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Impact of Anthocyanin Fractionate of Maize Seed Extract on the Microanatomy of the Testes and Epididymis in Adult Male Wistar Rats Animal Models

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ABSTRACT

This study evaluated the effects of anthocyanin fractionates from maize seed (*Zea mays*) extracts on the microanatomy of the testes and Epididymis of male Albino Wistar rats. Twenty-one (21) adult male Wistar rats were assigned to 3 groups randomly (n = 7). Group 1 (control) received water and food only, Group 2 received 200 mg/kg of the anthocyanin fraction of maize seed extract, and Group 3 received 400 mg/kg of anthocyanin. At the end of treatment, rats were euthanized and the testes and epididymis excised for histological analysis. Body weight results showed a significant increase in final mean body weight across all groups ($P < 0.05$). Testes of control rats displayed normal seminiferous tubules with intact germ cell layers and organized spermatogenesis. Low-dose anthocyanin induced mild germ cell regeneration, while the high dose caused mild to moderate germ cell degeneration. Periodic acid–Schiff (PAS) staining demonstrated intact basement membranes and glycogen-rich Sertoli cells in control tissues. In contrast, the high-dose group showed disrupted tubular integrity and reduced PAS-positive material, reflecting glycoprotein depletion. Epididymal sections in all groups revealed intact pseudostratified epithelium with normal stromal architecture, without inflammation or fibrosis. These findings suggest that maize-derived anthocyanin fractions exert dose-dependent effects on spermatogenesis, with minimal influence on epididymal maturation.

Keywords: anthocyanin, testes, epididymis, spermatogenesis

INTRODUCTION

Male fertility is a complex physiological process that can be significantly compromised by Oxidative stress (OS), which is characterized by an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defense mechanisms[1]. ROS are naturally occurring byproducts of cellular metabolism and include free radicals such as superoxide anions, hydroxyl radicals, and non-radical species such as hydrogen peroxide. However, when produced in excess, they overwhelm the detoxification capacity of seminal plasma and reproductive tissues, leading to oxidative damage at the cellular and molecular levels[2]. This oxidative stress is particularly detrimental to spermatozoa, which are highly susceptible due to their limited cytoplasmic antioxidant enzymes and high content of polyunsaturated fatty acids (PUFAs). Elevated ROS levels can induce sperm lipid peroxidation, DNA fragmentation, and mitochondrial dysfunction, contributing to decreased sperm motility, viability, and fertilization ability[3]. Consequently, OS is a major contributing factor to male infertility worldwide.

Infertility in men is multifactorial, arising from a range of physiological, genetic, and environmental causes. Among these, disruptions in the function of the testes and epididymis play critical roles[3]. The testes are the primary male reproductive organs responsible for spermatogenesis (the production of sperm cells) and the synthesis of testosterone, the key androgen hormone regulating sex drive, secondary sexual characteristics, and muscle and bone mass development[4]. The epididymis, a convoluted duct located on the posterior surface of the testes, is essential for sperm maturation, storage, and transport. It secretes proteins and factors that are crucial for sperm motility and the acquisition of fertilizing capacity[3,4]. Despite their importance, the testes and epididymis remain relatively understudied in the context of male infertility, highlighting the need for further research into their roles and vulnerabilities, particularly under oxidative stress conditions.

In recent years, attention has turned to natural antioxidants as potential therapeutic agents to counteract oxidative damage in male reproductive tissues. Anthocyanin, a family of flavonoids responsible for the brilliant red, purple, and blue hues in many fruits, vegetables, and grains, has received significant interest[5]. Anthocyanins are powerful antioxidants that scavenge free radicals, chelate metal ions, and modulate cellular antioxidant enzyme systems, thereby protecting cells from oxidative stress and inflammation[6]. Their health benefits extend beyond antioxidant activity, encompassing anti-inflammatory, anticancer, anti-obesity, and cardiovascular protective effects[5,6]. These multifunctional properties make anthocyanins promising candidates for improving reproductive health, especially in conditions where oxidative stress is a key pathological factor.

