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Article

Protective Effects of Tigernut (*Cyperus esculentus*) on Bisphenol A- Induced Testicular Toxicity in Wistar Rats

Efectos protectores de la Chufa (*Cyperus esculentus*) sobre la toxicidad testicular inducida por bisfenol A en ratas Wistar

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ABSTRACT

Bisphenol A (BPA) have been reported to induced reprotoxicity in rats. This study was conducted to find out the ameliorative properties of aqueous extract of *Cyperus esculentus* (tigernut) on BPA induced testicular toxicity in Wistar rats.

Methods: Twenty male rats were divided randomly into 4 groups (n=5): group A: (Control); group B: Bisphenol A (BPA) (25 mg/kg b.w/day); group C: tigernut (200 mg/kg b.w); group D: (25 mg/kg of BPA+ 200 mg/kg of tigernut extract. 25 mg/kg of BPA was dissolved in 0.2 ml of olive oil as vehicle and administration was given by oral gavage for 4 weeks. The body weights were measured. Blood were collected for the testosterone (T) and luteinizing hormone (LH) assays; the epididymis were processed for sperm count, sperm motility, sperm viability and sperm abnormality test; while the testes were harvested for histology

Results: There was a significantly ($p < 0.05$) decreased in body weight; reduced (sperm count, motility, viability, serum testosterone and luteinizing hormone) in BPA compared with control group. These parameters however increased significantly ($p < 0.05$) in tigernut (200mg) and BPA + tigernut (200mg) compared with BPA. Also, histological examination showed widened interstitial spaces, some distorted seminiferous tubules, degeneration of basement membrane, scanty Leydig cells, fewer spermatozoa and vacuolation While BPA + tigernut (group D); showed improved testicular architecture (preserved interstitial spaces and interstitial cells. restoration of the loss of the basement membrane and closely packed seminiferous tubules with well-arranged germinal

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epithelium. Supplementation with tigernuts following BPA administration produces a reversal of the deleterious effect of BPA on the testis

Keywords: Bisphenol (BPA); Tigernut, Testes; Histology; Sex Hormones

1. Introduction

Urbanisation, consumerism, and progressive industrialization have all contributed to environmental degradation, which has an impact on public health. For a number of years, worries about the health impacts of chemicals on humans have arisen due to the large-scale manufacture of synthetic and biological compounds that produce undesired contaminants (Kawa, et al., 2021) Endocrine-disrupting chemicals (EDCs) such as Bisphenol A (BPA) are external substances that can target many organs and systems within the human body, disrupting the endocrine system and causing several detrimental consequences on health (Abu Hasan, et al., 2023; Fonseca, et al., 2022). According to Aghajani, et al. (2023), BPA is a known endocrine disrupting (ED) substance that has estrogenic activity. It was first created as a synthetic oestrogen, meant to resemble the body's natural oestrogen and obstruct the endocrine system's regular operations. It's used to make epoxide resins and polycarbonate plastics, as well as to speed up the growth of cattle and poultry (Im & Löffer, 2016). It is a widely used chemical that is produced in vast quantities all over the world and is found in a wide range of items, including food packaging, dental sealants, thermal paper receipts, epoxy resins, and polycarbonate plastics, dental filling material, water and food plastic containers, infant bottles and feeders, medical tubing, and the metal lining of beverage and food cans (Kurniawan, et al., 2020; Moussavi & Haddad, 2019). BPA exposure can occur in humans and animals through a variety of pathways, such as ingestion, inhalation, and skin contact. However, the primary human exposure route is by ingestion or oral intake of food and water (Akash, et al., 2023). According to studies, BPA lowers plasma testosterone levels, induces structural abnormalities in rodent sperm, decreases testicular and epididymal sperm counts and weight (Santiago, et al 2021). In addition to the traditional genomic and non-genomic mechanisms, oxidative stress and the dynamic balance of enzymatic antioxidants are the main causes of the harmful effects of BPA (Amjad, et al., 2020). Hence, one of the most frequent ways that BPA damages sperm is through inducing oxidative stress (Sidorkiewicz, et al. 2017).

Cyperus esculentus L. varsativus (*C. esculentus*) commonly known as tigernut, is a perennial plant species and a tuberous plant that belongs to the Cyperaceae family (Nwakanma, et al., 2022)According to Adam, et al. (2020), the Hausas refer to it as "Aya," the Yorubas as "Ofio," and the Igbos as "Aki Hausa." There are three recognised variations of tiger nut that are grown in Nigeria: the yellow, brown, and black types. The brown and yellow variants are more widely grown, although the yellow variety is favoured due to its larger size, appealing appearance, and meaty body (Oyedepo & Odoje, 2014). Tigernuts have been found to include phytochemicals such as cyanogenic glycoside, alkaloid, glycoside, resin, flavonoid, tannins, sterols, and saponins (Nwosu, et al., 2022). The different biological actions associated with tigernuts could be attributed to these phytochemicals; also due to their high fat, carbohydrate, mineral, and vitamin content, tiger nuts have the potential to be a valuable food source for both people and animals (Ofem, et al., 2023). Tigernuts provide a number of additional functions besides eating. It promotes skin suppleness, minimises wrinkles, and slows down the ageing process of bodily cells; certain cosmetic items are also made from the milk concentrate of tigernuts (Sánchez-Zapata, et al., 2012). Studies on tigernuts have revealed that they are beneficial and can be used as an antimicrobial agent, to prevent and treat urinary tract infections and other bacterial infections, as well as to activate blood circulation and manage a number of pathophysiological conditions like coronary heart disease, thrombosis, and obesity (Zhang, et al., 2022). Following tigernut administration,

improvements have also been noted in sperm count, motility and morphology, testicular architectural maintenance, and enhanced testosterone concentration (Gbotolorun, et al., 2022).

