World Journal of Advanced Pharmaceutical and Medical Research

Journal homepage: https://zealjournals.com/wjapmr/

ISSN: 2799-0656 (Online)

(RESEARCH ARTICLE)

Check for updates

Annona muricata (Soursop) leaves extract mitigates cyclophosphamide induced reproductive toxicity in male Wistar rats

Ejovwoke Marcellinus Arhoghro [1,*], Ebitimi Peter Berezi [2], Sylvanus Beredugo [3] and I Omeodu Steve [4]

[1] *Department of Biochemistry, Faculty of Basic Medical Sciences, College of Health Science, Niger Delta University, Wilberforce Island, Amassoma, Bayelsa State, Nigeria.*
[2] *Department of Chemistry, Isaac Jasper Boro College of Education, Sagbama, Bayelsa State, Nigeria.*
[3] *Department of Medical Laboratory Science, Faculty of Basic Medical Sciences, College of Health Science, Niger Delta University, Wilberforce Island, Amassoma, Bayelsa State, Nigeria*
[4] *Department of Biochemistry, University of Port Harcourt, P.M.B 5323, Port Harcourt, Nigeria.*

World Journal of Advanced Pharmaceutical and Medical Research, 2023, 04(01), 007–016

Publication history: Received on 30 November 2022; revised on 07 January 2023; accepted on 09 January 2023

Article DOI: https://doi.org/10.53346/wjapmr.2023.4.1.0054

Abstract

Background: Cyclophosphamide (CP) is an anticancer and immunosuppressive agent commonly used in men of reproductive age. *Annona muricata*(soursop) is a tropical plant with a widely acclaimed history of various therapeutic properties.

Objective: This study was aimed at determining the effect of the ethanolic leaf extract of *Annona muricata* on the reproductive system of male rats treated with cyclophosphamide.

Materials and Methods: Twelve (n-12) male wistar rats were randomly divided into three groups of four rats each. Group 1 served as control and received only feed and water. Group 2 was administered a single dose of CP (100 mg/kg body weight) intraperitoneally on the 15th day, while group 3 was administered 100 mg/kg body weight of ethanolic extract of *Annona muricata* for 14 days and a single intraperitonealdose of CP on the 15th day. At the end of the experiment, the animals were sacrificed under anaesthesia, blood and tissue samples were collected for biochemical and histological evaluations respectively.

Result: The result showed that serum concentrations of LH and FSH were significantly increased ($p<0.05$) in group 2 (5.53±1.28 and 10.95±1.44) when compared to the control group (1.38±1.02 and 4.97±1.53) and decreasedin group 3(1.14±0.93 and 4.97±1.45) when compared to the control group (1.38±1.02 and 4.97±1.53). Serum testosterone level decreased significantly in group 2 (1.31±1.08) when compared to the control group (5.08±1.02**)** andincreasedin group 3 **(**4.95±1.23) when compared to the control group (5.08±1.02). Testicular SOD and MDA was significantly decreased in group 2 (3.23±0.41 and 1187.71±1.21) when compared to the control group (6.00±1.65 and 371.91±1.47) and slightly elevated in group3 (6.05±0.97 and 371.97±1.47). The histology of the testicular tissue shows markedly shrunken seminiferous tubules with severe germ cell aplasia and basement membrane thickening following cyclophosphamide administration which was restored to normal after treatment with aqueous extract of *Annona muricata*.

Conclusion: The study reveals that *Annona muricata* possess protective effect against cyclophosphamide-induced reproductive toxicity in male wistar rats.