Corn (*Zea mays*), a staple food crop worldwide, is a rich source of anthocyanin, particularly in pigmented varieties where these compounds contribute to kernel coloration[7]. The anthocyanin profile of maize includes cyanidin, pelargonidin, and peonidin derivatives, which have demonstrated strong antioxidant activities in vitro and in vivo[7]. An experimental study reported the effects of anthocyanin treatment in varicocele-induced male models (a condition known to cause oxidative stress and impaired fertility), revealing significant improvements in testis weight, sperm motility, and spermatogenic cell density, despite no notable changes in sperm count[8]. These results indicate that anthocyanins may improve testicular function and sperm quality by mitigating oxidative damage and supporting spermatogenesis.

Building on this emerging evidence, the present study aims to investigate the influence of anthocyanin fractionates extracted from maize seeds on the reproductive profile of male experimental models. By assessing parameters such as sperm quality, hormonal profiles, and testicular histology, this research seeks to elucidate the potential of maize-derived

anthocyanins as natural antioxidants capable of improving male fertility. Given the global prevalence of OS-related infertility and easy accessibility of maize as a dietary source, this study holds promise for developing affordable and effective interventions to support reproductive health.

MATERIALS AND METHODS

Ethical approval

The Faculty of Basic Medical Ethical Committee granted ethical permission for the experiment's use of experimental animals, and an ethical certificate with the number FBMS/2023/05/1022 was given. The rules governing the use of animals in research were appropriately observed.

Plant materials

The maize seeds (*Zea mays* L.) were obtained from the Okuku Community Market in Yala, Cross River State, Nigeria. The seeds were thoroughly cleaned, air-dried at approximately 25 °C for three weeks, and then milled to obtain the extract. After the plant material was verified by the University of Calabar, Nigeria's Department of Botany, a voucher specimen with the number UC/Herb/2025/032 was placed in the departmental herbarium for future use.

Extract preparation

Zee Mays (maize) powder was dissolved in a plastic container with 9000 ml of bottled table water. The mixture was thoroughly stick-stirred and allowed to sit for a full day before being filtered through a cotton sieve. To create a crude solid extract, the filtrate was evaporated for three (3) weeks at 45°C in a water bath. Before being administered, the extract was stored in a refrigerator.

Partitioning/fractionating of crude plant extract

(10 grams g) of the crude extract was removed from the refrigerator and exposed to microwave extraction using a closed system with automatic moderate stirring. Different crude extractions using water were carried out for 1 – 3 minutes. Extraction solvent was a mixture of water-saturated ether (equal volume of anhydrous diethyl ether + distilled water) at a ratio of 5/100m/v (5g of crude sample with 100mls of solvent) and methanol.

Methanol was evaporated under decreased pressure at 40 degrees Celsius. Following methanol evaporation, the remaining water volume was hydrolyzed with two drops of HCl (6N) to separate the phenolic and non-phenolic phases. The extraction process was then carried out using 3· 5 mL of diethyl ether. After the diethyl ether evaporated, the residue was diluted in 5 mL of methanol. The non-phenolic phase (organic phase), which contained some oligomers of phenolic compounds and sugars, was dried with anhydrous sodium sulfate. After being regrouped, the anthocyanin-containing phenolic phase (aqueous phase) was diluted with 10 milliliters of distilled water. Anthocyanin has an absorption of 452nm (mass spectrophotometer). Ten grams (10g) of anthocyanin was obtained as the final fractionate.

Experimental animals

The Okuku Campus of the University of Cross River State's (UNICROSS) Department of Human Anatomy and Forensic Anthropology's animal housing provided twenty-one (21) mature male Wistar rats, which were employed in this investigation. Three (3) groups of seven (7) rats each were randomly selected from among the animals. Group 1 is the control group; Group 2 is the low dosage group; and Group 3 is the high dose group. To give the animals time to acclimate, they were housed for two weeks. Before the administration started, they were fed ordinary growers' vita feed and provided water in plastic cages with lighting controls (12-hour intervals of sunshine and darkness).

Experimental design

The twenty (21) male Wistar rats were grouped into three cages, each with seven (7) rats per group: Control, Low dosage, and High dose, respectively.

Group A (the control) only received normal diet and water, group B (low dose) received anthocyanin fractionate of corn seed extract at a dose of approximately 200 mg/kgBw[20], while group C (high dose) received 400 mg/kg anthocyanin fractionate. Administration was orally by oral cannula once daily for 14 days.