Additionally, studies have shown that tigernuts have significant antioxidant activity, demonstrating the presence of minerals like zinc, potassium, and phosphorus as well as antioxidants like vitamin E, vitamin C, and quercetin (Yu, et al., 2020; Saeed, et al., 2022). This antioxidant activity could help treat a variety of disorders linked to oxidative stress. Furthermore, it has been shown that administering tigernuts methanolic extract to male rats enhances sperm motility and count, which is linked to elevated serum levels of testosterone and gonadotropins. According to Hassan, et al. (2018), tigernuts has been shown to increase testicular activity and lessen the negative effects of flutamide on the testis. The purpose of this study was to examine the preventive effect of tigernuts in bisphenol A-induced damage testes on serum hormonals and testicular histology in Wistar rats.

Figure 1: Picture of yellow fleshly Tigernut (*Cyperus esculentus* Linn)

2. Materials and Methods

Collection of plant material

The fresh tigernuts (*Cyperus esculentus* L.) were purchased from Mararaba Market Abuja, Federal Capital Territory of Nigeria. The tiger nuts were taken to a taxonomist in the Department of Botany, University of Lagos, Akoka, Lagos State, Nigeria, for authentication. The voucher, LUH 8022, specimen was deposited at the herbarium for the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, University of Lagos, Akoka, Lagos State, Nigeria.

Extraction of tiger nuts:

Fresh tigernuts were washed to get rid of dirt and sand, they were allowed to air dry and then processed with a grinder to a fine powder. Three litres of distilled water were used to soak about 700 grammes of powdered nuts for a duration of 48 hours. It was filtered through Whatman filter paper No. 1 and evaporated to dryness using a rotary evaporator under reduced pressure at 45 °C. The yield of the extract was 140g. The extract was placed in an airtight container and refrigerated at 4°C until it was needed.

Chemical

BPA (bisphenol A 99.9%) of analytical grade were obtained from Sigma-Aldrich, USA and olive oil produced by Goya En Espana S.A.U. Sevilla Spain. Olive oil was used to dissolve BPA.

Experimental Animals

Twenty mature male Wistar rats weighing between 162-194 g were acquired from the Animal House of the College of Health Sciences, Bingham University Karu, Nasarawa State, Nigeria. The animals were housed in an animal facility and maintained in ventilated cages with a light-dark cycle of 12 hours and conventional laboratory conditions of 25 ± 2°C. Before the trials started, the animals were given two weeks to acclimatize. They had unrestricted access to water ad libitum and a regular laboratory rat chow

The animals were split into four groups, each with five rats, at random: The oral dosage of 25mg/kg of Bisphenol A was dissolved in olive oil (0.2 ml) (Munir, et al., 2017); while tigernut extract was administered daily at a rate of 200 mg/kg body weight for a duration of 4 weeks respectively.

Group A: control (normal saline)

Group B: BPA (25 mg/kg/day) dissolved in olive oil (0.2 ml) as vehicle

Group C: Aqueous extract of tigernut at 200mg.kg

Group D: BPA (25 mg/kg/day) dissolved in olive oil (0.2 ml) + aqueous extract of tigernut at 200mg/kg

The Wistar rats were weighed to determine their final body weight on the last day that they received bisphenol A and tiger nut extract. The rats were sacrificed under chloroform anaesthesia 24 h after the last treatment. Rats' hearts were punctured to obtain blood samples for the test of luteinizing and testosterone hormones. The epididymis was removed, and the sperm was subjected to tests for sperm abnormalities, motility, count, and viability; while the testes was taken out for histological examination.

Semen parameter analysis

Sperm count

The left cauda epididymis of each rats was excised and placed in a small clean Petri dish containing 2 mL of normal saline and the concentration was allowed to settle at 37 °C for 10 minutes. After collecting the sperm, it was suspended in normal saline and used a Pasteur pipette to transfer 200 µL of the sperm suspension to the improved Neubauer hemocytometer. Chamber (Deep1/10mm, LABART Germany). The sperm were then counted in five large squares using a microscope (Leica DM 750, Switzerland) and was expressed as million/mL of suspension (Cheng, et al., 2007)

Sperm Motility

Ten microliter of the sperm suspension was placed in a microscope slide and covered with a coverslip and examined at x40 and x 100 magnifications with the help of a light microscope (Leica DM 750, Switzerland). The number of progressively motile sperm cells were counted and divided by the total number of spermatozoa counted and was expressed as a percentage (Ekaluo, et al., 2013).

Sperm Morphology

One drop of the sperm suspension was smeared on a glass slide and stained with 1 % eosin Y solution (10:1) for 30 min and air-dried smears were prepared on glass slides for the sperm abnormality

test. A light microscope (Leica DM 750, Switzerland) was then used to view it at x400 magnification. For each rat, 200 sperm were screened and five air-dried smears were prepared on glass slides for each sample. The percentage of total abnormalities of head, middle piece and tail was calculated (Ekaluo, et al. 2009).

Sperm viability

Twenty microliter of 0.05 % eosin Y–nigrosine was briefly added to equal volume of sperm suspension and five air-dried smears were prepared on glass slides for each sample. The slides were viewed microscopically (Leica DM 750, Switzerland) at x100 and x400 magnifications within 15 min for percentage viability. Normal live sperm cells were unstained and appeared whitish, whereas dead sperm cells appeared pinkish. Percentage viability was calculated based on the number of live sperm cells out of the total number of sperm cells observed (Bjorndahl, et al., 2003).

