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Research Article

Protective Effects of Purple Onion Extract on Malondialdehyde Levels in the Spleen of Wistar Albino Rats with Ibuprofen-Induced Oxidative Stress

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Abstract: Oxidative stress, characterized by an imbalance between reactive oxygen species (ROS) production and the antioxidant defence system, plays a pivotal role in drug-induced tissue injury.Ibuprofen, is known to generate ROS, deplete endogenous antioxidants, and induce oxidative damage in various tissues, including the spleen. This study investigates the protective effects of *Allium cepa* (purple onion) extract on ibuprofen-induced oxidative stress in Wistar albino rats. A total of 25 Wistar albino rats, weighing approximately 200 g each, were divided into five groups (n = 5). Group 1 served as the normal control and received only food and water. Group 2 was administered ibuprofen to induce oxidative stress without treatment. Groups 3 and 4 received ibuprofen alongside low-dose (250 mg/kg) and high-dose (1000 mg/kg) *Allium cepa* extract, respectively. Group 5 served as the standard control and received ibuprofen with Vitamin C (1000 mg/kg). Biochemical parameters, including malondialdehyde (MDA) levels, were analysed to assess lipid peroxidation, while histological examination of the spleen was performed to evaluate tissue-specific effects. The results demonstrated that ibuprofen administration significantly elevated MDA levels, indicating increased oxidative stress. However, treatment with *Allium cepa* extract, particularly at a high dose, significantly reduced MDA levels and preserved spleen histology compared to the ibuprofen control group. Vitamin C also showed notable protective effects. The findings highlight the potent antioxidant properties of *Allium cepa,* suggesting its potential as a natural therapeutic agent for managing drug-induced oxidative damage.

Keywords: *Allium cepa*, Biochemical parameters, Histopathological Study Ibuprofen-Induced Oxidative Stress, Malondialdehyde Levels, Spleen, Vitamin C, Wistar albino rats

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1. Introduction

Oxidative stress is a physiological condition that arises from an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defence systems. While ROS are naturally produced during normal cellular metabolism, excessive levels can result in significant damage to biomolecules such as lipids, proteins, and DNA. This damage is a key driver of cellular dysfunction, inflammation, and the pathogenesis of numerous diseases, including cardiovascular disorders, neurodegenerative diseases, diabetes, and cancer [1]. ROS include free radicals like superoxide anions (O_2^-), hydroxyl radicals ($OH\cdot$), and non-radical species like hydrogen peroxide (H_2O_2). Under normal physiological conditions, endogenous antioxidants such as glutathione, superoxide dismutase, and catalase effectively neutralize these reactive species, maintaining cellular homeostasis. However, when ROS production exceeds the antioxidant capacity, oxidative stress ensues, leading to a cascade of molecular damage and impaired cellular function. Among the exogenous factors contributing to oxidative stress, the administration of nonsteroidal anti-inflammatory drugs (NSAIDs) like ibuprofen has received significant attention. NSAIDs are widely used for their analgesic, anti-

inflammatory, and antipyretic properties, making them a cornerstone of treatment for conditions such as arthritis, musculoskeletal injuries, and chronic pain. Despite their clinical utility, NSAIDs are associated with adverse effects, including gastrointestinal irritation, renal toxicity, and oxidative damage in various tissues [2]. Ibuprofen, a commonly prescribed NSAID, exerts its therapeutic effects by inhibiting cyclooxygenase (COX) enzymes, thereby reducing the production of prostaglandins responsible for inflammation and pain. However, this inhibition also disrupts the protective functions of prostaglandins in the gastrointestinal tract, kidneys, and other organs, leading to side effects such as gastric ulcers, nephrotoxicity, and hepatotoxicity [3]. Furthermore, ibuprofen generates ROS during its metabolism, depleting endogenous antioxidants like glutathione and catalase. This results in oxidative stress, which exacerbates tissue injury, particularly in organs such as the liver, kidneys, and spleen [4]. The deleterious effects of ibuprofen-induced oxidative stress underscore the need for effective therapeutic interventions to mitigate these damages. While synthetic antioxidants have been explored, their long-term use is often limited by side effects, cost, and accessibility. As a result, there is growing interest in natural antioxidants derived from plants due to their affordability, safety, and multifaceted therapeutic potential. *Allium cepa*, commonly known as purple onion, is a widely consumed vegetable renowned for its medicinal properties. It has been extensively studied for its rich content of bioactive compounds, including flavonoids (e.g., quercetin), phenolic acids, sulphur-containing compounds, and saponin. These compounds exhibit potent antioxidant, anti-inflammatory, and antimicrobial activities, making *Allium cepa* a promising candidate for managing oxidative stress [5]. Quercetin, the most abundant flavonoid in *Allium cepa*, is a well-documented antioxidant that neutralizes ROS, inhibits lipid peroxidation, and enhances the activity of endogenous antioxidant enzymes such as superoxide dismutase and catalase. Its ability to protect cellular components from oxidative damage has been demonstrated in both in vitro and in vivo studies [6]. In addition, sulphur-containing compounds in *Allium cepa*, such as allyl sulphides and thiosulfates, play a crucial role in modulating redox signalling pathways and enhancing cellular antioxidant defences [7]. Beyond its antioxidant properties, *Allium cepa* exhibits anti-inflammatory effects by downregulating pro-inflammatory mediators like tumour necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6). These combined properties make it a suitable candidate for addressing the dual challenge of oxidative stress and inflammation associated with ibuprofen use [8]. The use of natural antioxidants such as *Allium cepa* aligns with the broader effort to explore safer and more accessible therapeutic options for mitigating oxidative stress-induced damage. The affordability and widespread availability of purple onion further enhance its appeal, particularly in resource-constrained settings where access to synthetic antioxidants may be limited. This study aimed to investigate the protective effects of *Allium cepa* extract on oxidative stress induced by ibuprofen in Wistar albino rats. By evaluating biochemical markers such as malondialdehyde (MDA) and conducting histological analyses of the spleen, this research seeks to elucidate the potential of *Allium cepa* as a natural therapeutic agent for managing drug induced oxidative damage. Furthermore, the study explores the comparative efficacy of *Allium cepa* and Vitamin C, a well-established antioxidant, in reducing oxidative stress and preserving tissue integrity.The findings of this research will contribute to the growing body of evidence supporting the use of plant-based antioxidants in mitigating oxidative damage. It is anticipated that this study will provide valuable insights into the role of *Allium cepa* in oxidative stress management, paving the way for its potential integration into therapeutic strategies for drug-induced tissue injury.

