Original Research Article

Histomorphological Assessment of Adrenal Glands in Wistar Rats Following Myricetin Treatment Against MNU-Induced Oxidative Stress

ABSTRACT

Aim: This study aimed to investigate the protective effects of myricetin against oxidative stress induced by N-Methyl-N-Nitrosourea (MNU) in Wistar rats, emphasizing its impact on adrenal gland histomorphology and antioxidant enzyme activities.

Study Design: The study utilized a randomized controlled trial design with seven groups: normal control (Group A), MNU (Group B), MNU + Casodex (Group C), 100mg myricetin (Group D) and varying doses of myricetin (Groups E-F) administered to rats that had been subjected to MNU-induced oxidative stress.

Place and Duration of Study: The experiment was conducted at the University of Calabar between February 2024 and October 2024.

Method: A total of 42 male Wistar rats were used in this study. Oxidative stress was induced in the experimental groups through intraperitoneal administration of MNU (50 mg/kg). Following MNU exposure, varying doses of myricetin (75 mg/kg, 150 mg/kg, and 300 mg/kg) were administered orally for three weeks. Biochemical assays measured antioxidant enzyme activities, namely Superoxide Dismutase (SOD), Catalase (CAT), and Glutathione Peroxidase (GPx). Histological evaluations of adrenal glands were performed using hematoxylin and eosin (H&E) staining.

Results: Findings indicated that antioxidant and oxidative stress markers (superoxide dismutase, catalase, malondialdyhide) was significantly higher ($p<0.05$) after induction while (glutathione) also showed slight significant increase in the induction group when compared to normal control group. Results after treatment with myricetin showed significant reduction in oxidative stress markers (superoxide dismutase, catalase). Malondialdyhide showed statistically ($p<0.05$) significant difference in groups D & E. However, there was no statistically significant difference in glutathione level across the groups following treatment with doses of myricetin, but the group induced with 50mg of nitrosourea only showed a slightly increase in the mean expression of glutathione. Histopathological analysis revealed that myricetin treatment preserved the structural integrity of the adrenal glands, showcasing a dose-dependent restoration of normal architecture and improved vascular arrangement, particularly in the highest treatment group.

Conclusion: This study suggests that myricetin has promising therapeutic potential for protecting adrenal gland integrity from MNU-induced oxidative stress. The observed

improvements in antioxidant enzyme activities and histological preservation underscore myricetin's role as a natural product for alleviating oxidative damage in endocrine dysfunction. These findings support the integration of myricetin into treatment strategies for conditions associated with oxidative stress, highlighting the importance of natural compounds in modern medicine.

Keywords: Myricetin, Antioxidant, Histomorphology, Catalase, Hematoxylin

1. INTRODUCTION

The adrenal glands, critical components of the endocrine system, are responsible for the synthesis and release of various hormones that play essential roles in regulating metabolic processes, immune responses, and stress management. Dysfunction of these glands can lead to significant health issues, including adrenal insufficiency and hyperaldosteronism (1). One of the primary contributing factors to impaired adrenal function is oxidative stress, a condition resulting from an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defenses (2).

N-methyl-N-nitrosourea (MNU) is a well-established carcinogen that induces oxidative stress and is frequently used in experimental models to study the mechanisms of carcinogenesis and oxidative damage (3). Exposure to MNU has been shown to cause significant histopathological changes in various tissues, including the adrenal glands, thereby disrupting their normal structure and impairing hormone production (4).

Myricetin, a flavonoid found in numerous fruits, vegetables, and herbs, has gained attention for its antioxidant, anti-inflammatory, and anticancer properties (5). Research has demonstrated that myricetin can protect tissues from oxidative damage by scavenging free radicals and modulating cellular signaling pathways involved in stress responses (6). However, the specific effects of myricetin on the adrenal glands in the context of MNU-induced oxidative stress remain inadequately explored.

The present study aimed to conduct a histomorphological assessment of the adrenal glands in Wistar rats subjected to MNU-induced oxidative stress and subsequently treated with myricetin.

By examining the histological alterations, the study sought to elucidate the potential protective effects of myricetin on adrenal tissue.

The adrenal glands are vital to the endocrine system, where they produce hormones that regulate numerous physiological functions, including metabolism, immune response, and the body's reaction to stress. Disruption in adrenal function can lead to various conditions, including adrenal insufficiency and hyperaldosteronism, significantly impacting health and well-being (7).

Oxidative stress has emerged as a critical factor contributing to endocrine dysfunction, occurring when there is an excess of reactive oxygen species (ROS) that overwhelms the body's antioxidant defenses (2). MNU, a potent chemical carcinogen, is known to induce oxidative stress and has been utilized in numerous studies to investigate the effects of oxidative damage on various biological systems, including the adrenal glands (3; 4).

Research highlights that myricetin holds promise as a therapeutic agent owing to its strong antioxidant properties, which may counteract oxidative stress by enhancing the body's natural defense mechanisms and reducing inflammation (8). While the protective effects of myricetin have been documented across various organ systems, its specific impact on the adrenal glands under oxidative stress conditions induced by MNU requires further investigation.

2. MATERIALS AND METHODS

2.1 Experimental Design

This study involved an experimental design with a total of seven groups of rats to assess the effects of N-Methyl-N-Nitrosourea (NMU) and myricetin on antioxidant levels, oxidative stress markers, and the histological integrity of the adrenal glands. Each group consisted of six rats.

2.2 Materials

2.2.1 Animals

Forty two male Wistar rats were used for this study, with a weight range of approximately 200-250 grams. The rats were housed under standard laboratory conditions, which included a controlled temperature maintained at 21-23°C and a 12-hour light/dark cycle. Throughout the duration of the study, the animals had unrestricted access to standard pellet feed and water. This controlled environment ensured the well-being of the rats and contributed to the validity of the experimental results.