Blood Sampling and Hormonal Analysis

Blood was withdrawn via cardiac puncture from anaesthetized rats and allowed to clot then centrifuged at 3000 rpm for 15 min (Laboratory centrifuge CD-0412-50; PHOENIX Instrument GmbH, Garbsen, Germany) to obtain the serum. Serum samples were assayed for levels of testosterone and luteinizing hormones using the Microwell (solid phase) enzyme linked immunosorbent assay (ELISA) kits, according to the manufacturer's instructions and guidelines.

Histological Examination

Testicular tissues were fixed in 10% buffered formalin for 48 hours. Testicular tissues that had been fixed were dehydrated in ethanol concentrations of 30, 50, 75, 95, and 100% before being cleared in xylene and embedded in paraffin wax. Using a microtome, 5 µm thick paraffin slices were cut. The obtained sections were routinely deparaffinized and stained with hematoxylin and eosin (H&E) for light microscopy for histopathological analysis

Statistical analysis

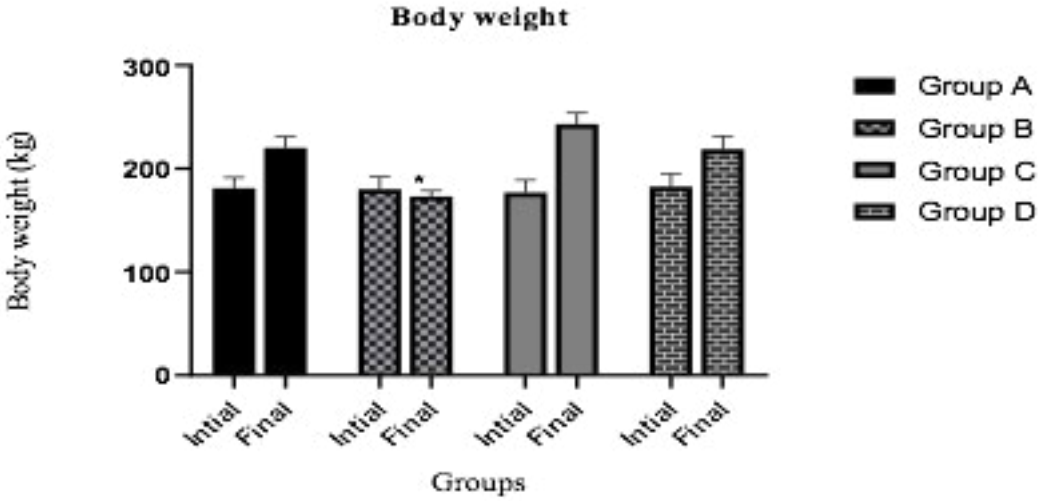
Data that was obtained from this study, was analyzed using GraphPad Prism software, (Version 6.0. San Diego, USA). One way analysis of variance (ANOVA) was done; which was followed by Bonferroni post hoc- test. The confidence limit of statistically significance was set at $P<0.05$. The result therefore was presented as mean ± standard deviation (SD).

Results

Effects of BPA and tigernut on body weight of rats

Figure 2 shows body weights in the different experimental groups (A, B, C, and D). The initial body weight showed no significant difference among the different groups. The final body weight was significantly ($p < 0.05$) decreased in the group B (BPA alone) when compared with the group A (Normal control). The final body weight was significantly ($p < 0.05$) increased in group C (200 mg of tigernut) and group D (BPA + 200 mg of tiger nut), when compared with group B (BPA). Also the final body weight was increased in group C (200 mg of tigernut) and group D (BPA + 200 mg of tiger nut), which was comparable with group A (Control).

Figure 2: Values represented as M± SD. N=5; *(P< 0.05) when compared to control. Group A= Control; Group B= BPA; Group C=Tigernut; Group D= BPA+Tigernut



Effect of BPA and Tigernut on seminal parameters in male Wistar rats

The result showed a significant (p<0.05) decrease in sperm motility in the BPA alone group when compared to the tigernut alone and control groups. There was no significant increase in tigernut group when compared with control group. Also, there was significantly (P < 0.05) increased in BPA + tigernut group when compared with BPA. Sperm count significantly (p<0.05) reduced in the BPA alone treated group when compared to all the groups; but when the BPA + tigernut (group D) were compared to the tigernut alone group, sperm count was reduced but was not significant. Percentage of sperm abnormal morphology significantly decreased (P < 0.05) in tigernut alone (group C), BPA + tiger nut (group D) and control group (group A) but increased (P < 0.05) in BPA alone (group C). Sperm viability was significantly decreased (P < 0.05) in BPA alone (group B); and increased (P < 0.05) in tigernut alone (group C) when compared with normal control (group A). It was also significantly (P < 0.05) increased in tigernut + BPA (group D) compared with BPA (group B) as shown in Table 1.

Table 1: Effect of BPA and tigernut on seminal parameters in adult male Wistar rats