2. Materials and Methods

Collection of Samples

Plant Collection and Authentication

Fresh bulbs of *Allium cepa* (purple onion) were obtained from Choba Market, Port Harcourt, Rivers State, Nigeria. The onions were selected based on their quality, ensuring they were free from visible defects such as bruising, fungal infections, or sprouting. This careful selection process was crucial, as poor-quality samples could affect the chemical composition and potency of the extract [9]. The plant material was authenticated by a certified botanist at the Department of Plant Science and Biotechnology, University of Rivers State, Port Harcourt. The onions were assigned a unique herbarium number for future reference.

Drug Procurement

Ibuprofen, the agent used to induce oxidative stress, and Vitamin C, the standard drug used for comparison, were purchased from a reputable pharmaceutical outlet in Choba, Rivers State. Both drugs were procured in tablet form, ensuring that they met the required pharmaceutical standards for use in experimental studies. The drugs were stored in their original containers at room temperature, protected from light and moisture, to maintain their stability and ensure the integrity of the substances throughout the experimental procedures.

Determination of Onion Concentration

Purple Onion Extraction

The plant bulb homogenization method was utilized to extract the fresh plant juice. The fresh bulbs from the plant were ground into fine particles using a blender, a total of 3,000 g was placed in a maceration jar, and 2.4 litres (2,400 ml) of ethanol was added. The jar was left for maceration for 72 hours, with daily agitation to enhance the extraction of bioactive compounds. After 72 hours, the juice was extracted and filtered through a clean white handkerchief. The resulting juice was collected meticulously and stored in clean reagent bottles, which were then refrigerated for preservation.

Determination of Onion Concentration

In this process a crucible (an evaporating dish) kept at room temperature was weighed and its weight noted to be 52.98g. 1ml of the onion aqueous extract was introduced into the crucible and its weight with the liquid extract was also noted to be 55.16g. This was placed over a warm beaker of water on a hot plate set at 40oC. The content of the dish was allowed to dry completely and was removed from the beaker and allowed to cool back to room temperature. Its content and the crucible was weighed again and their weight noted to be 53.22g. The weight of the extract in the crucible was determined by subtracting the weight of the crucible from the weight of the crucible plus weight of extract as shown below.

WEIGHT DETERMINATION	PURPLE ONION EXTRACT
Weight of Dish + Liquid extract	55.16g
Weight of Dish+ Dry extract	53.02g
Weight of Dish	52.98g
Weight Difference	0.04g
Weight in mg	**0.04x1000 = 40mg**

Since the volume of the extract used was 21 ml, it implies that the concentration of the extract is 40mg/ml

Dosing Regimen

The doses of onion extract were categorized into two levels:

Low dose: 250 mg/kg = Low Dose: 1.25 ml

High dose: 1,000 mg/kg = 5.0 ml

Oral Toxicity Testing (Ld_{50} Determination)

In this study, Bruce, method of 1985 was employed in determining the LD_{50}, with all the animals used weighing 180g. With this method, a nulliparous and non-pregnant female Wistar rat, fasted overnight (food but not water was withheld) prior to dosing, was orally given a single dose by gavage using a suitable stomach intubation cannula, starting with a dose of 120.5mg/kg of onion aqueous extract (i.e. 21.69mg/180g rat i.e. 1.05ml/180g animal). The female specie was chosen in order to reduce variability and as a means of minimizing the number of animals used. After the onion aqueous extract was administered, food was still withheld for a further 3-4 hours. The animal was observed for death for a period of 48 hours. This dose was chosen since there was no knowledge of the probable toxicity of the extract.

At this dose, no death was observed. Since no death was observed, the dose for the next animal was increased by a factor of one half log times the original dose; (Note: 3.2 is the default factor corresponding to a dose progression of one half log unit). This was calculated to be 385.6mg/kg or 69.408mg/180g i.e. (1.74ml/180g animal) of the extract. The animal was observed carefully for up to 48 hours before making a decision on whether and how much to dose the next animal, and still there was no death.

The process of progressive increment was continued with the following doses of 1233.92mg/kg or 222.106mg/180g, i.e. (5.6ml/180g of animal) extract. Again, another animal was treated with 3948.48mg/kg or 473.87mg/180g or (11.85ml/180g) of the onion aqueous extract, and was again observed for 48 hours still there was no death observed. Since there was no observed death, the dose was increased to 5000mg/kg because, it is scientifically accepted that a substance is said to be non-toxic at a dose of 5000mg/kg. This 5000mg/kg or 900mg/180kg i.e. (22.5ml/180g animal), still caused no death and three other animals were treated with this same dose of 5000mg/kg and still there was no death in all any of the animals, thus it was concluded that the extract is safe. It therefore implies that the onion aqueous extract is said to be safe, using this method of LD_{50} determination. Where death occurs, the LD50 is usually determined using the geometric mean of the last two doses, the highest dose that did not cause death and the lowest dose that caused death as follows;

The LD_{50} is determined from the formula

$$LD50 = \sqrt{D1 \times D2}$$

Where D1 is the highest dose that did not cause death and D2 is the lowest dose that caused death in the test animals.