Sacrifice of experimental animals

Three Wistar rats from each group were randomly chosen at the end of the fourteen (14) day dosing period, and they were sacrificed by cervical dislocation one day later. In order to determine the impact of the extract given to each of the study groups, the testes and epididymis from each animal in each group were collected, stored in 10% formalin, and then assessed.

Morphological studies

A sensitive weighing balance was used to weigh each animal before the experiment began and after the anthocyanin fractionate of corn seed extract was administered. This enabled the determination of the morphological changes.

Tissue/Slide preparation

The animals' testes and epididymis were taken out and stored in containers with labels that contained 10% neutral buffer formalin. To get adequate tissue penetration and efficient fixation, this was done for seventy-two (72) hours. They were then put in increasing ethanol grades to dehydrate them. They had two hours of 70% ethanol treatment apiece, followed by an hour of 95% ethanol and then an hour of absolute alcohol. Three fifteen-minute xylene changes were used to remove the tissues after they had been dehydrated.

The tissues were impregnated overnight in molten paraffin at 58°C, and the next morning, it was embedded in wax to create blocks. Using a microtome, these tissue blocks were cut into sections that ranged in thickness from 3-5µm. The portions were taken up on albumenized glass slides after floating in heated water (45°C). They were allowed to air dry before being dyed with the Periodic Acid-Schiff staining procedure (Me Manus, 1946) and cut into 5 mm sections with a rotary microtome. They were dewaxed for two minutes during each change in xylene. Xylene was cleaned in 95% alcohol for one minute every two changes, followed by another minute in 70% alcohol. The parts were then separated in 1% alcohol for 5–10 seconds after being rehydrated in running tap water. The parts were thoroughly cleaned in hot running water until they became blue. Following this, they were counterstained with 1% alcohol, eosin for 1 minute, rapidly dehydrated using an escalating alcohol grade, cleaned in xylene, and mounted with DPX mounting. Slide sections were examined and photographed using a light microscope.

Statistical analyses

The mean standard error is reported as a result of the experiment's descriptive statistics (Mean ±SEM). Paired sample T-test was considered statistically significant at $p<0.05$

RESULTS

Effect of Anthocyanins fractionate extracts on the body weight of animals

Morphological observations revealed a significant ($P < 0.05$) increase in the final mean body weights of all experimental groups compared to their respective baseline values. In the control group, the mean body weight increased to a significantly higher value at the end of the study **($P < 0.05$).** Similarly, rats in the low-dose group demonstrated a significant ($P < 0.05$) increase post-treatment. The high-dose group also showed a significant ($P < 0.05$) increase at the end of the experimental period.

Table 1: Morphological observation of body weight

Groups	Initial body weight (g)	Final body weight (g)
Control	76.40 ± 7.402^{a}	107.50 ± 7.187^{b}
Anthocyanin fraction Low Dose (200 mg/kg BW)	107.57 ± 8.038^{a}	141.75 ± 14.453^{b}
Anthocyanin fraction High Dose (400 mg/kg BW)	176.75 ± 6.627^{a}	180.00 ± 30.670^{b}

Values are presented as Mean ± SEM, n = 7. Superscripts with different letters (^{a},^{b}) within the same row indicate significant differences at **$P < 0.05$** (Paired t-test). No significant differences were observed between final body weights across groups (One-way ANOVA, $P > 0.05$).

Testicular Morphology

Figure 1 presents a Photomicrograph of the testes of the control group (H&E) stain, showing well-preserved seminiferous tubules (ST) with intact germ cell layers (arrow) and adjoining interstitial tissue (IT). The lumen contains abundant spermatozoa, and the overall parenchyma demonstrates normal architecture without evidence of degeneration. The low-dose anthocyanin group (200 mg/kg BW) showed mild germ cell layer regeneration, evidenced by increased spermatogonial proliferation and enhanced tubular epithelial thickness. While the high-dose anthocyanin group (400 mg/kg BW) exhibited mild to moderate germ cell degeneration, characterized by vacuolization, spermatid sloughing, and reduced epithelial height.