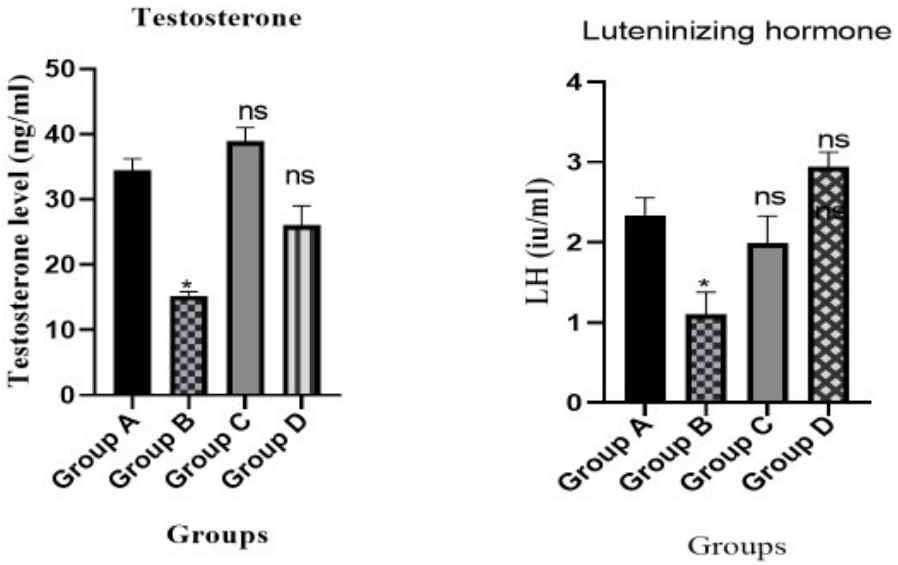
	Group A	Group B	Group C	Group D
Sperm motility (%)	83 ± 2.1	40 ± 11.5*	92 ± 2.0	70 ± 12.0
Sperm count (10^6/ml)	85 ± 3.00	50 ± 5.7*	96 ± 2.5	80 ± 6.3
Abnormal Morphology %	5.0 ± 2.1	12 ± 4.7 *	4.0 ± 2.2	7.0 ± 1.7
Sperm viability %	97 ± 3.3	60 ± 8.8*	98 ± 3.3	84 ± 3.3

Values are Mean ± SD, Values represented as M ± SD. N=5; *(P< 0.05) when compared to control. Group A= Control; Group B= BPA; Group C=Tigernut; Group D= BPA+Tigernut

Effects of BPA and tigernuts on the levels of testosterone and luteinizing hormones

The result showed serum testosterone and luteinizing hormone (LH) levels were significantly ($p < 0.05$) decreased in BPA alone treated group compared with normal control. There was also a significant ($P < 0.05$) decrease in serum testosterone and LH levels in BPA compared with tigernut. There was an increase in serum testosterone and LH levels in tigernut group and tigernut + BPA when compared with the control group, but the increase was not significant ($p < 0.05$); however, the increase was significant when compared with BPA group (Figure 3).

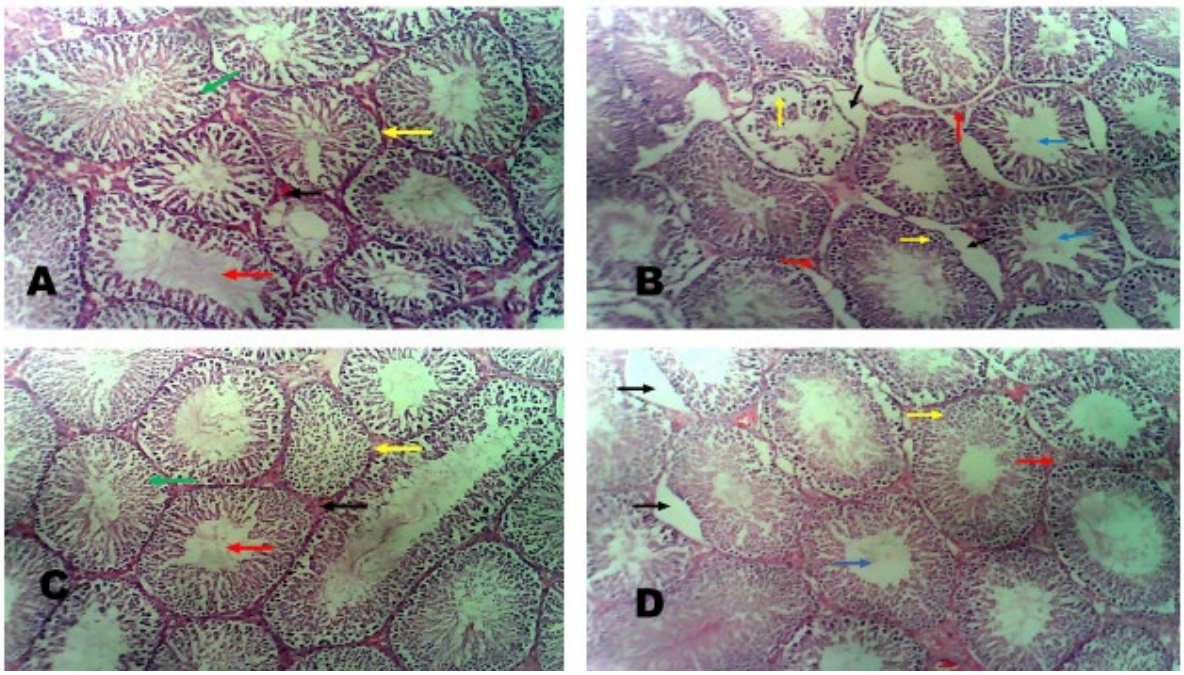
Figure 3: Graphical representation of testosterone and luteinizing hormone levels of BPA and aqueous extract of tiger nut-treated rats. *P<0.05 indicates a significant difference from the control. ^{ns} Not statistically significant difference from the control; ^{ns} Not statistically significant compared to control group



Histology of Testes

The histological feature of Groups A and C shows normal circular and closely packed seminiferous tubules, with well-arranged germinal epithelium. The interstitial spaces containing the Leydig cells appeared normal, basement membranes also appeared normal and intact. Large numbers of normally dividing spermatids were seen extending their cellular processes into the lumen of the seminiferous tubules (Figure 4A and 4C). Photomicrograph of Group B (BPA) showed widened interstitial spaces, some distorted seminiferous tubules, and degeneration of basement membrane, scanty Leydig cells and fewer spermatozoa (Figure 4B). Sections from group D (BPA + tigernut) showed better-preserved interstitial spaces and interstitial cells, than the BPA group and the histoarchitecture of the tubules appeared similar to the control group. There was restoration of the loss basement membrane in group D (Figure 4D),