Experimental Animal Procurement

Twenty-five albino Wistar rats, each weighing approximately 200 g, were obtained from the animal house at the College of Basic Medical Sciences, University of Port Harcourt. The rats were acclimatized in well-ventilated cages lined with wood shavings for 14 days before the start of the experiment. Standard grower's mash (UAC Vital Feed, Grand Cereals, Jos, Nigeria) and tap water were provided ad libitum. The environmental conditions were maintained at 26 ± 2°C with a 12-hour light/dark cycle.

Experimental Design

The 25 Wistar albino rats were divided into five groups, each consisting of five rats (n = 5). The groups were treated as follows:

- Group 1 (Control Group): Rats were given only standard food and water, with no additional treatment.
- Group 2 (Ibuprofen-Induced Oxidative Stress Group): Rats in this group were
- administered ibuprofen to induce oxidative stress.
- Group 3 (Ibuprofen + Low Dose Purple Onion Extract): Rats were given ibuprofen followed by the administration of a low dose of purple onion extract.
- Group 4 (Ibuprofen + High Dose Purple Onion Extract): Rats received ibuprofen along with a high dose of purple onion extract.
- Group 5 (Ibuprofen + Vitamin C): Rats in this group were treated with ibuprofen and the standard antioxidant, Vitamin C.

Each animal received a specific dose of the treatment according to their group. The **Ibuprofen** was administered at a dose of 60mg/ kg, which corresponds to 12 mg for a 200 g rat. The ibuprofen was prepared as a solution at 6.8 mg/mL and was administered at a volume

of 0.5 mL per animal. This dosage was designed to induce oxidative stress by elevating the production of reactive oxygen species (ROS).

For the **Vitamin C** treatment, the standard dose of 1000 mg/70 kg (2.9 mg for a 200 g animal) was used. Vitamin C was prepared at a concentration of 5.8 mg/mL and was administered to the rats at a volume of 0.5 mL per animal. Vitamin C serves as the standard antioxidant agent in this study, known for its protective role against oxidative damage. The **purple onion extract** was administered at two doses based on the LD50 of 5000 mg/kg. The low dose was calculated as 1/20 of the LD50, which equals 250 mg/kg, and the high dose was 1/5 of the LD50, which equals 1000 mg/kg. These doses were selected to assess the potential antioxidant properties of purple onion at varying concentrations. The treatments were administered once daily for a period of 14 days. Rats were monitored throughout the study for any signs of adverse effects or unusual behaviour. At the end of the treatment period, animals were sacrificed for further analysis of oxidative stress markers, Malondialdehyde levels, and other relevant biomarkers.

Methods for Analysis of Parameters

Biochemical Analysis

The primary biochemical marker for evaluating oxidative stress in this study is malondialdehyde (MDA), which is a secondary product of lipid peroxidation and a well-established marker of oxidative damage. MDA is produced when reactive oxygen species (ROS) interact with polyunsaturated fatty acids, leading to the formation of lipid hydroperoxides that are further degraded into MDA [10]. Elevated MDA levels in tissues are indicative of increased oxidative stress and cell membrane damage.

In this study, MDA will be measured in the stomach and other relevant tissues to evaluate the degree of oxidative damage caused by ibuprofen and the protective effects of *Allium cepa* (purple onion) extract. The thiobarbituric acid reactive substances (TBARS) assay will be employed to measure MDA concentration, as it is one of the most reliable methods for detecting lipid peroxidation products [11]. The assay involves reacting MDA with thiobarbituric acid, forming a pink complex that can be quantified spectrophotometrically at 532 nm [12].

In addition to MDA, the antioxidant status of the rats will be assessed by measuring glutathione (GSH) and catalase (CAT) levels. Glutathione is a key intracellular antioxidant that protects cells against oxidative damage by neutralizing ROS and maintaining redox balance [1]. Catalase, an enzyme that decomposes hydrogen peroxide (a ROS), will also be measured to assess the ability of the tissues to combat oxidative damage [13]. Increased oxidative stress typically leads to a depletion of GSH and reduced catalase activity, which will be countered by the administration of Allium cepa extract, known for its antioxidant properties [6].

Histological Analysis

Histological analysis will be conducted to evaluate the structural integrity of the spleen, which plays a critical role in immune function and may show alterations in response to oxidative stress. After the rats are sacrificed, spleen tissues will be carefully excised and processed for histopathological examination. The tissues will be fixed in 10% formalin to preserve cellular structure and prevent enzymatic degradation [14].

Following fixation, the tissues will be embedded in paraffin wax, and thin sections (approximately 4–5 micrometres thick) will be cut using a microtome. The sections will be stained with haematoxylin and eosin (H&E), a commonly used staining technique for visualizing cellular and tissue morphology [15]. Haematoxylin stains cell nuclei blue, while eosin stains the cytoplasm and extracellular matrix pink, allowing for the detailed observation of tissue structure and pathology.

The primary goal of histological analysis is to assess cellular morphology, mucosal integrity, inflammatory infiltration, and the extent of tissue damage. Histopathological changes associated with oxidative stress, such as necrosis, apoptosis, cellular swelling, and fibrosis, will

be evaluated under light microscopy [16]. The severity of these changes will be graded using a histopathological scoring system that considers the number of damaged cells, the degree of inflammation, and the overall tissue architecture [17]. This scoring system will help quantify the extent of tissue damage and the protective effects of Allium cepa extract and Vitamin C in comparison to ibuprofen-induced damage.