2.3 Treatment Groups

1. Group A (Normal Control - NC): Rats received standard feed and water.

2. Group B (NMU): Rats were administered a single dose of 150 mg/kg body weight NMU intraperitoneally.

3. Group C (NMU + Casodex): Rats received NMU (150 mg/kg) and were treated with 50 mg/kg body weight of Casodex (bicalutamide).

4. Group D (NMU + Myricetin, 100 mg): Rats were treated with NMU followed by 75 mg/kg body weight of myricetin.

5. Group E (NMU + Myricetin, 75 mg): Rats were treated with NMU and 100 mg/kg body weight of myricetin.

6. Group F (NMU + Myricetin, 150 mg): Rats were administered NMU and 150 mg/kg body weight of myricetin.

7. Group G (NMU + Myricetin, 300 mg): Rats received NMU followed by 300 mg/kg body weight of myricetin.

2.4 Methods

2.4.1 Drug Procurement

Myricetin was obtained from Source Natural in the United States, while N-Methyl-N-Nitrosourea (MNU), a carcinogenic agent used in laboratory animal studies, was purchased

from Syncro Systems, a licensed scientific laboratory store in Calabar, Cross River State, based on a prescription. Additionally, Casodex was sourced from the University of Calabar Teaching Hospital.

2.4.2 Preservation of MNU

To maintain its potency and prevent contamination, MNU was stored at low temperatures in a dark environment, as recommended by Li et al. (**Error! Reference source not found.**).

2.4.3 Induction of Cellular Damage with MNU

After two weeks of acclimatization, 150 mg of MNU was dissolved in 27 ml of sodium citrate buffer. Treatment group rats received an intraperitoneal injection of 0.9 ml of this MNU solution every Friday at 11:30 am for four weeks. This method was chosen to effectively simulate cellular damage in adrenal tissues, leading to oxidative stress and inflammation (**Error! Reference source not found.**).

2.4.4 Administration of Myricetin

Myricetin was administered via the oral route for three weeks, as this method is preferred due to its higher bioavailability. The compound was dissolved in water before administration (**Error! Reference source not found.**).

2.4.5 Antioxidant and Oxidative Stress Marker Measurements

Following the treatment period, rats were sacrificed. Ketamin injection was used to anesthetize the animals first. Blood samples were collected for serum analysis, and the adrenal glands were excised for histological evaluation.

2.4.6 Biochemical Assays

The concentrations of Superoxide Dismutase (SOD), Catalase (CAT), and Glutathione Peroxidase (GPx) were determined spectrophotometrically using commercial kits and protocols. Malondialdehyde (MDA) levels were measured as an indicator of lipid peroxidation.

Superoxide Dismutase (SOD) Activity: SOD activity was measured spectrophotometrically by evaluating its ability to inhibit the auto-oxidation of epinephrine, with the change in absorbance read at 480 nm after a 5-minute reaction. The reaction mixture included sodium carbonate buffer, liver homogenate, and epinephrine.

Lipid peroxidation (MDA): MDA, an indicator of lipid peroxidation, was quantified using the Buege and Aust (**Error! Reference source not found.**) method. The supernatant was mixed with a TCA-TBA-HCl reagent, boiled, and the absorbance measured at 532 nm.

Glutathione Peroxidase (GPx) Activity: GPx activity was assessed by adding tissue homogenate to a Tris buffer containing EDTA and sodium azide, followed by GSH and H2O2. The reaction was stopped with TCA, and GSH consumption was measured, expressed as µg of GSH consumed per minute per mg of protein (**Error! Reference source not found.**).

2.4.7 Histological Analysis

Adrenal glands were fixed in 10% formalin and subsequently processed for histopathological examination. Tissue sections (5 µm) were sliced using a microtome, stained with haematoxylin and eosin (H&E), and mounted on glass slides. Slides were examined under a light microscope. Observations were made on the structural integrity of the adrenal gland, including the zona reticularis, vascular sinusoids, chromaffin cell orientation, and the presence of inflammatory cells and lipofuscin.

2.4.8 Statistical Analysis

Results were expressed as mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) was used to assess the significance of differences among groups, with a significance level set at $p < 0.05$.

3. RESULTS

3.1 Antioxidant and Oxidative Stress Marker Concentrations

Table 1 presents the concentrations of key antioxidants and oxidative stress markers in different experimental groups after treatment. The measured parameters include Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx), and Malondialdehyde (MDA).

1. Normal Control (NC): The normal control group exhibited baseline levels of SOD (33.46 ± 2.4 µmol/ml), CAT (60.05 ± 1.98 µmol/ml), GPx (4.39 ± 0.25 µmol/ml), and MDA (4.63 ± 0.21 µmol/ml).

2. NMU (N-Methyl-N-Nitrosourea): The NMU group demonstrated elevated levels of SOD (67.36 ± 3.68 µmol/ml) and CAT (87.9 ± 2.16 µmol/ml), indicating increased oxidative stress, with a significant rise in MDA (7.26 ± 0.39 µmol/ml). GPx levels (5.11 ± 0.08 µmol/ml) were also elevated compared to the NC group.

3. NMU + Casodex (50 mg): In this treatment group, SOD (51.56 ± 1.71 µmol/ml) and CAT (63.67 ± 1.12 µmol/ml) levels were significantly lower than in the NMU group, while GPx was similar to the NC group (4.42 ± 0.33 µmol/ml). MDA levels (5.67 ± 0.13 µmol/ml) were also lower compared to the NMU group.

4. Myricetin (100 mg): This group showed SOD levels of 56.92 ± 2.52 µmol/ml and CAT levels of 74.53 ± 1.23 µmol/ml, suggesting moderate antioxidant activity. GPx (4.89 ± 0.46 µmol/ml) was comparable to NMU+CDX, and MDA (6.55 ± 0.31 µmol/ml) remained elevated but lower than the NMU group.