Figure 4: A photomicrograph of the testes of Wistar rat from group A: Control, group B: BPA, group C: tiger nut, group D: BPA and tigernut. Red arrow head shows the spermatozoa. Green arrow head shows spermatogonia. Black arrow shows Leydig cells in the interstitial space surrounded by connective tissue. Yellow arrow head shows a well-defined Basement membrane. Magnification-H & E stain X100



3. Discussion

Reproductive health and chemical pollutants are related and have long been public health issues. Endocrine disruptors like BPA lower oestrogen synthesis and may contribute to male infertility (Lahimer, et al., 2023). The purpose of this work was to examine the impact of tigernut aqueous extract on testicular dysfunction in Wistar rats caused by BPA. According to our findings, rats exposed to BPA (group B) saw a modest decrease in body weight when compared to the control group A. However, some research has found that at low doses of BPA, rats' body weight did not alter much (Yamasaki, et al., 2002). Rats in the tigernut treated group D, however, had lower body weights than those in groups A (control) and C (tigernut alone), but somewhat higher body weights than those in group B (BPA alone). Tigernuts are rich in nutrients, including protein and fat and carbohydrates, which provide energy; and are attractive because of their crude fibre content (Nwosu, et al., 2022). Thus, foods high in energy provide the body with fuel and can aid in a healthy weight gain.

A decline in semen quality has been documented, and exposure to environmental toxins may be the cause of unfavourable trends in male reproductive health. Damage to the sperm cell can arise from physiological, cytotoxic, or genetic mechanisms (Qiu, et al., 2020). Chemical exposure may result in pituitary hypothalamus or sex hormone impacts, which may impact spermatogenesis and lead to anomalies in seminal fluid that impair sperm's ability to function (Bakare, et al., 2005). According to studies, long-term exposure to BPA in adult male Wistar rats results in testicular oxidative stress, which disrupts spermatogenesis processes by reducing sperm production and causing structural changes in testicular tissue as well as endocrine changes (Ullah, et al., 2018; Olukole, et al., 2020).

The study's evaluation of sperm parameters revealed a negative effect after BPA was administered, suggesting that sperm function is compromised by BPA exposure. The findings are consistent with those of Kumar, et al. (2020), who after BPA treatment observed a disruption in spermatogenesis and a decrease in sperm count, concentration, vitality, and aberrant morphology in mice. When compared to the normal control rats, the group C (given tigernut extract) had considerably higher sperm counts, motility, and viability as well as decreased aberrant sperm morphology. These results suggest that the extract can enhance sperm function. Additionally, BPA treated with tiger nuts demonstrated an enhancement in the sperm (count, motility, viability, and abnormalities).

The male reproductive organs, including the testis and prostate, develop primarily in response to testosterone; rats exposed to BPA showed a significant reduction in their levels of both LH and testosterone. According to Smith & Walker, (2014), spermatogenesis is impaired when these hormone levels drop. The Leydig cells secrete testosterone, which is necessary for the testicular germinal cells to proliferate and divide. The sperm count decrease in the BPA group of rats may have been caused by the BPA's direct harmful action on the hypothalamic-pituitary axis, which affects the endocrine control of reproduction. The anterior pituitary gland secretes LH, which causes the Leydig cells to release testosterone. Therefore, LH functions through a feedback mechanism to maintain testosterone production since the amount of testosterone released increases roughly in direct proportion to the amount of LH available [Gbotolorun, et al., 2022]. It has been observed that BPA in rats results in pituitary and hypothalamic deformities that impact LH secretion and reduce the amount of testosterone secreted by Leydig cells (Shamhari, et al., 2021).

Furthermore, research indicates that BPA may affect androgen and oestrogen receptors, as well as obstruct LH binding and testosterone production in Leydig cells (Li, et al., 2020). In contrast to natural androgens, BPA interacts with cell androgen receptors with a lesser affinity as an endocrine disruptor. According to Lahimer et al. (2023) BPA has the ability to bind to androgen receptors and impede or militate specific androgenic responses. This might potentially cause changes in hormone signalling, including the manufacture of testosterone.

These results are comparable to the study (Takahashi & Oishi, 2003) that demonstrated a drop in testosterone following BPA treatment. However, the group D (BPA and tiger nuts), showed an increase in Testosterone and LH; but it was not significant. When compared to normal control rats, rats given the tigernut alone (group C) experienced a considerable increase in serum testosterone and LH levels. It's possible that the tigernut stimulated the production of testosterone by acting directly on the Leydig cells. Antioxidants like quercetin, vitamins C and E, and trace elements may be the reason why tigernut extract can raise testosterone levels (Allouh, et al., 2015; Udefa, et al., 2020). Consequently, the rise in LH and testosterone levels points to a potential androgenic quality of tiger nuts.

According to reports, the testis of rats given BPA treatment showed signs of spermatogenic arrest in a number of seminiferous tubules, decreased in the diameter of the tubules, abnormalities in the formation of spermatids and cellular changes such as edema between the tubules, a lack of cellular components, a wide empty lumen, degeneration of germinal epithelial cells, and aspermatogenesis (Norazit, et al., 2012). The control and tigernut alone groups' histological photomicrographs display the typical testicular architecture, with the spermatozoa, Leydig cell, and basement membrane arranged in an orderly fashion. On the other hand, the testicular histoarchitecture of the testes rats treated with BPA alone was only aberrant, exhibiting degradation of the basement membrane and a reduction in the number of spermatozoa. Exposure to environmental toxins like BPA can induce Endoplasmic reticulum (ER) stress and contribute to testicular cell damage leading to impaired spermatogenesis and apoptosis (Yin, et al., 2017). The testicular histoarchitecture was normal upon co-administration of tigernut and

BPA, as evidenced by normal seminiferous tubules, an intact basement membrane, normal interstitial spaces, and a lumen filled with spermatids. This suggests that the extract could potentially mitigate the impaired histoarchitecture and decreased spermatogenesis resulting from the BPA administration. The antioxidant found in tigernuts may have a beneficial effect on the synthesis of LH and testosterone as well as an improvement in testicular histology. As a result, giving tigernuts had ameliorated effects on the effects of BPA-induced spermatotoxicity.