Keywords: Cyclophosphamide; *Annona muricata*; Testosterone; LH; FSH

*Corresponding author: Arhoghro Ejovwoke Marcellinus

1. Introduction

Humans employ a variety of chemotherapeutic medications to treat various diseases, however many of these drugs have side effects which are harmful and destructive. Many human malignant tumors are treated with cyclophosphamide (CP), an alkylating drug of the oxazaphosphorine class, on a regular basis and in large quantities [1,2]. CP is a drug of choice for the treatment of non-Hodgkin's lymphoma, acute myeloid leukemia, chronic myelogenous leukemia, acute lymphoblastic leukemia, solid tumors, and breast cancer. Pharmacologically, it is categorized as a cytotoxic alkylating drug with anti-tumor and immunosuppressive potentials [3,4]. Nevertheless, despite its therapeutic significance and advantages, it has a variety of negative side effects, including reproductive toxicity and leucopenia in both humans and animals[1,2]. Additionally, it causes oxidative stress and has cytotoxic effects on healthy cells, particularly those found in the reproductive organs [5].

Despite the emergence of modern drugs, medicinal plants continue to be advantageous to man [6]. Around 5.2 billion people worldwide, or 80% of the population, rely only on medicinal plants for their healthcare needs [7]. Secondary metabolites, a class of chemically active plant constituents, are what give these plants their therapeutic value [8]. Secondary metabolites, or phytochemicals, have amazing therapeutic benefits, such as anti-oxidant, anti-allergic, antibacterial, hypoglycemic, anti-carcinogenic, sedative, analgesic, antipyretic, cardioprotective, and antiviral characteristics [9]. Significant scientific interest in the biological activities of these phytochemicals have been sparked in recent times to investigate the cardinal therapeutic roles of these active components of plants in both agriculture and medicine [10]. Despite these investigations, only a small number of plant species have undergone thorough scientific examination, and our understanding of their possible function in nature is very limited. A significant number of physiologically active compounds in medications or drugs are obtained from plants. Traditional medicinal plants offer an intriguing and still remains a largely untapped source for the creation of new drugs [11]. Since the dawn of medicine, natural products, particularly those made from plants, have been used to support human health. Plant phytochemicals have played a key role in pharmacological discoveries throughout the past century [10].

Numerous medical uses have been ascribed to *Annona muricata* which also have a long history of pharmacological impact. The decoction of bark, root, seed, or leaf with a variety of applications is the most popular preparation in traditional medicine. Malaysian natives use the leaves of *A. muricata* to treat both internal and external parasites. In South America, the fruit is valued not just as food but also for its juice, which is used as a galactagogue to cure diarrhea, heart and liver ailments, as well as intestinal parasites [12].For cancer treatment, some patients take *A. muricata* decoctions or capsules. As biopesticides, bioinsecticides, and topical insect repellents, unripe fruit, seeds, leaves, and roots are also employed. The fruit is also consumed to enhance lactation in breastfeeding women after childbirth, and it is used as a natural remedy for rheumatism, neuralgia, arthritis, dysentery, fever, malaria, and skin rashes [13].The leaves are also used to treatcystitis, diabetes, headaches, insomnia and abscesses[14].

In pharmacological landscape, plants with a long history of use in ethnomedicine are a rich source of active phytoconstituents that provide medicine or health benefits against various ailments and diseases. One of such plant with extensive traditional use is *Annona muricata.* This plant has received a lot of interest lately because of its possible implications on sexual function [15]. It is against this background; the researchers wish to evaluate the effect of aqueous extract of *Annona muricata* leaves on fertility parameters in cyclophosphamide induced-reproductive toxicity in male wistar rats.

2. Material and methods

2.1. Chemicals

All chemicals used were of analytical grade quality.

2.2. Preparation of extracts

Fresh leaves of *Annona muricata* were collected from a residential quarter in Niger Delta University (N.D.U.), Wilberforce Island, Amassoma, Bayelsa State, Nigeria. After being botanically identified, the leaves were deposited in the herbarium of the Department of Biological Science at N.D.U., Wilberforce Island, Amassoma, Bayelsa State, Nigeria. The leaves were thoroughly cleaned with distilled water to get rid of any dirt or contaminants, dried in an oven at 40°C andpulverized into powder with an electric blender. 1350ml of 75% ethanol was used to extract about 450grams of the powdered leaves. The resultant decoction was filtered, treated to a low but thorough solvent evaporation using a water bath at a temperature of 60°C and the filtrate was then stored in an airtight container with a lid for later administration. The protocol outlined by [16] was used to prepare *Annona muricata* leaf extract.