5. NMU + Myricetin (75 mg): In this group, SOD (51.61 ± 1.35 µmol/ml) and CAT (73.55 ± 1.07 µmol/ml) levels were similar to the NMU+CDX group. GPx levels (4.62 ± 0.27 µmol/ml) were the lowest among treatment groups, and MDA (6.35 ± 0.71 µmol/ml) was higher than in both the normal and NMU+CDX groups.

6. NMU + Myricetin (150 mg): This treatment resulted in decreased SOD (48.45 ± 1.18 µmol/ml) and CAT levels (65.66 ± 1.51 µmol/ml) compared to the previous two groups. GPx concentrations (4.59 ± 0.2 µmol/ml) were close to the lowest levels seen, while MDA (5.87 ± 0.26 µmol/ml) decreased compared to NMU.

7. NMU + Myricetin (300 mg): The highest dose of myricetin led to a further decline in SOD (39.79 ± 2.02 µmol/ml) and CAT (63.95 ± 1.12 µmol/ml) levels. Moreover, GPx (4.46 ± 0.3 µmol/ml) was among the lowest recorded, while MDA (5.16 ± 0.01 µmol/ml) values were significantly reduced compared to the NMU group.

Table 1: Anti-Oxidant and oxidative stress marker concentrations of experimental rats

Anti-oxidants and oxidative stress marker concentrations in experimental groups after treatment

Groups	SOD (µmol/ml)	CAT (µmol/ml)	GPx (µmol/ml)	MDA (µmol/ml)
NC	33.46 ± 2.4 [a]	60.05 ± 1.98 [a]	4.39 ± 0.25 [a]	4.63 ± 0.21 [a]
NMU	67.36 ± 3.68 [b]	87.9 ± 2.16 [b]	5.11 ± 0.08 [b]	7.26 ± 0.39 [b]
NMU+CDX (50mg)	51.56 ± 1.71 [c]	63.67 ± 1.12 [a]	4.42 ± 0.33 [a]	5.67 ± 0.13 [c]
MYR (100mg)	56.92 ± 2.52 [d]	74.53 ± 1.23 [c]	4.89 ± 0.46 [c]	6.55 ± 0.31 [b]
NMU+MYR (75mg)	51.61 ± 1.35 [c]	73.55 ± 1.07 [c]	4.62 ± 0.27 [d]	6.35 ± 0.71 [b]
NMU+MYR (150mg)	48.45 ± 1.18 [c]	65.66 ± 1.51 [a]	4.59 ± 0.2 [d]	5.87 ± 0.26 [c]
NMU+MYR (300mg)	39.79 ± 2.02 [a]	63.95 ± 1.12 [a]	4.46 ± 0.3 [a]	5.16 ± 0.01 [c]

Values are presented as Mean ± SEM, n=6. Using one-way analysis of variance. Different superscripts (a, b and c) denote significant difference among experimental groups. **NC**: Normal Control; **NMU**: n-Methyl-n-nitrosourea; **NMU+CDX**: N-methyl-n-nitrosourea(50mg) + Casodex; **MYR**: Myricetin; **NMU+MYR**: n-Methyl-n-nitrosourea + myricetin.

3.2. Histological Observations

The histological examination of adrenal gland sections across different experimental groups revealed notable variations in tissue morphology and cellular integrity following N-Methyl-N-Nitrosourea (MNU) exposure and subsequent myricetin treatment.

1. Group A (Control): The normal control group exhibited healthy adrenal gland structure, characterized by a well-defined zona reticularis populated with lipofuscin cells, vascular sinusoids in the cortical area, and normal orientation of chromaffin cells and blood vessels within the medulla.

2. Group B (MNU): The negative control group, administered 150 mg/kg of Nitrosourea, displayed moderate histopathological changes, including dilated vascular sinusoids, infiltrating inflammatory cells, and degenerating lipofuscin cells, while chromaffin cells and blood vessels in the medullary area maintained normal orientation.

3. Group C (Nitrosourea + Casodex): The treatment group receiving 50 mg of Casodex showed moderate alterations with similar histopathological features as the negative control, including dilated vascular sinusoids and inflammatory cell infiltration but preserved orientation of the chromaffin cells and blood vessels.

4. Group D (Nitrosourea + 100 mg Myricetin): The group treated with 75 mg/kg body weight of myricetin exhibited moderate alterations, with evidences of dilated vascular sinusoids, inflammatory cell infiltrates, and degenerating lipofuscin cells, while chromaffin cells and blood vessels retained normal orientation.

5. Group E (Nitrosourea + 75 mg Myricetin): Administration of 100 mg/kg body weight of myricetin resulted in mild alterations, characterized by infiltrating inflammatory cells in the zona reticularis while chromaffin cells and blood vessels remained normally oriented.

6. Group F (Nitrosourea + 150 mg Myricetin): The group given 150 mg/kg body weight of myricetin displayed almost normal histological features with well-preserved cells in the zona reticularis, also showing normal orientation of chromaffin cells and blood vessels.

7. Group G (Nitrosourea + 300 mg Myricetin): The highest treatment group receiving 300 mg/kg body weight of myricetin demonstrated a well-defined zona reticularis with populated lipofuscin cells and vascular sinusoids, along with normal orientation of the chromaffin cells and blood vessels.