4. Conclusion

According to the current study, tigernuts can shield rats from the spermatotoxicity caused by BPA. The hazardous damage caused by BPA was somewhat mitigated by tigernut extract, including changes in body weights, sperm parameters, testicular histology, and hormonal parameters.

Ethical aspects

All procedures relating to animal care and use were implemented in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory animals (Guide for the Care and Use of Laboratory Animals., 2011) and were approved by the Local Ethics Committee of the of College of Health Sciences, Bingham University Karu, Nasarawa State. Nigeria

Declaration of Interests

The authors declare that there is no conflict of interests

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Contribution by Authors

AA conceptualization and design of the research, AA and ICA interpreted the data, writing—review and editing manuscript. ICA and AA both critically revised the article for important intellectual content.

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References

Abu, H. H., Muhamad, M. H., Budi, K. S, Buhari, J., & Husain, A. O. (2023). Managing Bisphenol A Contamination: Advances in Removal Technologies and Future Prospects. Water., 15(20):3573. https://doi.org/10.3390/w15203573

Adam, M., Esievo, K. A. N, (2020). Bisalla, M., Ayo, J. O., Aliyu, A.I. , Akanbi, O. B., Baba, A.Y., Raji, L. O., & Saleh, A. Reproduction Pathology of Experimentally Induced Lead Poisoning in Red Sokoto Buck: Protective Effect of Tiger Nut (Cyperus Esculentus) Assiut Veterinary Medical Journal., 66(165): 67-79,

Aghajani M. M. R., Shafi, H., Gholamitabar, T. M., Moslemi, L., Aghamohammadi, A., Hajitabar, F., Mirabi, P., & Mehdinezhad, G. N. (2023). The Correlation of Urine Bisphenol A with Semen Parameters in Men Referred to Infertility Centers: A Cross-Sectional Study. Int J Fertil Steril., 7;17(4):292-298. doi: 10.22074/ijfs.2023.559352.1341. PMID: 37577915; PMCID: PMC10439992.

Akash, M. S. H., Rasheed, S., Rehman, K., Imran, M., & Assiri, M. A. (2023). Toxicological evaluation of bisphenol analogues: preventive measures and therapeutic interventions. RSC Adv., 19;13(31):21613-21628. doi: 10.1039/d3ra04285e. PMID: 37476040; PMCID: PMC10354593.

Allouh, M. Z., Daradka, H. M. & Ghaida, J. H. A. (2015). Influence of *Cyperus esculentus* tubers (Tiger Nut) on male rat copulatory behavior. BMC Complement Altern Med 15, 331. https://doi.org/10.1186/s12906-015-0851-9

Amjad S., Rahman, M. S., & Pang, M. G. (2020). Role of Antioxidants in Alleviating Bisphenol A Toxicity. Biomolecules., 25;10(8):1105. doi: 10.3390/biom10081105. PMID: 32722388; PMCID: PMC7465987

Bakare, A. A., Mosuro A. A., & Osibanjo, O. (2005). An in vivo evaluation of induction of abnormal sperm morphology in mice by landfill leachates. Mutat. Res., 582: 28-34.

Bjorndahl, L., Sodourlund, I., & Kvist, U. (2003). Evaluation of the one-step eosin-nigrosin staining technique for human sperm vitality assessment. Hum. Reprod., 18: 813-816.

Cheng, Y., Buffone, M. G., Kouadio M., Goodheart, M., Page, D. C., Gerton, G. L, Davidson, I., & Wang, P. J. (2007). Abnormal sperm in mice lacking the Taf7l gene. Mol Cell Biol., 27:2582–2589. doi: 10.1128/MCB.01722-06.

Ekaluo, U. B., Ikpeme, E.V., & Udokpoh, E. A. (2009). Sperm head abnormality and mutagenic effects of aspirin, paracetamol and caffeine containing analgesics in rats. Int. J. Toxicol., 7: 1-9

Ekaluo, U. B., Ikpeme, E.V., Ibiang Y.B., & Amaechina, O.S. (2013). Attenuating role of vitamin C on sperm toxicity induced by monosodium glutamate in albino rats. J. Biol. Sci., 13: 298-301

Fonseca, M. I., Lorigo. M., & Cairrao, E. (2022). Endocrine-Disrupting Effects of Bisphenol A on the Cardiovascular System: A Review. Journal of Xenobiotics., 12(3):181-213. https://doi.org/10.3390/jox12030015

Gbotolorun, S. C., Salako, A. A., & Ogunlade, B. (2022). Tiger nut: Antidote for alcohol-induced testicular toxicity in male Sprague-Dawley rats. JBRA Assist Reprod., 17;26(2):222-231. doi: 10.5935/1518-0557.20210061. PMID: 34672482; PMCID: PMC9118967.

Guide for the Care and Use of Laboratory Animals. (8TH ed.). (2011). National Academies Press

Hassan, L. A., Anyanwu, G. E., Nto, N. J., Obikili, E. N., Finbarrs-Bello, E., & Abireh, L. (2018). Curative effect of aqueous extract of Cyperus esculentus on flutamide-induced testicular dysfunction in male Wistar rats. Journal of Experimental and Clinical Anatomy, 17:13 - 17.