2.3. Experimental Animals

Twelve (12) healthy adult male wistar rats weighing between 117 g and 250 g were purchased from the animal house of the Faculty of Pharmacy at Niger Delta University, Wilberforce Island, Amassoma, Bayelsa State, Nigeria. The rats were housed in cages made of metal nesting in the animal house of the Department of Medical Biochemistry, Niger Delta University, Wilberforce Island, Amassoma, Bayelsa State, Nigeria where they were allowed two (2) weeks to acclimatize under standard environmental conditions of a 12hr light/dark cycle and were fed with grower's mash and distilled water *ad libitum.* The beddings were changed and the cages cleaned every morning and disinfected at interval of three days. All procedures were carried out in compliance with the Institutional Animal Ethical Committee's (IAEC) directives for the control and supervision of animal experimentation (CPCSEA).

2.4. Experimental design

Twelve (12) adult wistar rats weighing between 117 g and 250 g were used in this research work.The animals were divided into three groups:

- **Group 1:**served as negative control and was fed with pelleted growers feed and distilled water for 14 days.
- **Group 2:**served as positive control and received a single dose of 100mg/kgbody weightof cyclophosphamide intraperitoneally on the 14thday and left for 24 hoursbefore sacrificing.
- **Group 3**: Received aqueous extract of *Annona muricata* leaves (100mg/kgbody weight)orally for 14 days and a single intraperitonealdose of CP on the 15th day.

2.5. Estimation of Reproductive Hormones

The levels of follicle stimulating hormone (FSH), luteinizing hormone (LH), and testosterone in theserum were estimated usingstandard operating procedure and specific commercial kits(IBL-Hambug GmbH, Germany) based on the ELISA technology described by [17]. The assays were performed in accordance with the manufacturer's protocols as stated in the manual. The absorbances were measured using a microtitre plate reader using the appropriate wavelength for each analyte, and the associated concentrations were determined.

2.6. Estimation of Superoxide Dismutase (SOD)

The protocol by[18] was used to quantify superoxide dismutase (SOD) activity. It is based on the capacity of superoxide dismutase to suppress epinephrine autoxidation at pH 10.2. Procedure: After being adjusted to 420 nm, the spectrophotometer was zeroed using a blank comprised of 3.0 ml of distilled water. The reference tube received 0.2ml of pure water, while the test tube received 0.2 ml of the appropriate enzyme extracts. Each of these tubes received 2.5ml of phosphate buffer, and after equilibration at room temperature, 0.3 ml of the 0.3mM adrenaline solution was dispensed to the reference and each of the test solutions. This was then allowed to mix, and the absorbance was measured at 420 nm.

2.7. Determination of Malondialdehyde

The technique by [19] was used to measure lipid peroxidation product as an index of MDA production. Malondialdehyde was determined to be a conjugate with TBA. TCA was used to precipitate proteins, which were then centrifuged to remove them. The MDA-TBA complex was measured at 534 nm. The procedure was as follows: two test tubes were labelled as test and blank, respectively. 1ml of the test solution was poured into the test tube and 1ml of distilled water into the blank tube. Reagents 1, 2, and 3 were added to the test and blank in 1 ml amounts and mixed. The tubes were incubated in a boiling bath for 15 minutes before cooling for 20 minutes at room temperature.After 15 minutes of centrifuging the test tubes at 2000 rpm, the supernatant layer was read at 534 nm. The following formula was used to compute the MDA concentration (nmol/ml): Abs (test) - Abs (blank) / 1.56 x 1000000 is the test's concentration.