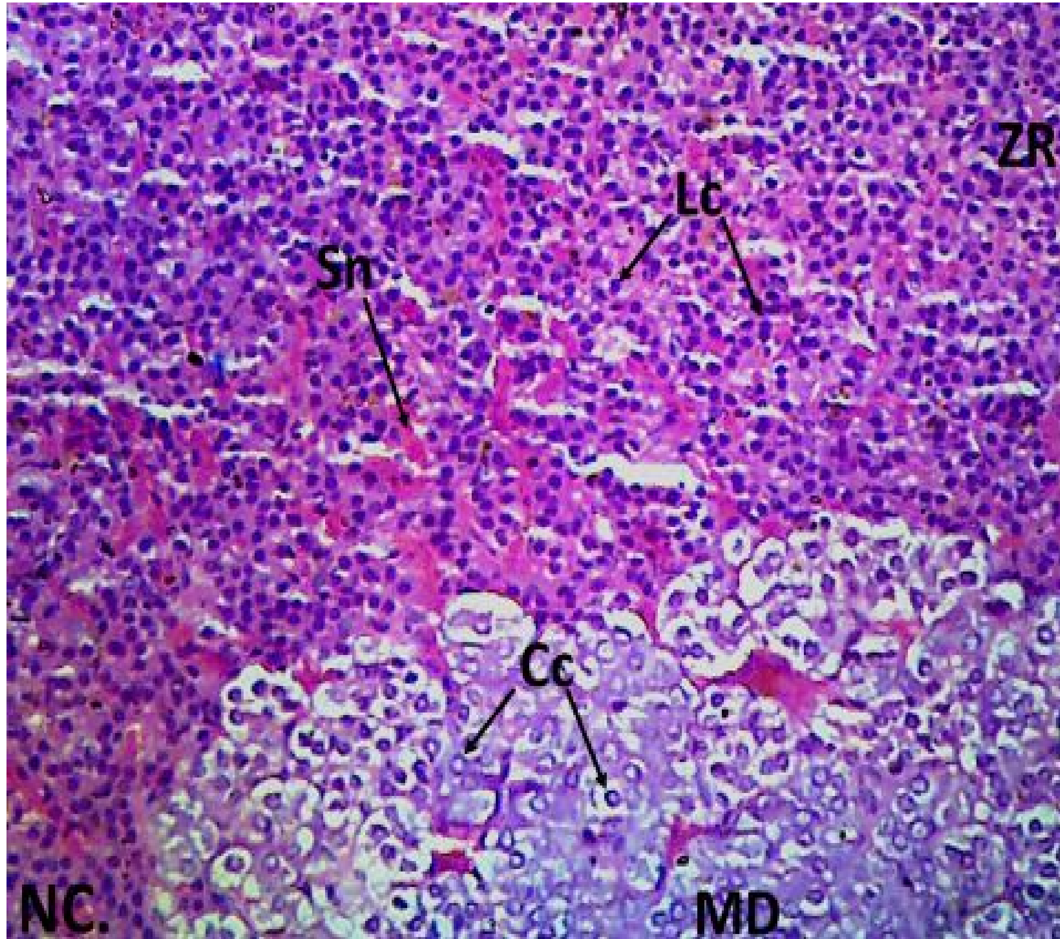

Plate 1: Photomicrograph of a longitudinal section of a Normal control adrenal tissue demonstrating histo-architecture with well-presented zona reticularis with populated lipofusin cells and vascular sinusoids within the cortical area, and normal orientation of the chromaffin cells (Cc) and blood vessels within the medullary (H&E x400).