Im, J., & Löffler, F. E. (2016). Fate of bisphenol A in terrestrial and aquatic environments. Environmental science & technology., 50(16): 8403-8416.

Kawa, I. A., Akbar M., Fatima, Q, Mir, S. A., Jeelani, H., Manzoor, S., & Rashid, F. (2021). Endocrine disrupting chemical Bisphenol A and its potential effects on female health. Diabetes Metab Syndr., 15(3):803-811. doi: 10.1016/j.dsx.2021.03.031. Epub. PMID: 33839640.

Kumar, M., Sarma, D. K., Shubham, S., Kumawat, M., Verma, V., Prakash, A., & Tiwari, R. (2020). Environmental Endocrine-Disrupting Chemical Exposure: Role in Non-Communicable Diseases. Frontiers in Public Health., 8

Kurniawan, S. B., Abdullah, S. R. S., Imron, M. F., & Ismail, N. (2020). Current state of marine plastic pollution and its technology for more eminent evidence: A review. J. Clean. Prod., *278*, 123537.

Lahimer, M., Abou, D. M., Montjean, D., Cabry, R., Bach, V., Ajina, M., Ben, A. H, Benkhalifa, M., & Khorsi-Cauet, H. (2023). Endocrine disrupting chemicals and male fertility: from physiological to molecular effects. Front Public Health., 10;11:1232646. doi: 10.3389/fpubh.2023.1232646. PMID: 37886048; PMCID: PMC10598475.

Li. X., Wen, Z., Wang, Y., Mo, J., Zhong, Y., & Ge, R-S. (2020). Bisphenols and Leydig cell development and function. Front Endocrinol., 11:447. doi: 10.3389/fendo.2020.00447

Moussavi, G., & Haddad, F.A. (2019). Bacterial peroxidase-mediated enhanced biodegradation and mineralization of bisphenol A in a batch bioreactor. Chemosphere., *222*, 549–555.

Munir, B., Qadir, A., & Tahir, M. (2017). Negative effects of bisphenol A on testicular functions in albino rats and their abolitions with Tribulus terristeris L. Braz. J. Pharm. Sci., 53(3):e00104

Norazit, A., Mohamad, J., Razak, S. A., Abdulla, M. A,, Azmil, A., & Mohd, M. A. (2012). Effects of Soya Bean Extract, Bisphenol A and 17β-Estradiol on the Testis and Circulating Levels of Testosterone and Estradiol Among Peripubertal Juvenile Male Sprague-Dawley Rats. Sains Malaysiana., 41(1): 63–69

Nwakanma, A. A., Ekong, M., Ngwuben, I. C, Idaguko, C. A., & Elemuo, C.O . (2022). Cyperus esculentus L. Protects Testis and Sperm Morphology of Hyperglycaemic Rats. International Journal of Medical and Surgical Sciences., *9*(3), 1–16. https://doi.org/10.32457/ijmss.v9i3.1924

Nwosu, L.C., Edo. G. I., & Ozgor. E. (2022). The phytochemical, proximate, pharmacological, GC-MS analysis of Cyperus esculentus (Tiger nut): A fully validated approach in health, food and nutrition. Food Biosci., 46:101551. doi: 10.1016/j.fbio.2022.101551.

Ofem, O. E., Udonkang, M. I., Bassey, I. E., & Okechi, O. O. (2023). Cyperus esculentus (Tiger Nut) Improves Fertility and Testicular Histology in Male Sprague Dawley Rats. Tropical Journal of Natural Product Research (TJNPR)., 7(12), 5670–5676

Olukole, S. G., Ola-Davies, E. O., Lanipekun, D. O., & Oke, B. O. (2020). Chronic exposure of adult male Wistar rats to bisphenol A causes testicular oxidative stress: Role of gallic acid. Endocr Regul., 1;54 (1):14-21. doi: 10.2478/enr-2020-0003. PMID: 32597147.

Oyedepo, T.A., & Odoje, O.F. (2014). Hepato-protective Activities of Tiger Nut (Cyperus esculentus) Against Hepatotoxicity Induced by Carbon Tetrachloride in Rats. J Pharm Toxicol Stud, V. 2(4), P. 37 – 41.

Qiu, Y., Yang, H., Li, C., & Xu, C. (2020). Progress in Research on Sperm DNA Fragmentation. Med Sci Monit., 22;26:e918746. doi: 10.12659/MSM.918746. PMID: 32319429; PMCID: PMC7191954

Saeed, M. M., Fernández-Ochoa, Á., Saber, F. R., Sayed, R. H., Cádiz-Gurrea, M. L, Elmotayam, A. K, Leyva-Jiménez, F. J., Segura-Carretero, A., & Nadeem, R. I. (2022). The Potential Neuroprotective Effect of *Cyperus esculentus* L. Extract in Scopolamine-Induced Cognitive Impairment in Rats:

Extensive Biological and Metabolomics Approaches. Molecules., 21;27(20):7118. doi: 10.3390/molecules27207118. PMID: 36296710; PMCID: PMC9606906

Sánchez-Zapata, E., Pérez-Álvarez, J. A., & Fernández-López, J. (2012). Effects of tiger nut (Cyperus esculentus) milk liquid co-products on the quality of pork burgers. International Journal of Food Science & Technology,, 47(10):2198-2204

Santiago, J., Silva, J. V., Santos, M. A. S., & Fardilha, M. (2021). Fighting Bisphenol A-Induced Male Infertility: The Power of Antioxidants. Antioxidants (Basel)., 15;10(2):289. doi: 10.3390/antiox10020289. PMID: 33671960; PMCID: PMC7919053.

