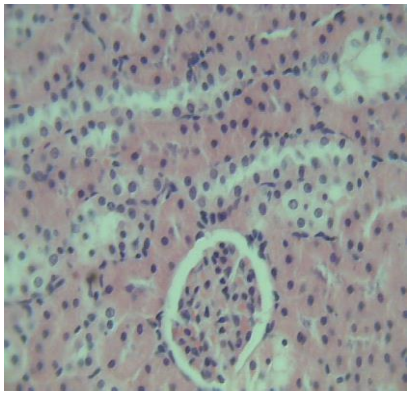

Plate 14: photomicrograph of kidney from acetaminophen (400 mg/kg b.w.t) and Vitamin C (100 mg/kg b.w.t) treated group

Histopathological analysis of the liver tissues reveals distinct electron microscopy findings for each group, outlined as follows:

Plate 1 represents the photomicrograph of the livers removed from rats in the control group. The liver tissue in this group exhibits a typical hepatic architecture with well-organized hepatic lobules.

Plate 2 shows the photomicrograph of the livers obtained from rats treated with 100 mg/kg body weight of acetaminophen. Observations indicate slight hepatic alterations, including mild inflammation and minimal hepatocellular necrosis.

Plate 3 displays the photomicrograph of the livers of rats treated with 100 mg/kg body weight of acetaminophen and 100 mg/kg body weight of vitamin C. Notably, there were reductions in hepatocellular necrosis and inflammation.

Plate 4 illustrates the photomicrograph of the livers derived from rats treated with 200 mg/kg body weight of acetaminophen. In this case, there was moderate hepatic necrosis, hepatic degeneration, vacuolation and inflammation.

Plate 5 shows the photomicrograph of the livers of rats treated with 200 mg/kg body weight of acetaminophen and 100 mg/kg body weight of vitamin C. Hepatocellular damages were significantly mitigated, with reductions in both necrosis and inflammation.

Plate 6 reveals the photomicrograph of the livers taken from rats treated with 400 mg/kg body weight of acetaminophen. Notably, there are severe hepatocellular necrosis, extensive vacuolation, and inflammation.

Plate 7 presents the photomicrograph of the livers derived from rats treated with 400 mg/kg body weight of acetaminophen and 100 mg/kg body weight of vitamin C. Remarkably, reductions are observed in hepatocellular necrosis and inflammation, indicating a protective effect.

Histopathological examination of kidney tissues reveals distinct electron microscopy findings for each group, explained as follows:

Plate 8 captures the photomicrograph of kidneys extracted from rats in the control group. Renal tissues in the control group exhibited normal histology, featuring intact glomeruli and tubules.

Plate 9 exhibits the photomicrograph of kidneys obtained from rats treated with 100 mg/kg body weight of acetaminophen. Slight alterations in renal tissue, such as mild inflammation and minimal tubular injury, were observed.

Plate 10 showcases the photomicrograph of kidneys from rats treated with 100 mg/kg body weight of acetaminophen and 100 mg/kg body weight of vitamin C. This group exhibits reduced tubular damage and inflammation in comparison to the acetaminophen-only treated group.

Plate 11 shows the photomicrograph of kidneys derived from rats treated with 200 mg/kg body weight of acetaminophen. In this scenario, there were moderate tubular epithelial cell damage, vacuolation and signs of interstitial inflammation in the kidney when compared to the 100 mg/kg acetaminophen group.

Plate 12 presents the photomicrograph of kidneys from rats treated with 200 mg/kg body weight of acetaminophen and 100 mg/kg body weight of vitamin C. Mild tubular injuries

and interstitial inflammation were observed compared to the group treated with acetaminophen alone.

Plate 13 reveals the photomicrograph of kidneys obtained from rats treated with 400 mg/kg body weight of acetaminophen, demonstrating severe tubular necrosis, loss of cellular integrity and marked interstitial inflammation.

Plate 14 displays the photomicrograph of kidneys derived from rats treated with 400 mg/kg body weight of acetaminophen and 100 mg/kg body weight of vitamin C. Tubular injuries, and interstitial inflammation were ameliorated.

Discussion

In animal models, acetaminophen has been shown to induce hepatic and renal injury, commonly characterized by apoptosis and necrosis in these tissues (Elsafty *et al.,* 2024; Hamid *et al.,* 2018). The study seeks to assess the degree of liver and kidney damage caused by acetaminophen, examining dose-dependent effects to gain insights into optimal dosages for protection. Furthermore, this investigation explores the potential hepato- and reno-protective effects of vitamin C in averting such injuries. Vitamin C has been documented to offer pharmacological protection due to their antioxidant properties and diverse roles in cell regulation (Doseděl *et al.,* 2021).