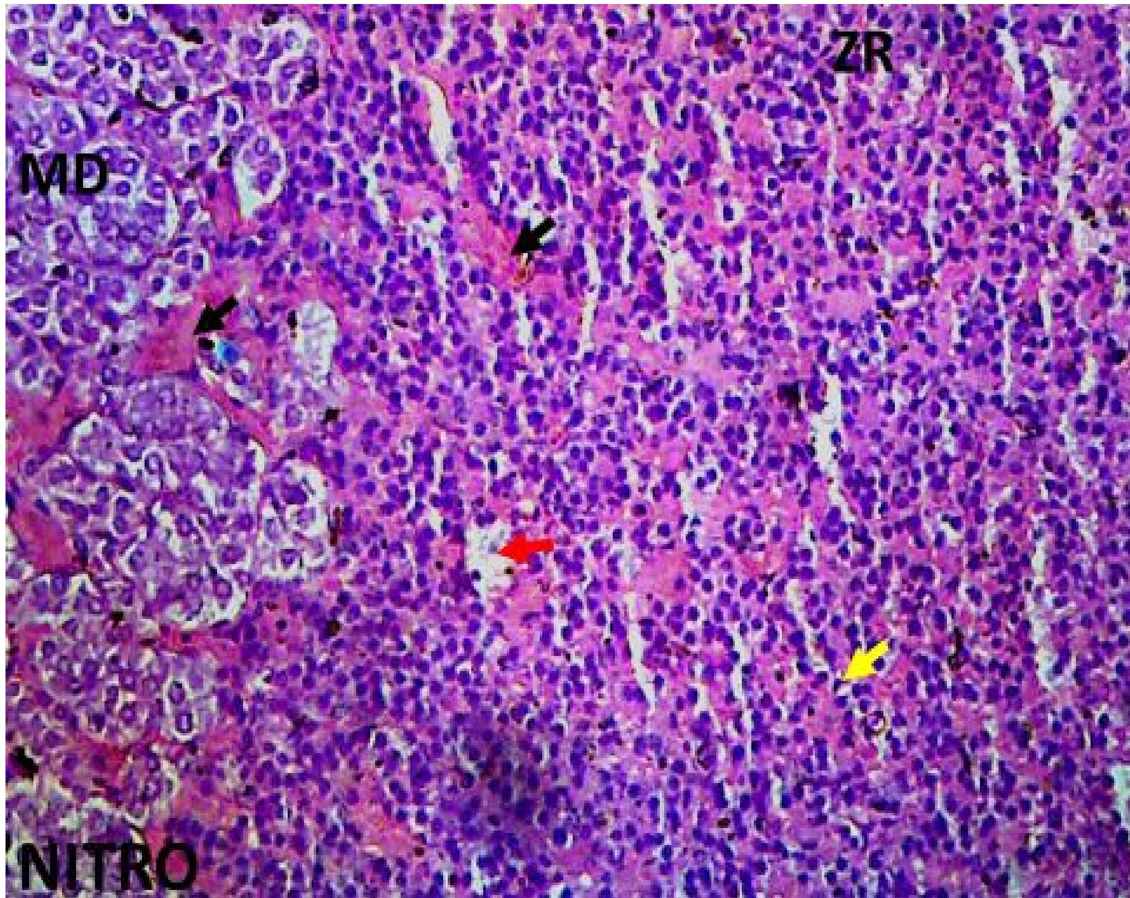

Plate 2: Photomicrograph of a longitudinal section of a Nitrosourea treated adrenal tissue demonstrating moderate histo-architectural alteration with the zona reticularis having dilated vascular sinusoids, infiltrating inflammatory cells (red arrow) and degenerating lipofusin cells within the cortical area, and normal orientation of the chromaffin cells (Cc) and and dilated blood vessels within the medullary area (H&E x400).

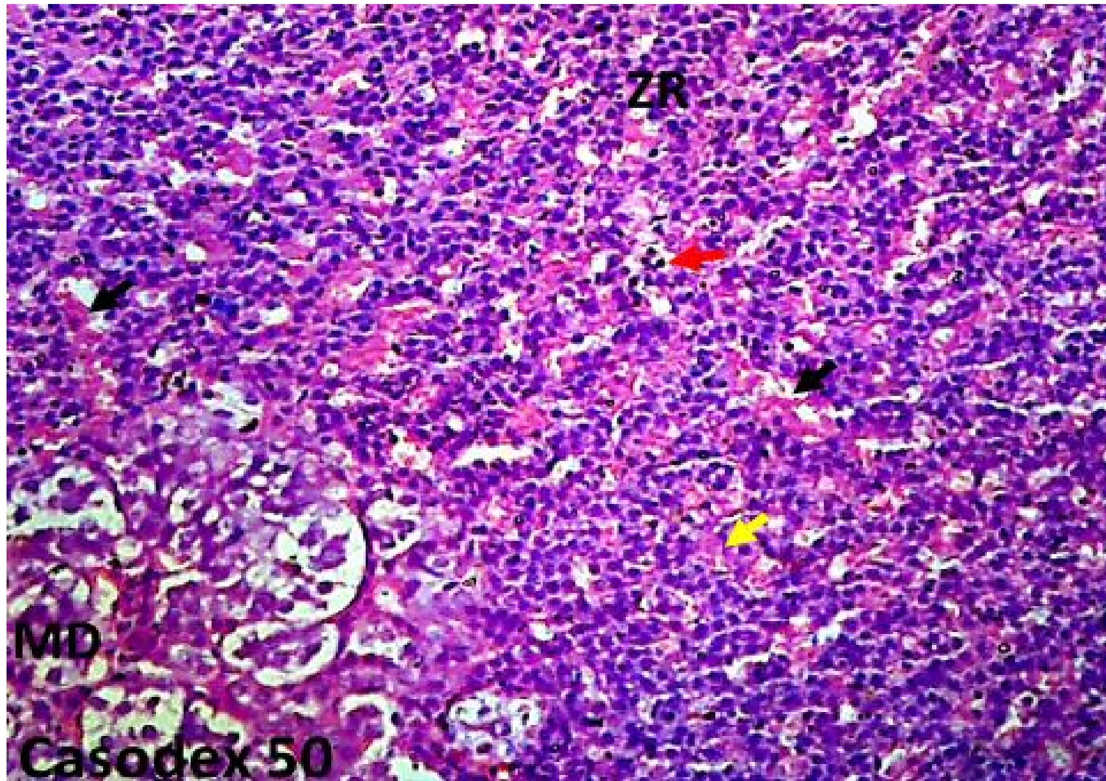

Plate 3: Photomicrograph of a longitudinal section of a 50 mg Casodex treated adrenal tissue demonstrating moderate histo-architectural alteration with the zona reticularis having dilated vascular sinusoids, infiltrating inflammatory cells (red arrow) and degenerating lipofusin cells within the cortical area, and normal orientation of the chromaffin cells (Cc) and dilated blood vessels within the medullary area (H&E x400).