2.8. Histopathological Analysis

At the end of the experimental period, animals were sacrificed using chloroform inhalation method and the testes of control and treatment groups were harvested and fixed in 10%Bouin's fluid. Routine tissue processing was carried out using automatic tissue processor; Histokinette (LEICA TP 1020). The tissues were embedded in paraffin wax in tissue embedder (LE1CA EG 1160) and trimmed with a rotary microtome (LEICA P.M 2125 RTS) at 20 microns and sectioned at 5 microns thickness. The sectioned tissues were attached to slides and subsequently dewaxed in xylene and stained in haematoxylin and eosin for general tissue architecture. The stained slides were then examined using compound light microscope at X400 magnification.

2.9. Statistical Analysis

All data obtained were presented as mean and standard deviation (Mean±SD).TheSPSS Software ofversion23.0 was used for the analysis of the data obtained. Comparison of result between control and test was done using one-way analysis of variance (ANOVA) and group means were compared using Bonferroni multiple comparison. Level of significance was determined at a probability level of $p<0.05$.

3. Results

Results presented in table 1 reveals that the serum levels of LH(5.53±1.28) and FSH(10.95±1.44) was significantly increased ($p<0.05$) while serum concentration of testosterone(1.31±1.08) decreased significantly following treatment with CP alone when compared to the normal (LH: 1.38±1.02; FSH: 4.97±1.53) and testosterone (5.08±1.02). The study also showed that following administration of *Annona muricata* (100mg/kg) and CP (100mg/kgbody weight),there was a decreased ($p<0.05$) in LH(1.14±0.93) andFSH(4.97±1.41) levels compared to the negative control (1.38±1.02 and 4.97±1.53) while serum testosterone level was increased (4.95±1.23) to near normal level (5.08±1.02). Hence, *Annona muricata* leaf extract reversed CP induced reproductive toxicity. Results in table 2 shows that the administration of CP alone significantly decreased($p<0.05$) the antioxidant activity of SOD (3.23±0.41)and MDA (1187.71±1.21) when compared to the negative control-group 1 (6.00±1.65 and 371.91±1.47).The study also observed a significant increase ($p<0.05$) in antioxidant activity of SOD(6.05±0.97) and MDA (371.97±1.47) in the rats administered with aqueous extract of *Annona muricata* leaves (100mg/kg) and CP (100mg/kgbody weight)when compared to rats in group 1 (6.00±1.65and 371.91±1.47).The study also observed normal morphology of the testes, seminiferous tubules (ST), sperm cell(C) and the lumen of rats in negative control group and a marked cytoarchitectural alteration which includes: shrunken seminiferous tubules with severe germ cell aplasia and basement membrane thickening in rats induced with 100mg/kg body weight of cyclophosphamide. Morphology of the testes after the administration of *Annona muricata* leaves extract (100ml/kgbody weight) for 14 days and a single dose of cyclophosphamide (100mg/kg body weight.) reveals a normal morphology of the testes, seminiferous tubules (ST), sperm cell(C) and the lumen (L).

Table 1 Effect of ethanolic leaf extract of *Annona muricata* on serum concentration of sex hormones

Groups	**Sex hormones**		**Testosterone** (mg/ml)
	LH(mg/L)	**FSH**(mg/L)	
Group 1 (Control)	1.38±1.02[a]	4.97±1.53[a]	5.08±1.02[a]
Group 2 (Positive Control)Cyclophosphamide	5.53±1.28[b]	10.95±1.44[b]	1.31±1.08[b]
Group 3 (Annona muricata + CP)	1.14±0.93[c]	4.97±1.41[a]	4.95±1.23[c]

Data are expressed as mean ± SD **(**n=4). Mean in the same column with different superscript letter(s) are significantly different; P<0.05 one way ANOVA followed by post-hoc and Tukey.