Additionally, the spleen's immune cell infiltration will be evaluated, as oxidative stress often triggers an inflammatory response involving increased immune cell recruitment. By assessing the number and distribution of lymphocytes, macrophages, and neutrophils, the histological analysis will provide further insights into the immunomodulatory effects of the treatments [8].

Statistical Analysis

All data were expressed as mean ± standard deviation (SD). Statistical analysis was conducted using Graph Pad Prism software (version X.X) to compare the means across treatment groups. One-way analysis of variance (ANOVA) was used to determine statistically significant differences among the groups, followed by Tukey's post hoc test for pairwise comparisons. A p-value < 0.05 was considered statistically significant, indicating a meaningful difference between the groups. Graphical representations, such as bar charts or line graphs, were utilized to illustrate trends and differences in biochemical and histological parameters. The use of ANOVA ensured that variability within and between groups was appropriately accounted for, while Tukey's test allowed for precise identification of significant differences between individual treatment groups. This approach provided robust and reliable insights into the protective effects of Allium cepa extract in mitigating ibuprofen-induced oxidative stress

3. Results

There was a significant increase in the MDA level of the negative control group (Treated with Ibuprofen only) when compared with Control group with a Mean + SEM of 123.98±1.41 and 87.85±1.31 respectively. However, in the low dose purple onion group, there was a significant reduction in MDA level with a mean + SEM of 87.86±4.63 when compared with the Ibuprofen only treated group. Also the high dose purple onion group and the group treated with Vitamin C also showed a significant reduction in MDA level when compared with the Ibuprofen only treated group with a Mean + SEM of 105.21±6.14 and 103.93±4.66 respectively as shown in table 1.

Table 1. Effect of purple onion extract on serum MDA levels of Ibuprofen-induced oxidative stress in Wistar rats.

Groups	*MDA (ng/ml)*
Control	87.85±1.31#
Neg. control (Ibuprofen only)	123.98±1.41*
250mg/kg (Low Dose Purple Onion)	87.86±4.63#
500mg/kg (High Dose Purple Onion)	105.21±6.14*#
Vit. C	103.93±4.66*#

Mean ± standard Error of mean (SEM) is used to represent values. At p<0.05, the *value differs substantially from the Control; at p<0.05, the #value differs significantly from the AlCl3 control group.

3.1. Effect of purple onion extract on serum catalase level levels of Ibuprofen-induced oxidative stress in Wistar rats.

An observable significant reduction of serum catalase level was recorded in the Ibuprofen only treated when compared with the control group with Mean + SEM values of 154.83±3.10 and 159.38±2.04 respectively. The low dose, high dose purple onion treated groups as well as the Vitamin C treated group all recorded a significant increase in serum catalase level when compared with the Ibuprofen only treated group with Mean + SEM values of 177.34±11.57, 210.43±6.38 and 204.90±2.99 respectively as shown in table 2

Table 2. Effect of purple onion extract on serum catalase level levels of Ibuprofen induced oxidative stress in Wistar rats.

Group	*CAT (U/ml)*
Control	159.38±2.04#
Neg. control (Ibuprofen only)	154.83±3.10*
250mg/kg (Low Dose Purple Onion)	177.34±11.57*#
500mg/kg (High Dose Purple Onion)	210.43±6.38*#
Vit. C	204.90±2.99*#

Mean ± standard Error of mean (SEM) is used to represent values. At p<0.05, the *value differs substantially from the Control; at p<0.05, the #value differs significantly from the AlCl3 control group.

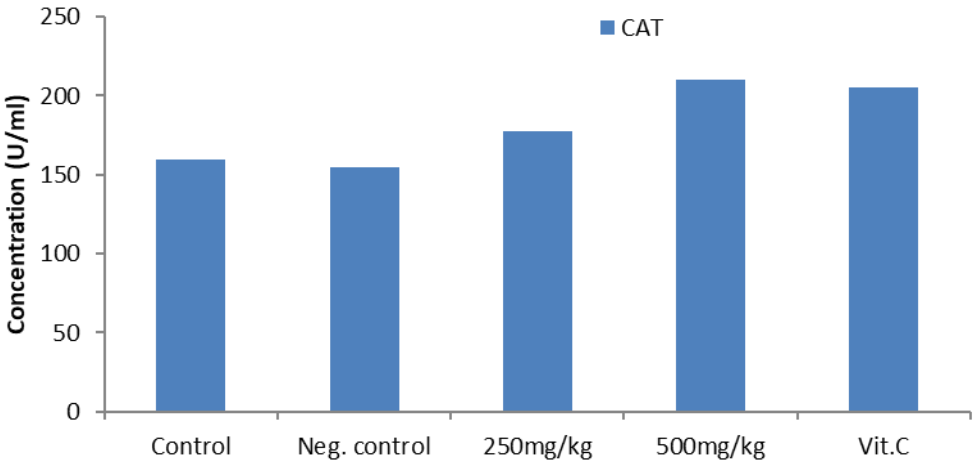

Figure 1: Effect of purple onion extract on serum catalase level of Ibuprofen-induced oxidative stress in Wistar rats

3.2. Effect of purple onion extract on serum GSH level of Ibuprofen-induced oxidative stress in Wistar rats.

As presented in Table 3, there was no significant difference in serum GSH level between the

Ibuprofen only treated group and the control group with Mean + SEM values of 69.42±3.59 and

68.80±2.28 respectively. However, the low dose, high dose purple onion treated groups as well as the Vitamin C treated group all recorded a significant increase in serum catalase level when compared with the Ibuprofen only treated group and the control group with Mean + SEM values of 75.63±3.49, 76.96±2.37 and 87.16±2.86 respectively

Table 3. Effect of purple onion extract on serum GSH level of Ibuprofen-induced oxidative stress in Wistar rats.