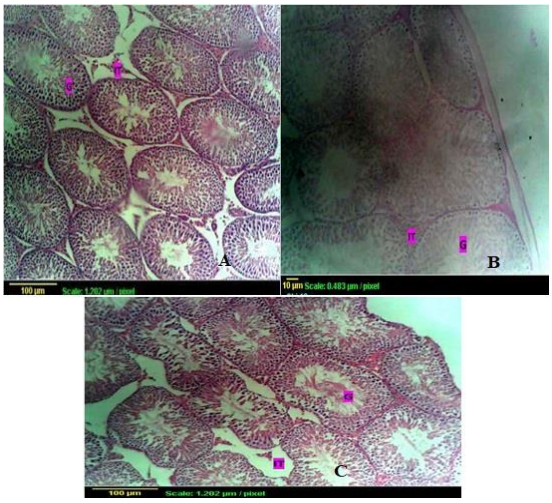

Figure 1: Photomicrograph of the testes of rats showing (A) Control group, (B) low dose Group, (C) High Dose Group (H&E, ×400). Seminiferous tubules (ST) germ cell layers GC, interstitial tissue (IT).

Figure 2 presents the histological sections of the Testes stained with Periodic Acid–Schiff (PAS), which provided concordant results. PAS staining highlighted intact basement membranes and glycogen-rich Sertoli cells in the control group. In contrast, the high-dose group showed disrupted tubular integrity and reduced PAS-positive material, consistent with glycoprotein depletion**.**

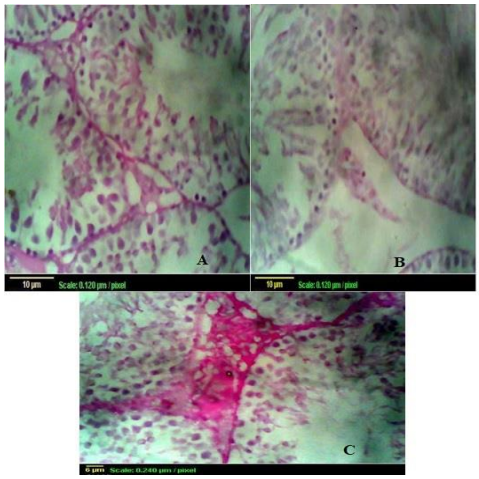

Figure 2: Photomicrograph of the testes of rats showing (A) Control group, (B) low dose Group, (C) High Dose Group (PAS, ×400**).**

Histological sections of the epididymis showed that all experimental groups, including control, low-dose, and high-dose, exhibited normal epididymal histology. The duct lumens were filled with mature spermatozoa (S), and the connective tissue stroma (CT) showed no signs of inflammation or fibrosis (Fig 3). The pseudostratified epithelium, critical for sperm maturation and storage, remained intact with characteristic principal and basal cells. The lack of significant epididymal changes suggests that anthocyanin fractionates may exert their primary effects on spermatogenesis rather than post-testicular sperm maturation.

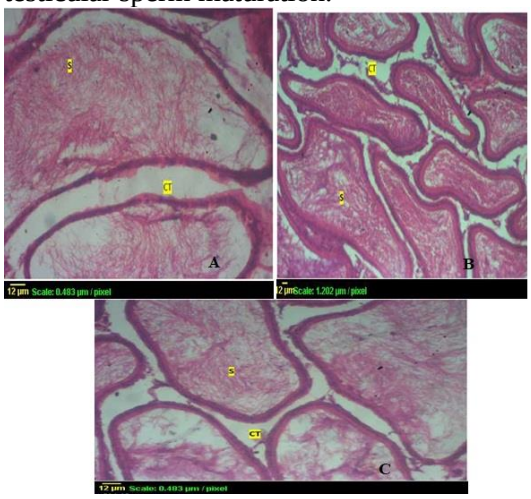

Figure 3: Photomicrograph of the testis from the high-dose anthocyanin group showing the control group as (A), low-dose group as (B), and high-dose group as (C). (H&E, ×400). Seminiferous tubules (ST) with germ cell layers (G) and adjoining interstitial tissue (IT).

DISCUSSION

This study investigated the effects of anthocyanin fractionates of maize seed extracts on some reproductive organs of male rat models, with a focus on body weight dynamics and histological changes in the testes and epididymis. The findings revealed dose-dependent and organ-specific responses to anthocyanin supplementation, offering insights into its potential benefits and risks for male reproductive health.