Table 2 Effect of ethanolic leaf extract of *Annona muricata* on superoxide dismutase (SOD) and malondialdehyde (MDA)

GROUPS	**Antioxidants**	
	SOD(U/mg protein)	**MDA**(nmol/g protein)
Group 1 (Control)	6.00±1.65[a]	371.91±1.47[a]
Group 2 (Positive Control)Cyclophosphamide	3.23±0.41[b]	1187.71±1.21[b]
Group 3 (*Annona muricata* + CP)	6.05±0.97[c]	371.97±1.47[c]

Data are Mean ± SD (n=4). Mean in the same column with different superscript letter(s) are significantly different, (P<0.05).

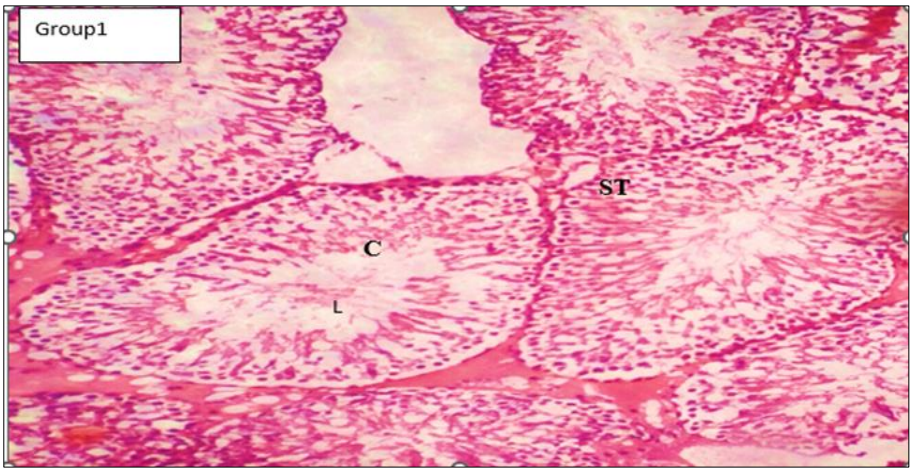

Figure 1 The morphology of the testis of control group. Slide shows normal morphology of the testes seminiferous tubules (ST), sperm cell (C) and the lumen (L) (X400)

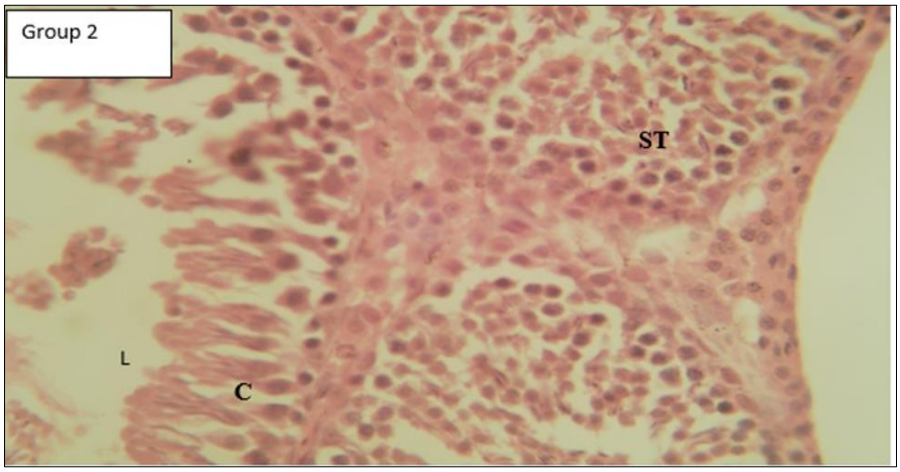

Figure 2 Photomicrograph of testicular section of Cyclophosphamide (100mg/kg bwt) treated rats reveals markedly shrunken semeniferous tubules with severe germ cell aplasia and basement membrane thickening, seminiferous tubules (ST), sperm cell (C) and the lumen (L) (X400)