Group	GSH (mmol/l)
Control	68.80±2.28
Neg. control (Ibuprofen only)	69.42±3.59
250mg/kg (Low Dose Purple Onion)	75.63±3.49*#
500mg/kg (High Dose Purple Onion)	76.96±2.37*#
Vit. C	87.16±2.86*#

Mean ± standard Error of mean (SEM) is used to represent values. At $p<0.05$, the *value differs substantially from the Control; at $p<0.05$, the #value differs significantly from the AlCl3 control group.

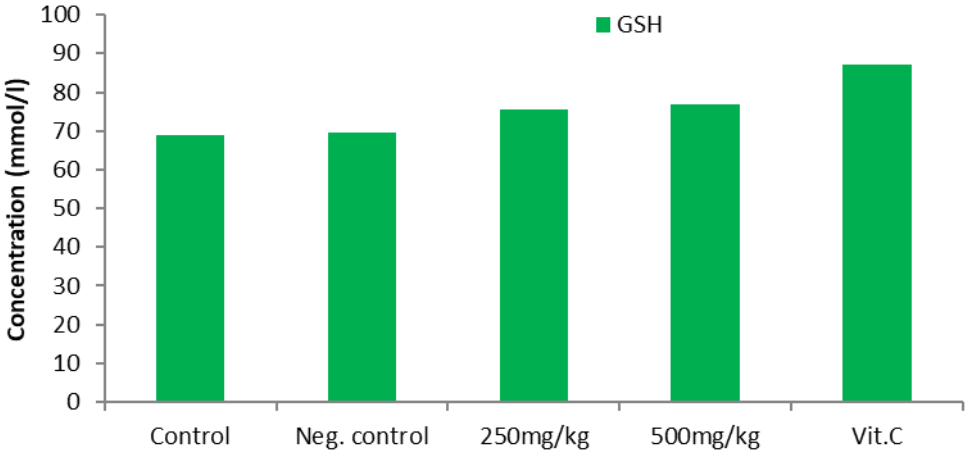

Figure 1: Effect of purple onion extract on serum Glutathione peroxidase (GSH) level of Ibuprofen-induced oxidative stress in Wistar rats

3.3. Histopathological Study

The histological examination of the spleen of rat in group one (1) shows a Normal spleen tissue displaying granulation of the lymphoid cells within white pulp with arterioles located at the mantle and marginal zone of the spleen tissue as shown on plate 1. Conversely, rats in

group 2 (treated with Ibuprofen only) showed severe degeneration and distortion of the spleen tissue with marked degeneration of lymphoid cells and disorganized with pulp and red pulp arterial atrophy within the mantle and marginal zone of the spleen tissue as shown in plate 2.

Then, Plate 3 is the histoarchitecture of the spleen of rat in group 3 showing degenerated and distorted granulation of lymphoid cells and disorganized white pulp with arterial atrophy tissue. While the tissue of rat in group 4 showed signs of moderate degeneration and distortion of the spleen evidenced by a disorganized white pulp with poorly marked regions with reduced lymphoid cells degeneration as shown in plate 4.

Examination of the spleen of rats in group 5 showed moderate degeneration and distortion of the spleen tissue evidenced by vacuolation and disintegration of the white pulp with distorted follicular structures from the red pulp and T-cell areas (Plate 5).

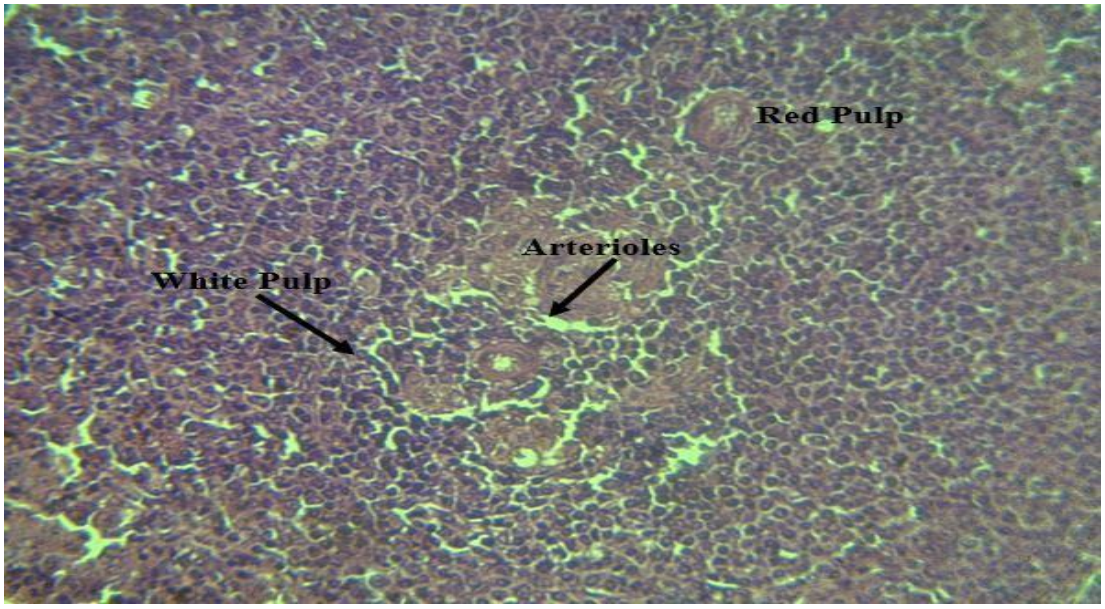

Plate 1. Photomicrograph (H&E X400) of the spleen tissue in control group showing normal tissue architecture with granulation of the lymphoid cells within white pulp as well as arterioles located at the mantle and marginal zone of the spleen tissue.