Liver transaminases, specifically ALT and AST, continue to serve as the primary indicators for evaluating liver damage and have been preferred biomarkers for an extended period (Fu *et al.,* 2020). These enzymes are discharged into the bloodstream following damage to liver cells, and heightened levels in serum signify cellular leakage and a compromise in the functional integrity of hepatocyte cell membranes (Contreras-Zentella and Hernández-Muñoz, 2016; Giannini *et al.,* 2005). The damage to hepatocytes caused by acetaminophen administration is attributed to the heightened production of reactive toxic metabolites, particularly NAPQI (Yoon *et al.,* 2016). Additionally, acetaminophen-induced hepatic injury may stem from mitochondrial permeability, leading to mitochondrial stress and ATP depletion (Jaeschke *et al.*, 2012).

In this study, the administration of acetaminophen at varying doses of 100, 200, and 400 mg/kg body weight, increased ALT and AST suggesting hepatocellular injury, as these enzymes are released into the bloodstream when liver cells are damaged, injured, or stressed. This finding mirrors the outcomes of a study conducted in 2013 by Jang *et al.* (2023). The observed decrease in ALT and AST activities in rats treated with vitamin C along with acetaminophen suggests a potential protective effect. Lower levels of these enzymes indicate reduced liver cell damage, implying that vitamin C may have a mitigating or preventive role against the hepatotoxic effects of acetaminophen. These findings are by the prior investigations conducted by Abdulkhaleq *et al.* (2018) and Adeneye and Olagunju (2008).

An increase in total bilirubin concentrations is indicative of impaired liver function, as the liver is responsible for processing and excreting bilirubin. Elevated bilirubin levels can result from liver dysfunction or obstruction of bile flow (Guerra Ruiz *et al.,* 2021). The decrease in total bilirubin concentration in vitamin C-treated rats further supports the notion that vitamin C supplementation may help preserve liver function and prevent the accumulation of bilirubin, contributing to a healthier hepatic state. A decrease in total protein concentration in the serum of the rats administered the varying doses of acetaminophen could be due to reduced protein synthesis or increased protein breakdown. The liver, being a significant organ responsible for protein synthesis, is known to be affected by acetaminophen toxicity (Yoon *et al.,* 2016). As a result, the decrease in total protein levels could serve as an indicator of liver damage or dysfunction. An increase in total protein concentration, caused by the administration of vitamin C to the acetaminophen-treated rats, may be indicative of improved protein synthesis or reduced protein breakdown. Vitamin C is known for its antioxidant properties which could contribute to improved protein metabolism.

The kidney plays a crucial role in removing waste products, maintaining fluid balance, and regulating electrolytes. Prior research has demonstrated that acetaminophen can induce acute kidney injury in both human subjects (Mour *et al.,* 2005) and animal models (Elsafty *et al.,* 2024). The administration of the varying doses of acetaminophen to male Wistar rats resulted in increased serum urea and creatinine concentrations. Urea and creatinine are important markers of kidney function. Elevated levels of urea and creatinine indicate impaired kidney function. Urea is a waste product that is normally excreted by the kidneys, and increased levels may suggest decreased filtration by the kidneys. Creatinine is another waste product, and high levels can indicate impaired kidney function as well (Brookes and Power, 2022). In this study, acetaminophen treatment appears to have negative effects on kidney function. These results align with earlier research indicating elevated serum urea and creatinine levels as indicators of acetaminophen-induced kidney injury (Sohail *et al.,* 2024; Abdeen *et al.,* 2019). On the other hand, vitamin C treatment seems to have a protective effect on the kidneys as indicated by the lower levels of urea and creatinine suggesting an improvement in kidney function. This demonstrates that vitamin C has a protective effect on the kidneys, promoting improved filtration and excretion of waste products. These findings are consistent with earlier research documented by Mamdouh and Tarek in 2000.

The liver and renal toxicity caused by NAPQI stems from oxidative stress, triggered by increased levels of reactive oxygen species (ROS). This surge in ROS leads to the oxidation of essential cellular components including lipids, proteins, and DNA, alongside provoking mitochondrial dysfunction (Sohail *et al.,* 2024; El-Maddawy and El-Sayed, 2018). The body's ability to counteract oxidative stress, which safeguards against cellular damage, is frequently assessed using biomarkers like SOD, GSH, CAT, and MDA. Oxidative stress is commonly identified by heightened lipid peroxidation levels and alterations in both enzymatic and non-enzymatic antioxidant systems. In our investigation, we examined alterations in liver and kidney levels of MDA and GSH, as well as CAT, and SOD activities as indicators of oxidative stress triggered by the oral administration of acetaminophen and the potential protective role of vitamin C against oxidative stress-induced alterations in these tissues. The administration of varying doses (100, 200, and 400 mg/kg body weight) of acetaminophen to male Wistar rats resulted in decreased superoxide dismutase (SOD) and catalase (CAT) activities, in both liver and kidney homogenates. SOD and CAT are crucial enzymes involved in scavenging reactive oxygen species (ROS) and protecting cells from oxidative damage. The decrease in their activities suggests that acetaminophen administration led to oxidative stress in the liver and kidneys of the rats. Additionally, acetaminophen administration resulted in reduced levels of glutathione (GSH) in both liver and kidney homogenates.