Shamhari, A., Abd Hamid, Z., Budin, S. B., Shamsudin, N. J., & Taib, I. S. (2021). Bisphenol A and Its Analogues Deteriorate the Hormones Physiological Function of the Male Reproductive System: A Mini-Review. Biomedicines., 9(11):1744. doi: 10.3390/biomedicines9111744. PMID: 34829973; PMCID: PMC8615890.

Sidorkiewicz, I., Zareba, K., Wolczynski, S., & Czerniecki, J. (2017). Endocrine-disrupting chemicals-Mechanisms of action on male reproductive system. Toxicol Ind Health., 33:601–9. doi: 10.1177/0748233717695160

Smith, L. B., & Walker, W. H. (2014). The regulation of spermatogenesis by androgens. Seminars in Cell & Developmental Biology, 30: 2-13

Takahashi, O., & Oishi, S. (2003). Testicular toxicity of dietary or parenterally administered bisphenol A in rats and mice. Food Chemistry Toxicology., 41: 1035-1044.

Udefa, A. L., Amama, E. A., Archibong, E. A., Nwangwa, J. N., Adama, S., Inyang, V. U., Inyaka, G. U., Aju, G. J., Okpa, S., & Inah, I. O. (2020). Antioxidant, anti-inflammatory and anti-apoptotic effects of hydro-ethanolic extract of Cyperus esculentus L. (tigernut) on lead acetate-induced testicular dysfunction in Wistar rats. Biomed Pharmacother., 129:110491. doi: 10.1016/j.biopha.2020.110491. PMID: 32768970.

Ullah, A., Pirzada, M., Jahan, S., Ullah, H., Turi, N., Ullah, W., Siddiqui, M. F., Zakria, M., Lodhi, K. Z., & Khan, M. M. (2018). Impact of low-dose chronic exposure to bisphenol A and its analogue bisphenol B, bisphenol F and bisphenol S on hypothalamo-pituitary-testicular activities in adult rats: A focus on the possible hormonal mode of action. Food Chem Toxicol., 121:24-36. doi: 10.1016/j.fct.2018.08.024.. PMID: 30120946.

Yamasaki, K., Sawaki, M., Noda, S., Imatanaka, N., & Takatsuki, M. (2002). Subacute oral toxicity study of ethynylestradiol and bisphenol A, based on the draft protocol for the 'Enhanced OECD Test Guideline no. 407'. Arch Toxicol., 76:65–74

Yin, L., Dai, Y., Cui, Z., Jiang, X., Liu, W., Han, F., et al.. (2017). The regulation of cellular apoptosis by the ROS-triggered PERK/EIF2α/chop pathway plays a vital role in bisphenol A-induced male reproductive toxicity. Toxicol Appl Pharmacol., 314:98–108. doi: 10.1016/j.taap.2016.11.013

Yu, Y., Lu, X., Zhang, T., Zhao, C., Guan, S., Pu, Y., & Gao, F. (2022). Tiger Nut (*Cyperus esculentus* L.): Nutrition, Processing, Function and Applications. Food., 19;11(4):601. doi: 10.3390/foods11040601. PMID: 35206077; PMCID: PMC8871521.

Zhang, S., Li, P., Wei, Z., Cheng, Y, L. J., Yang, Y., Wang, Y., & Mu, Z. (2022). Cyperus (*Cyperus esculentus* L.): A Review of Its Compositions, Medical Efficacy, Antibacterial Activity and

Allelopathic Potentials. Plants (Basel)., 21;11(9):1127. doi: 10.3390/plants11091127. PMID: 35567128; PMCID: PMC9102041.

RESUMEN

El bisfenol A (BPA) ha sido reportado por inducir reprotoxicidad en ratas. Este estudio se llevó a cabo para determinar las propiedades paliativas del extracto acuoso de Cyperus esculentus (chufa) sobre la toxicidad testicular inducida por BPA en ratas Wistar. Métodos: Veinte ratas macho fueron divididas aleatoriamente en 4 grupos (n=5): grupo A: (Control); grupo B: Bisfenol A (BPA) (25 mg/kg de peso corporal/día); grupo C: chufa (200 mg/kg de peso corporal); grupo D: (25 mg/kg de BPA + 200 mg/kg de extracto de chufa). Se disolvió 25 mg/kg de BPA en 0.2 ml de aceite de oliva como vehículo y se administró por gavaje oral durante 4 semanas. Se midieron los pesos corporales. Se recogió sangre para los ensayos de testosterona (T) y hormona luteinizante (LH); los epidídimos se procesaron para contar espermatozoides, motilidad espermática, viabilidad espermática y prueba de anormalidades espermáticas; mientras que los testículos se recolectaron para histología. Resultados: Hubo una disminución significativa ($p < 0.05$) en el peso corporal; reducción en el conteo de espermatozoides, motilidad, viabilidad, testosterona sérica y hormona luteinizante en el grupo BPA en comparación con el grupo control. Estos parámetros aumentaron significativamente ($p < 0.05$) en el grupo chufa (200 mg) y BPA + chufa (200 mg) en comparación con el BPA. Además, el examen histológico mostró espacios intersticiales ampliados, algunos túbulos seminíferos distorsionados, degeneración de la membrana basal, células de Leydig escasas, menos espermatozoides y vacuolización en el grupo BPA. En cambio, el grupo BPA + chufa mostró una mejora en la arquitectura testicular (preservación de los espacios intersticiales y células intersticiales, restauración de la membrana basal perdida y túbulos seminíferos cercanamente empaquetados con epitelio germinal bien organizado). Conclusión: La suplementación con chufas después de la administración de BPA produce una reversión del efecto perjudicial del BPA sobre el testículo.

Palabras clave: Bisfenol (BPA); Chufa; Testículos; Histología; Hormonas sexuales