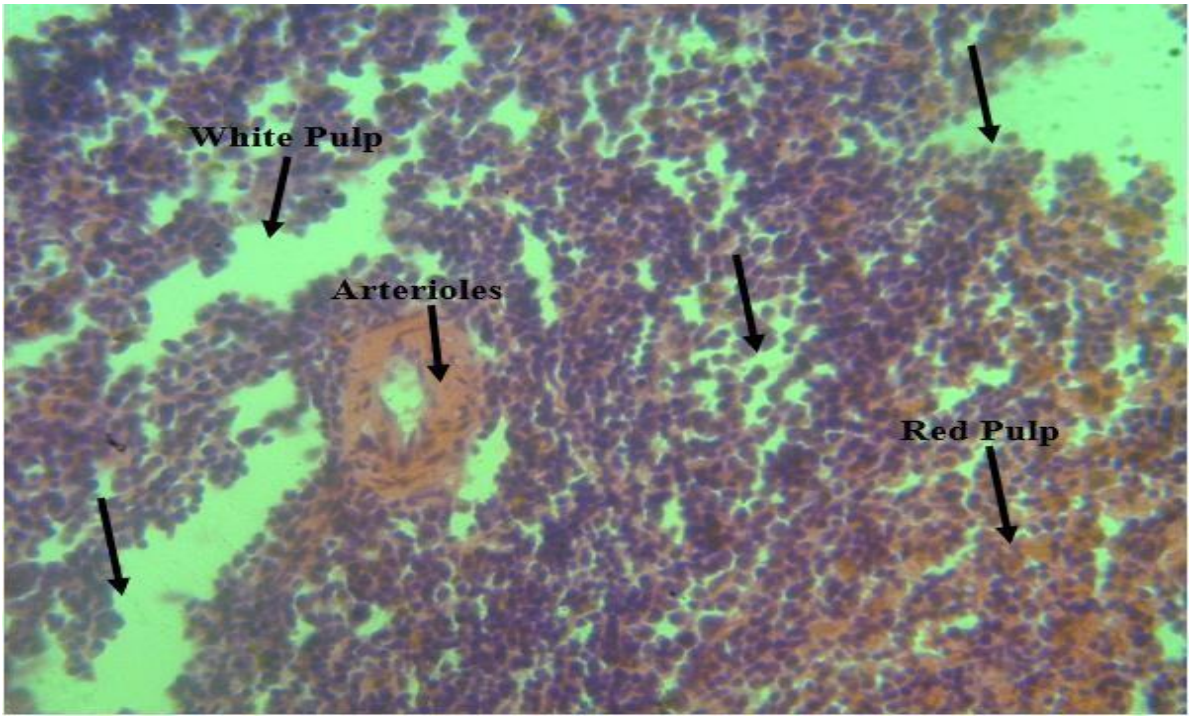

Plate 2 Photomicrograph (H&E X400) of the spleen showing severe degeneration of lymphoid cells and disorganized white pulp and red pulp Arterial atrophy within the mantle and marginal zone of the spleen tissue (arrows).

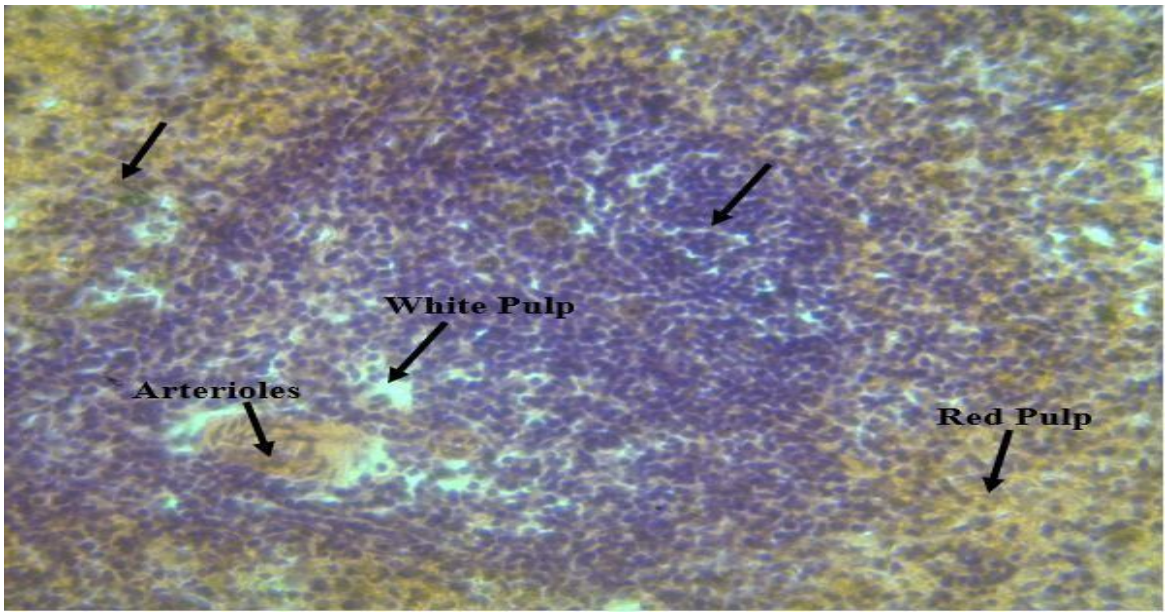

Plate 3. Photomicrograph (H&E X400) of the spleen showing distorted granulation of lymphoid cells and disorganized white pulp with arterial atrophy tissue (arrows).