GSH, a tripeptide present in various mammalian tissues, is an essential antioxidant molecule that plays a key role in neutralizing ROS and NAPQI, an active metabolite of acetaminophen. Serving as a vital element of the body's

antioxidant defence mechanism, it eradicates detrimental free radicals such as hydrogen peroxide and superoxide radicals, simultaneously safeguarding thiol groups in membrane proteins (Elsafty *et al.*, 2024; Yayla *et al.*, 2014). In this study, the decrease in GSH levels indicates a disruption in the antioxidant defence system, which may have contributed to oxidative damage in the liver and kidneys of the experimental animals.

Extensive documentation supports the notion that liver tissue possesses a notably elevated concentration of polyunsaturated fatty acids, which are highly susceptible to peroxidative harm (a process that occurs when ROS attack and damage cell membranes). This peroxidation process leads to the formation of MDA (a byproduct of lipid peroxidation), serving as a biochemical marker for necrosis. When present in serum, MDA serves as a biomarker for oxidative stress, signalling a state of oxidative damage to lipids and cellular structures. This research also noted an increase in malondialdehyde (MDA) levels in both the liver and kidney homogenates following acetaminophen administration. The increase in MDA levels indicates enhanced lipid peroxidation, suggesting that acetaminophen administration induced oxidative stress in the liver and kidneys of the rats. The decrease in SOD, CAT, and GSH levels, coupled with an elevation in MDA levels, corresponds with earlier studies investigating acetaminophen-induced nephrotoxicity (Abdeen *et al.*, 2019; Eshrati *et al.*, 2021; Wans *et al.*, 2021). The hepato- and nephroprotective benefits of vitamin C were demonstrated through its capacity to reduce MDA levels and inhibit lipid peroxidation, alongside enhancing GSH, SOD, and CAT levels in both tissues. The increase in SOD and CAT activities indicates that vitamin C may have helped restore the antioxidant defence system in these tissues, enhancing the removal of ROS and protecting the cells from oxidative damage. Vitamin C treatment also significantly increased GSH levels in the liver and kidney homogenates of acetaminophen-intoxicated rats. This elevation in GSH levels shows that vitamin C may have supported the regeneration of GSH and maintained its levels, improving the antioxidant capacity of the liver and kidneys. Decreased MDA levels in both liver and kidney homogenates were also observed after vitamin C treatment, indicating that vitamin C may have inhibited lipid peroxidation, reducing the damage to cellular structures caused by oxidative stress. These findings suggest that vitamin C played a crucial role in restoring the antioxidant defence system and mitigating oxidative damage caused by the administration of acetaminophen to the animals. These findings align with the earlier investigations conducted by Abdulkhaleq *et al.* (2018).

In this study, the ultrastructural changes induced by acetaminophen toxicity in the liver and kidneys of the experimental are characterized by hepatocellular degeneration and necrosis, tubular epithelial cell damage and necrosis, inflammation, and loss of cellular integrity. Vitamin C treatment appears to counteract these effects, exhibiting protective features such as reduced degeneration and necrosis, reduced tubular epithelial cellular injuries, anti-inflammatory actions, and preservation of cellular structures highlighting the potential therapeutic role of vitamin C in mitigating these ultrastructural damages caused by acetaminophen toxicity. These findings align with earlier research documenting renal histological alterations following APAP administration, as reported in studies by Sohail *et al.* (2024); Eshrati *et al.* (2021); Wans *et al.* (2021); El-Maddawy and El-Sayed (2018).

CONCLUSION

In conclusion, this study suggests that vitamin C exerts a protective effect against oxidative damage and mitigates the histopathological alterations induced by acetaminophen toxicity in the liver and kidneys. This protective mechanism might involve the inhibition of intracellular ROS production by vitamin C, as well as its contribution to cellular GSH synthesis.

AUTHORS' CONTRIBUTIONS

Experiment design, data collection, and statistical analysis were conducted by SOE, EVA, and HEI. Writing and drafting of the manuscript were primarily performed by SOE. All authors participated in the review and editing of the final version of the manuscript.

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