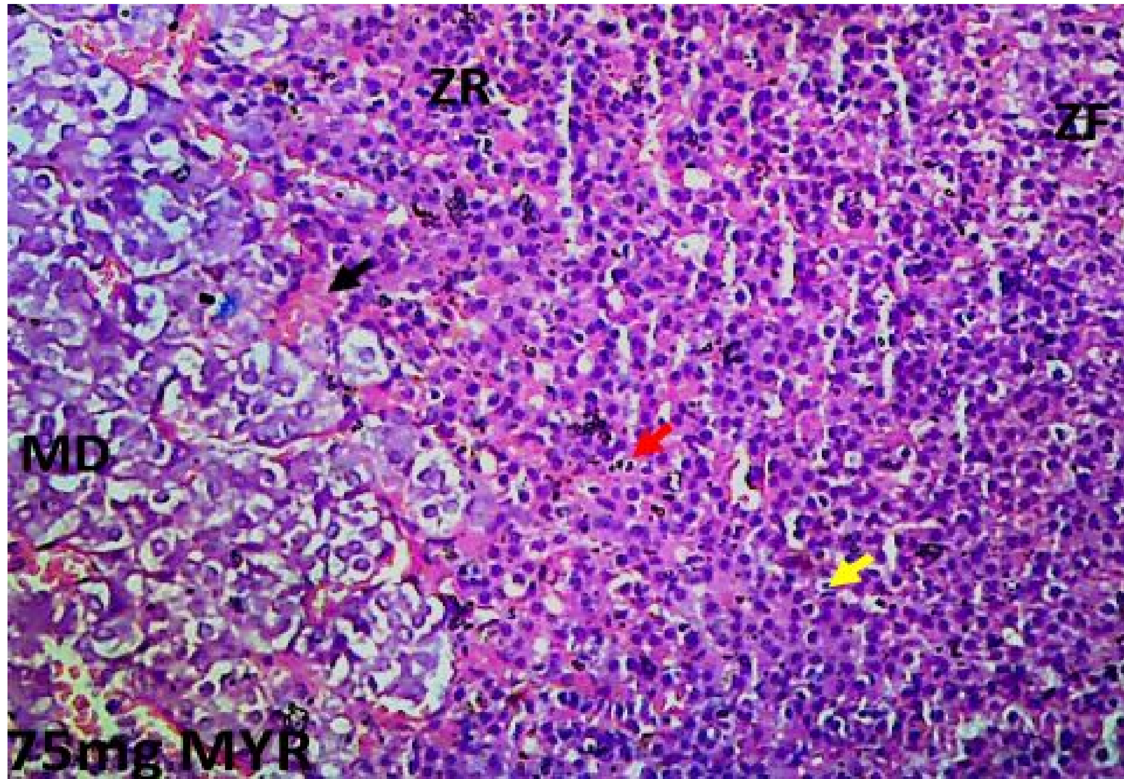

Plate 4: Photomicrograph of a longitudinal section of a 75mg *Myricetin* treated adrenal tissue demonstrating moderate histo-architectural alteration with the zona reticularis (ZR) having area of dilated vascular sinusoid, infiltrating inflammatory cells (red arrow) and degenerating lipofusin cells (yellow arrow) within the cortical area, and normal orientation of the chromaffin cells (Cc) and blood vessels within the medullary area. Zona fasciculata (ZF) (H&E x400).

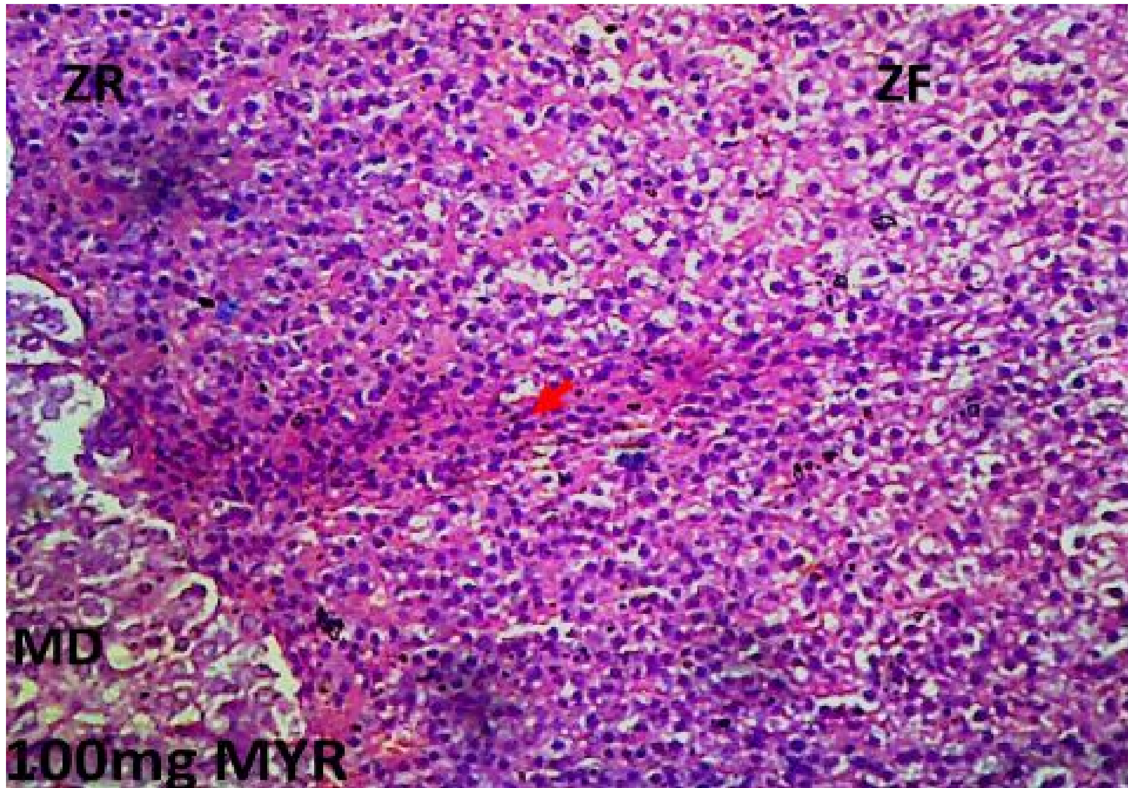

Plate 5: Photomicrograph of a longitudinal section of a 100 mg *Myricetin* treated adrenal tissue demonstrating mild histo-architectural alteration with the zona reticularis (ZR) having area of infiltrating inflammatory cells (red arrow) within the cortical area, and normal orientation of the chromaffin cells (Cc) and blood vessels within the medullary area. Zona fasciculate (ZF) (H&E x400).