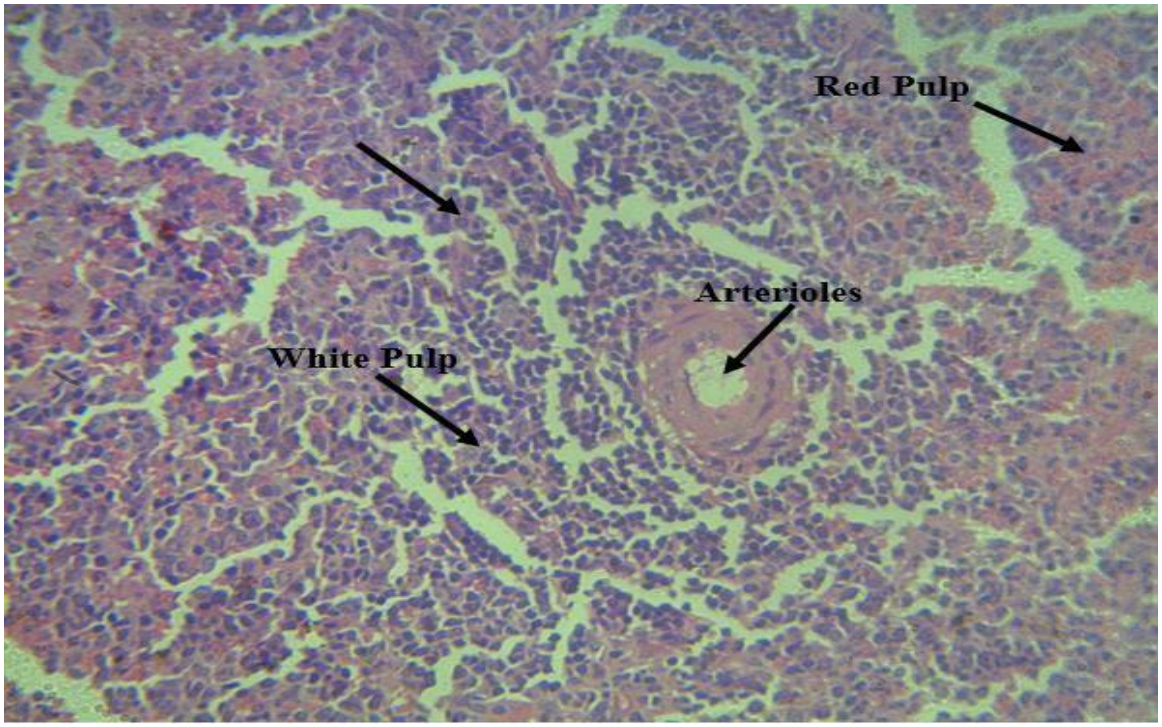

Plate 4. Photomicrograph (H&E X400) of the spleen showing disorganized white pulp with poorly marked regions with reduced lymphoid cells degeneration (arrows).

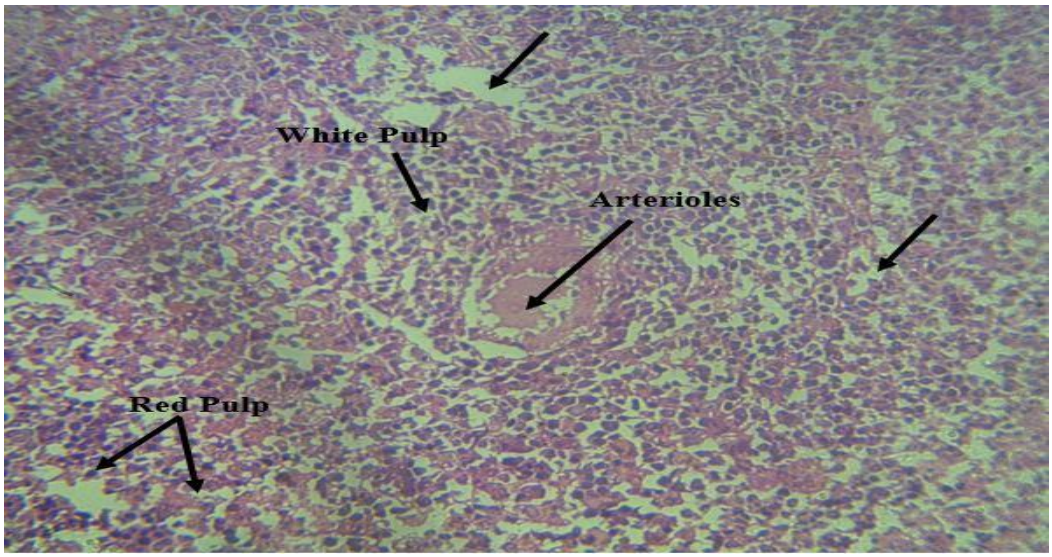

Plate 5. Photomicrograph (H&E X400) of the spleen showing Moderate degeneration and distortion of the spleen tissue evidenced by vacuolation and disintegration of the white pulp with distorted Follicular structures from the red pulp and T-cell areas (arrows).