The control group exhibited a significant increase in final mean body weight compared to initial weight, consistent with normal growth patterns in animals receiving adequate nutrition and care[9]. Similarly, both the low and high-dose anthocyanin groups showed significant weight gain over the study period. Interestingly, the final mean body weight of the low-dose group surpassed that of the control group, suggesting that anthocyanin at this dosage may enhance metabolic efficiency or nutrient absorption. This aligns with studies demonstrating that low-dose antioxidants, including flavonoids, can improve energy metabolism and reduce oxidative inhibition of anabolic pathways[10]. However, the high-dose group did not differ significantly from the control group in final body weight, indicating a non-linear, saturation-like response. This phenomenon, where higher doses fail to amplify effects, has been observed in other antioxidant interventions and may reflect adaptive downregulation of absorption mechanisms or a shift from antioxidant to pro-oxidant activity at elevated concentrations[11]. Such biphasic responses underscore the importance of dose optimization in therapeutic applications of phytochemicals.

Histological evaluation of the testes revealed distinct responses across groups. The control group displayed normal seminiferous tubule architecture, with well-organized germinal epithelium and active spermatogenesis. In contrast, the low-dose group exhibited mild regeneration of the germ cell layers, characterized by increased spermatogonial proliferation and enhanced tubular lumen

organization. This regenerative effect may be attributed to anthocyanin antioxidant and anti-inflammatory properties, which mitigate oxidative damage to testicular somatic and germ cells[12,13]. For instance, similar findings were reported in a study where anthocyanin-rich berry extracts restored spermatogenic activity in rodents exposed to environmental toxins[12]. Paradoxically, the high-dose group showed germ cell layer degeneration, marked by vacuolization, sloughing of spermatids, and reduced epithelial height. This adverse effect could result from pro-oxidant activity at excessive doses, as seen in studies where high concentrations of polyphenols induced oxidative stress via Fenton reactions or mitochondrial uncoupling[13]. These findings highlight the delicate balance between the beneficial and harmful effects of anthocyanins in testicular tissue, contingent on dosage.

These changes were time-dependent and associated with decreased seminiferous tubule diameter and disrupted spermatogenesis, confirming nickel's toxic impact on testicular structure and function[14]. A related study showed that exposure to $NiCl_2$ weakens the blood-testis barrier (BTB) by reducing Sertoli cell expression of the tight junction, adhesion junction, and gap junction proteins. This disruption was primarily caused by oxidative stress and the activation of the p38 MAPK signaling pathway. Oxidative stress's role in nickel-induced testicular toxicity was highlighted by the notable reduction of testicular histopathological damage and restoration of BTB protein expression following antioxidant therapy with N-acetylcysteine (NAC) [15].

A study on mice exposed to nickel chloride found increased lipid peroxidation and oxidative DNA damage in testicular tissue, evidenced by elevated single-strand DNA breaks. This oxidative stress was linked to increased apoptosis within the testes and a significant rise in abnormal sperm morphology. These findings suggest that nickel's reproductive toxicity involves ROS-mediated genotoxic effects, impairing sperm quality and testicular integrity [16].

Similar to anthocyanin, other antioxidant-rich natural extracts have been shown to mitigate testicular damage caused by environmental toxins. For example, berry-derived polyphenols restored spermatogenic activity and improved seminiferous tubule architecture in rodents exposed to oxidative stress-inducing agents, supporting the regenerative potential of antioxidants in testicular tissue [16].

Concerning the epididymal histology, all groups exhibited normal epididymal histology, with intact epithelial lining and luminal spermatozoa. This aligns with prior research indicating that the epididymis, while critical for sperm maturation, may be less susceptible to oxidative stress or anthocyanin modulation compared to the testes[14,15]. For example, a study on quercetin supplementation reported testicular improvements without epididymal changes, suggesting organ-specific antioxidant thresholds or differential expression of detoxification enzymes[14].

The organ- and dose-specific effects of maize-derived anthocyanin observed in this study underscore the complexity of translating phytochemical interventions into clinical practice. The regenerative effects on testicular germ cells at low doses align with the therapeutic potential of anthocyanins in oxidative stress-related infertility.

CONCLUSION

All groups showed significant increases in final body weight, with the low-dose group gaining more than the control, suggesting that anthocyanin fractionates may promote weight gain at lower doses. Histological analysis revealed mild germ cell regeneration in the low-dose group, while high doses caused degeneration, indicating potential dose-dependent adverse effects.

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