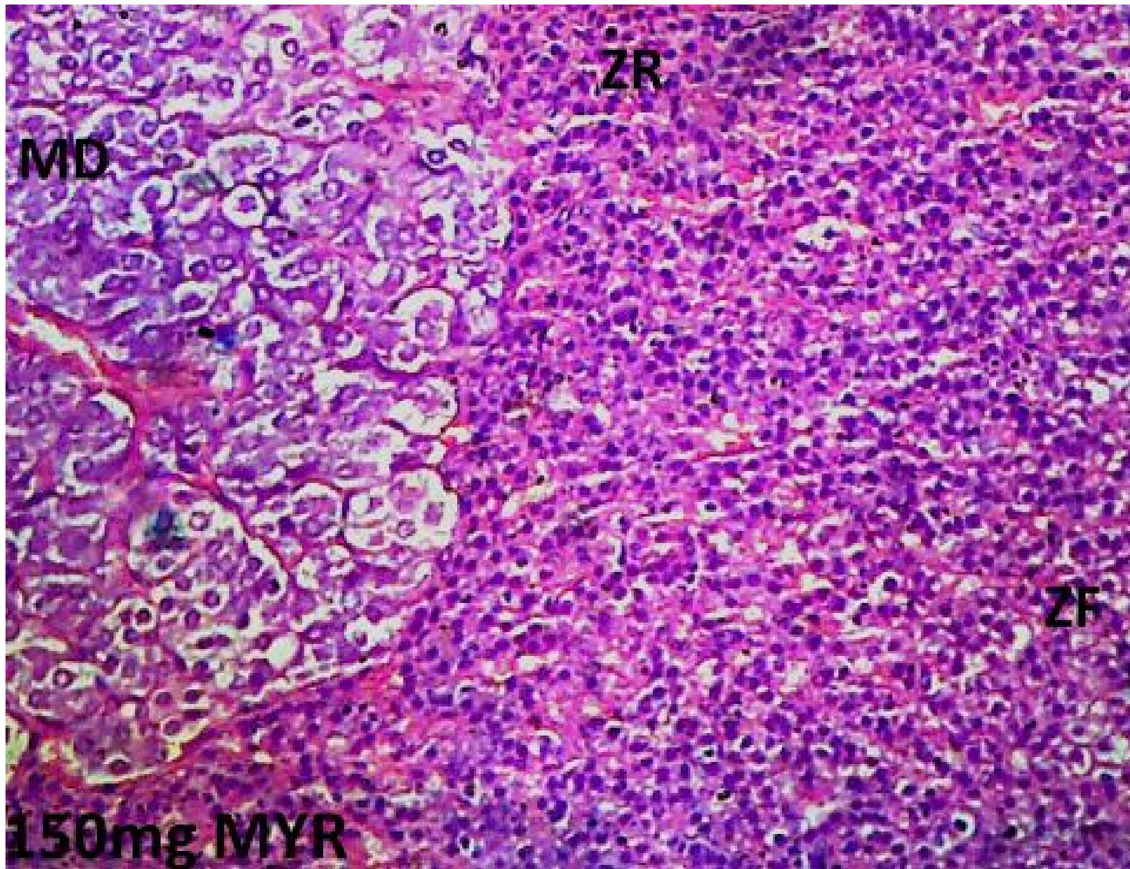

Plate 6: Photomicrograph of a longitudinal section of a 150 mg *Myricetin* treated adrenal tissue demonstrating normal histo-architectural with protected cells of the zona reticularis (ZR) within the cortical area, and normal orientation of the chromaffin cells and blood vessels within the medullary area. Zona fasciculata (ZF) (H&E x400).