4. Discussion

Oxidative stress is a significant factor in the pathogenesis of tissue damage caused by nonsteroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen. Ibuprofen, although widely used for its anti-inflammatory and analgesic properties, is known to induce oxidative damage, particularly in hepatic, renal, and gastrointestinal tissues [18]. This damage results from an overproduction of reactive oxygen species (ROS) and a concurrent decrease in the

activity of antioxidant defence systems. The exploration of natural antioxidants, such as purple onion extract, offers a promising approach to mitigate these effects. In this study,

Ibuprofen treated group was observed to induce oxidative stress in the spleen of the test animals. The is evident by the increase in MDA level, decrease in CAT levels of the Ibuprofen treated group when compared with the Control group. This is in line with a study by [19], which states that "Ibuprofen exerts its adverse effects by disrupting mitochondrial function and enhancing lipid peroxidation, as evidenced by elevated levels of malondialdehyde (MDA), a marker of oxidative stress. Additionally, ibuprofen reduces the activity of SOD, CAT, and glutathione peroxidase (GPx), compromising the body's ability to counteract ROS. This imbalance results in cellular damage and inflammatory responses"

The drug's interference with mitochondrial function is linked to a decrease in antioxidant enzyme activities, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), which are crucial for mitigating oxidative damage [20]. However, The GSH level of the Control group and the Ibuprofen treated group showed no significant difference as shown in Table 1.

In this study, there was a significant reduction in MDA level in both high and low dose treatment group. The groups also showed a significant increase in CAT and GSH level when compared with Ibuprofen treated group. These observations are in line with several studies. According to a comprehensive review by [21], which worked on Onion (*Allium cepa*) and its main Constituents as Antidotes or Protective Agents against Natural or Chemical Toxicities. The administration of purple onion extract has been associated with a significant increase in serum CAT levels. This enzyme plays a crucial role in detoxifying hydrogen peroxide (H_2O_2), thereby reducing oxidative stress and preventing cellular damage caused by ROS". Another review on the pharmacological Properties of *Allium cepa*, Preclinical and Clinical Evidences by [22] showed that Onion extract has been shown to enhance GSH levels in serum. This helped to restore the body's antioxidant capacity compromised by ibuprofen.

A study by [23], looked at the Cardio protective and Antioxidant activity of Onion *(Allium cepa)* Leaves Extract in Doxorubicin Induced Cardiotoxicity in Rats and showed that The extract significantly lowers MDA levels, indicating a decrease in lipid peroxidation. This reduction suggests that purple onion extract effectively protects against the oxidative damage typically induced by ibuprofen.

Research has shown that purple onion extract contains a variety of bioactive compounds, primarily flavonoids like quercetin, which exhibit strong antioxidant properties. These compounds can scavenge free radicals, reduce lipid peroxidation, and enhance the activity of endogenous antioxidants such as glutathione peroxidase and superoxide dismutase [24].

The spleen plays a crucial role in the immune system, particularly in filtering blood and facilitating the response to pathogens. However, various pathological conditions can lead to degeneration of lymphoid cells and disorganization within the white and red pulp. Histopathological assessment of the spleen tissue of rats in group 2 (treated with Ibuprofen only) showed Severe degeneration and distortion of the spleen tissue indicating a compromised immune response due to oxidative stress induced by ibuprofen when compared with the control group. In addition, the structural integrity of both white and red pulp is disrupted. Typically, the white pulp is responsible for immune functions while the red pulp aids in blood filtration; their disorganization suggests impaired functionality.

Furthermore, Notable atrophy of arteries within the mantle and marginal zones of the spleen was observed, which may contribute to reduced blood supply and further exacerbate tissue damage. Prolonged or high-dose ibuprofen use may impair normal immune functions of the spleen. Research indicates that by suppressing inflammatory mediators, ibuprofen can potentially reduce the spleen's ability to respond effectively to infections. This effect is particularly concerning for individuals with pre-existing conditions that compromise their immune system, as ibuprofen has been shown to significantly decrease antibody production in human cells, thereby affecting immune responses. According to a study by [25], Chronic ibuprofen use has been linked to oxidative stress in various tissues, including the spleen. Animal

studies have shown that prolonged NSAID exposure results in histopathological changes in the spleen, such as lymphocyte depletion and architectural disruption. These effects are attributed to ibuprofen induced mitochondrial dysfunction and the generation of free radicals, which contribute to tissue damage and compromised immune function.

The group 3 animals treated with Ibuprofen and low dose purple onion also reviewed tissue degeneration and distortion. The granulations of lymphoid cells are distorted, reflecting cellular stress and potential apoptosis. The presence of vacuolation and disintegration in white pulp areas indicates severe cellular distress, likely due to prolonged oxidative damage. On the other hand, Rats in group 4 and 5 that was treated with High Dose Purple Onion and vitamin C respectively, reveal a moderate degeneration and distortion of the spleen tissue. When compared with those in group one (Ibuprofen Only), there is a marked improvement. The improvement observed in the purple onion extract groups is due to the fact that Purple onion extract contains potent antioxidants that scavenge free radicals, reduce lipid peroxidation, and enhance endogenous antioxidant enzyme activity. This is in line with previous studies that states that Purple onion extract has been demonstrated to maintain normal tissue architecture in organs such as the liver and kidneys, effectively preventing structural damage caused by ibuprofen. The protective effect is particularly significant in mitigating ibuprofen-induced organ damage, highlighting the potential benefits of purple onion extract in therapeutic applications.

5. Conclusions

Purple onion extract exhibits protective effects against ibuprofen-induced oxidative stress in the spleen of Wistar rats. Its antioxidant properties mitigate cellular damage by enhancing the activity of antioxidant enzymes and reducing lipid peroxidation. These findings highlight the potential of purple onion extract as a natural therapeutic agent for managing drug-induced oxidative stress. Studies should be conducted to Investigate the molecular mechanisms underlying the antioxidative effects of purple onion extract. Studies should be carried out to explore the optimal dose and duration of administration for maximum protective benefits. Clinical trials should be to evaluate the efficacy and safety of purple onion extract in humans exposed to oxidative stress from ibuprofen or similar drugs. More studies should be considered to assess the protective effects of purple onion extract on other organs with the potentials of being affected by oxidative stress.

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