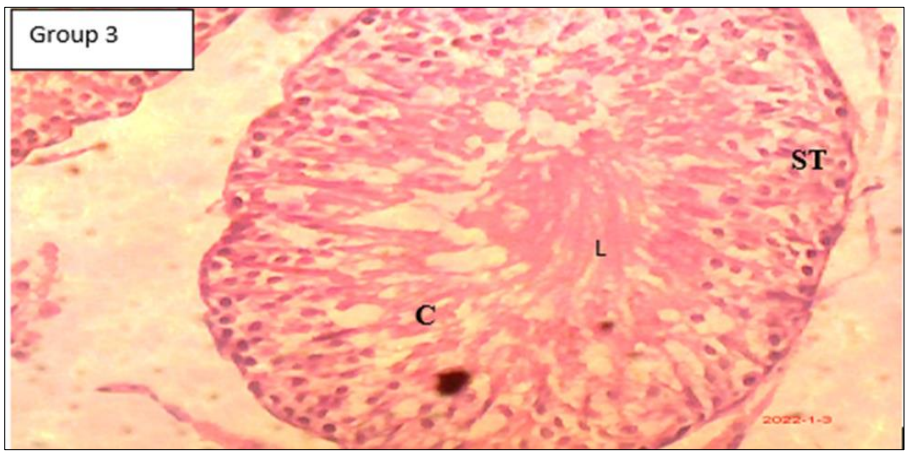

Figure 3 The morphology of the testes after the administration of *Annona muricata* leave extract and cyclophosphamide. Slide shows normal morphology of the testes seminiferous tubules (ST), sperm cell (C) and the lumen (L) (X400)

4. Discussion

The value of medicinal plants cannot be overestimated, since the majority of herbal plants are used to maintain good health and to treat medical conditions. The efficacy of herbal plants have been proven; and has triggered scientific interest and research in recent times [20, 21, 22]. It has been demonstrated that medicinal plants are useful for treating a variety of ailments. Their powerful phytochemical components which have strong antioxidant qualities are responsible for the pharmacological actions they exhibit [23, 24, 25 26].

Reproductive hormones significantly influenced the regulation of spermatogenesis. Male fertility depends on the production of sperm and testosterone, both of which are produced in the testes [27]. Endocrine hormones that are necessary for the synthesis of testosterone and sperm are produced by the testes and the hypothalamic-pituitary axis of the brain. LH activates the Leydig cells in the testes to produce testosterone, while hypothalamic GnRH (gonadotropin-releasing hormone) stimulates the pituitary gland to produce LH and FSH [28, 29, 30].

Cyclophosphamide (CP) is a chemotherapeutic drug used to treat a variety of cancers and autoimmune disorders [31, 32].According to [33] and [34], cyclophosphamide can disrupt the hypothalamic-pituitary axis and the testes with a resultant detrimental effect on the regulatory function of GnRH and affects the amount of male reproductive hormones. This present study observed that cyclophosphamide-treated rats had significantly lower testosterone levels and significantly higher LH and FSH levels (P 0.05). However, the administration of *Annona muricata* leaves extract significantly reduced serum levels of LH and FSH to within normal ranges while significantly increasing serum levels of testosterone in the treatment group, thus reversing the toxicity caused by CP and restoring the ability of the hypothalamic-pituitary axis of the brain to regulate hormone production (Table 1).The results of this investigation are consistent with the study by [35], who likewise noted a drop in the serum testosterone concentration in cyclophosphamide-induced rats. A possible explanation for the significant drop in serum testosterone in the CP-impaired group is the increased production of free radicals, which is one of the potential mechanisms underlying Leydig cell degeneration caused by CP. Additionally, it has been reported that CP induces cell death, which could be the source of the decreased number of epididymal sperm count seen in CP-treated rats [36, 37, 38].