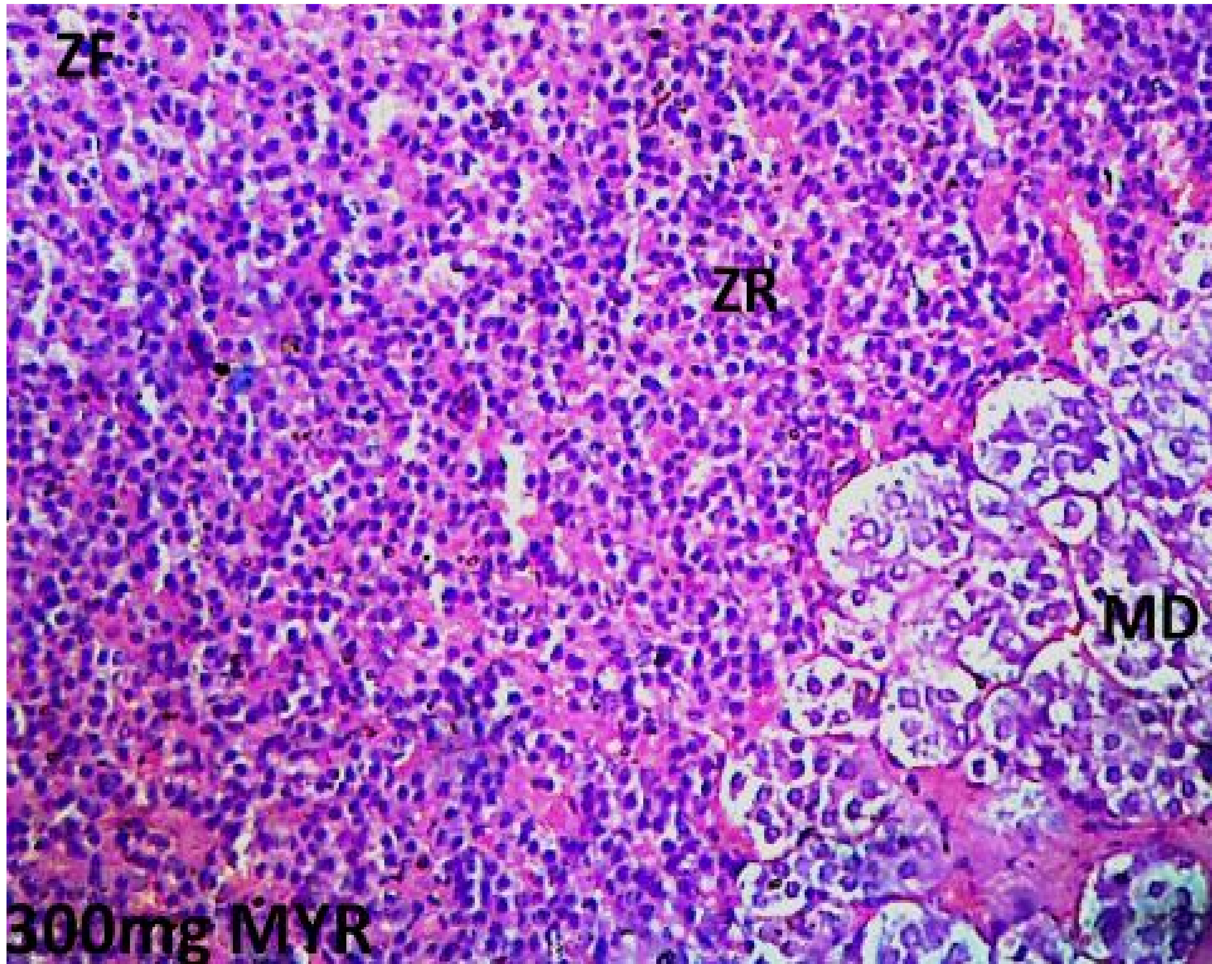

Plate 7: Photomicrograph of a longitudinal section of a 300 mg *Myricetin* treated adrenal tissue demonstrating histo-architecture with well-presented zona reticularis with populated lipofusin cells and vascular sinusoids within the cortical area, and normal orientation of the chromaffin cells (Cc) and blood vessels within the medullary (H&E x400).

Figure 1. Photomicrographs of the adrenal gland among the experimental groups (Plate 1-7)

4. DISCUSSION

The present study aimed to evaluate the protective effects of myricetin on MNU-induced oxidative stress and its impact on the histomorphology of the adrenal glands in Wistar rats. The findings from our research revealed significant insights into myricetin's role in mitigating oxidative damage and preserving adrenal structure, aligning with previous literature that highlights the antioxidant and anti-inflammatory properties of flavonoids.

The biochemical analysis demonstrated that myricetin treatment markedly influenced the levels of key oxidative stress markers. In our study, the administration of MNU resulted in increased levels of malondialdehyde (MDA), a byproduct of lipid peroxidation, thereby indicating enhanced oxidative stress. Similar observations have been made in previous research, where MNU was shown to induce oxidative damage in various organ systems, including the adrenal glands (14; 15). The rise in MDA levels was accompanied by a significant reduction in antioxidant enzyme activities, such as Superoxide Dismutase (SOD), Catalase (CAT), and Glutathione Peroxidase (GPx), underscoring the detrimental effects of MNU on the adrenal gland's ability to manage oxidative stress (16).

Conversely, myricetin treatment led to a notable reduction in MDA levels and an increase in the activities of SOD, CAT, and GPx. These findings are consistent with those reported in literature that indicates myricetin's capability to enhance the antioxidant defense system in various tissues, thereby counteracting oxidative damage (17; 18). Specifically, the ability of myricetin to scavenging free radicals reduces cellular oxidative stress by restoring the balance between ROS production and antioxidant defenses (19). This suggests that myricetin not only offers protection but may also enhance the endogenous enzymatic systems that safeguard adrenal function during oxidative insults.

Histopathological examination of adrenal tissues revealed significant differences among the treatment groups. The MNU group exhibited notable histological alterations, including dilated vascular sinusoids, inflammatory cell infiltration, and degenerating lipofuscin cells. These changes align with previous studies showing that MNU exposure results in functional and structural abnormalities in the adrenal glands (20).

In contrast, myricetin-treated groups exhibited a dose-dependent restoration of adrenal histomorphology. Particularly, higher doses of myricetin (150 mg/kg and 300 mg/kg) resulted in a nearly normal histological appearance, with well-formed zona reticularis and normal chromaffin cell orientation. This finding is consistent with existing literature that demonstrates myricetin's protective effects on cellular structures in various models of oxidative injury (17; 18).

It highlights myricetin's potential in promoting cellular repair processes and maintaining structural integrity in the adrenal glands amidst oxidative stress.

The mechanisms through which myricetin exerts these protective effects are multifactorial. Its antioxidant properties facilitate the depletion of reactive oxygen species, thus reducing oxidative stress-induced cellular injury (21). Additionally, myricetin's anti-inflammatory effects may also play a critical role in alleviating the inflammatory response triggered by MNU exposure, as evidenced by the reduced inflammatory cell infiltration observed histologically (18).

Furthermore, by modulating signaling pathways associated with cell survival and apoptosis, myricetin could help in the restoration of normal cellular functions within the adrenal glands. Research indicates that myricetin can inhibit pro-inflammatory pathways, thereby attenuating the inflammatory processes that contribute to cellular damage and dysfunction (22). This underscores its potential as a therapeutic agent for managing oxidative stress-related cellular injuries in endocrine organs.

5. CONCLUSION

In conclusion, the findings of this study provide compelling evidence for the protective effects of myricetin against MNU-induced oxidative stress in the adrenal glands of Wistar rats. The enhancement of antioxidant enzyme activities and the preservation of adrenal histomorphology in myricetin-treated groups support its potential utility in mitigating oxidative damage. The proposed mechanisms of action, centered around antioxidant and anti-inflammatory pathways, suggest that myricetin may be a viable candidate for future therapeutic strategies aimed at protecting adrenal function in oxidative stress-related conditions. Further research is warranted to investigate the long-term effects and clinical applicability of myricetin in endocrine health.

ETHICAL APPROVAL

Ethical clearance was obtained from the Faculty of Animal Research Ethics Committee, Faculty of Basic Medical Sciences (FAREC-FBMS), University of Calabar (approval no. 344ANA1024).

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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