Some studies have also shown that CP can reduce testicular weight, induce disturbance in markers of oxidative stress, induce oligospermia and azoospermia, alter gonadotrophin levels and because permanent infertility at higher doses in both human and animal models [39, 40, 42, 43]. It has also been established that CP mostly causes oxidative stress in biological systems by producing reactive oxygen species [35, 41]. This study revealed that rats given CP had considerably lower SOD and MDA activity compared with the control group. But the administration of *Annona muricata* leaf extract caused a significant increase in SOD and MDA levels to within normal range when compared to the CP treated group. The outcome of this research demonstrates that the administration of Annona muricata was effective in preventing or lessening testicular damage and hormonal disruption after exposure to CP, possibly as a result of its antioxidant properties, which is consistent with the findings of [44] and other studies involving curcumin plantain stem juice, which reduced the negative effects of cyclophosphamide toxicity and increased fertility in rats [30, 45, 46,]. Antioxidants effectively scavenge ROS produced in tissues through oxidative stress; and herbs with antioxidant properties have the ability to reduce the negative effects caused by ROS formation [47].

It has been established that several drugs cause infertility by generating ROS in the testes. Although the precise mechanism of CP-induced testicular toxicity is unknown, it may be related to the action of highly reactive metabolites that are mutagenic to mammalian cells [48, 35, 41]. Cytochrome P450 (CYP) enzymatically converts the cyclophosphamide to produce toxic metabolites [49]. The two active metabolites of CP are phosphoramide mustard and acrolein. While acrolein is linked to harmful consequences such as cell death, apoptosis, oncosis, and necrosis [44, 50], phosphoramide mustard has antineoplastic properties. The majority of the oxidative damage linked to the administration of CP has been primarily attributed to acrolein, a reactive electrophilic molecule [51]. Exposure to CP results in the accumulation of ROS, the production of oxidative stress, and the suppression of antioxidant enzymes by interfering with the tissue antioxidant defense system [52, 53, 54].

Studies have shown that cyclophosphamide can harm nearly all bodily organs, including the testes, liver, lung, spleen, kidneys, and heart [55, 56]. This is due to the drug's ability to produce toxic reactive oxygen species, which interact with protein and amino acids to alter their structural and functional properties [57, 58] additionally, in the testes and epididymis of experimental animals, [59] and [60] reported DNA reduction, impaired cytoarchitecture, and germ cell apoptosis. Our study also reveal that cyclophosphamide-induced rats had a noticeable cytoarchitectural modification of the testes, including constricted seminiferous tubules, severe germ cell aplasia, and thickened basement membranes. The administration of *Annona muricata* leaves extract resulted in the restoration of the normal morphology of the testes.

The results of this study showed that compounds in the *A. muricata* mitigated the destructive effects of cyclophosphamide and its metabolites.

5. Conclusion

The study revealed that oral administration of ethanolic extract of *Annona muricata* exhibited protective and therapeutic effects against cyclophosphamide-induced reproductive toxicity in male wistar rat model. Thus, *A. muricata* leaves possesses potent antioxidants that combats reproductive toxicity inducedby cyclophosphamide, and may therefore serve as a potential source of safe, effective and affordable therapy for infertility and other reproductive disorders.

Compliance with ethical standards

Acknowledgments

The authors acknowledge and express their appreciation to all the laboratory staff who were involved in this research work for their various supportive roles.

Disclosure of conflict of interest

The authors declare that there is no conflict of interest.

Statement of ethical approval

The study protocol was approved by the Ethical and Research Committee of the Niger Delta University, Wilberforce Island, Amassoma, Bayelsa State, Nigeria. The ethical principles for medical research involving animal subjects as outlined in the Helsinki declaration in 1975 and subsequent revisions were strictly adhered to in the course of this study.

References

[1] Mahmoud A. M., Germoush M. O., Alotaibi M. F., Hussein O.E.(2017). Possible involvement of Nrf2 and PPARy upregulation in the protective effect of umbelliferone against cyclophosphamide-induced hepatotoxicity. *Biomedicine & Pharmacotherapy*,86:297-306

[2] Omole J. G., Ayoka O. A., Alabi Q. K., Adefisayo M. A., Asafa M. A., Olubunmi B. O., Fadeyi B. A. (2018). Protective Effect of Kolaviron on Cyclophosphamide-Induced Cardiac Toxicity in Rats. J Evidence Based Integrative Medicine.

[3] Khan J, Shahdad S, MakhdoomiM,Hamid S, Bhat M, Jan Y. et.al. Effect of Cyclophoshamide on the microanatomy of liver of albino rats. International Journal of Research and Medical Science. 2014; 2:1466-9.

[4] Emadi A., Jones R. J., and Brodsky R. A.(2009). Cyclophosphamide and cancer: golden anniversary. Nature Reviews Clinical Oncology, 6(11):638-647.

[5] Arhoghro E.M. and Sule O.J (2012). Effect of *costusafer* on fertility parameters I cyclophosphamide induce reproductive toxicity in male albino rats. *European journal of biochemical and pharmaceutical sciences,4* (10), 119-125.

[6] Singh, V., & Kumar, R. (2017). Study of phytochemical analysis and antioxidant activity of Allium sativum of Bundelkhand region. *International Journal of Life-Sciences Scientific Research*, *3*(6), 1451-1458.

[7] Narendiran, S., Janani, D., Keerthana, M., Nivethitha, K. S., Nirmala Devi, S., Padmavathy, S., &Yasaswini, K. G. (2016). Comparative Studies on in-vitro Phytochemicals Analysis and Larvicidal Efficacy of Medicinal Plant Extracts against Culexquinquefasciatus. *International Journal of Life Science and Scientific Research*, *2*(6), 742-748.

[8] Yadav, R., Khare, R. K., &Singhal, A. (2017). Qualitative phytochemical screening of some selected medicinal plants of shivpuri district (mp). *International Journal of Life Science and Scientific Research*, *3*(1), 844-847.

[9] Quilez, A., Montserrat-de la Paz, S., De la Puerta, R., Fernández-Arche, M., and García-Giménez, M. (2015). Validation of ethnopharmacological use as anti-inflammatory of a decoction from Annona muricata leaves. *Afr. J. Tradit. Complement. Altern. Med. 12*, 14–20. doi: 10.4314/ajtcam.v12i4.3

[10] Moghadamtousi, S. Z., Fadaeinasab, M., Nikzad, S., Mohan, G., Ali, H. M., and Kadir, H. A. (2015). Annona muricata (Annonaceae): a review of its traditional uses, isolated acetogenins and biological activities. *Int. J. Mol. Sci. 16,* 15625–15658. doi: 10.3390/ijms160715625

[11] Cos, P., Vietneik, A.S. Berghe, D.V. and Maes, L. (2006). Anti- infective potential of natural products; How to develop a stronger in vitro pro concept. *Ethno. Pharmacology, 106:* 290 – 302.

[12] Badrie, N., Schauss, A.G., (2009). Soursop (Annona muricata L.) composition, nutritional value, medicinal uses, and toxicology. In Watson, R.R., Preedy. V.R, Bioactive Foods in Promoting Health. Oxford, pp `621-643

[13] Isman, M. B., and Akhtar, Y. (2007). "Plant natural products as a source for developing environmentally acceptable insecticides," in Insecticides Design Using Advanced Technologies, eds I, Ishaaya, F. Nauen, A. R Horowitz (Berlin; Heidelberg: Springer-Verlag Neitherland), 235–248.

[14] Soheil ZorofchianMoghadamtousi, Mehran Fadaeinasab, Sonia Nikzad, Gokula Mohan, HapipahMohd Ali, Habsah Abdul Kadir, (2015). Annona muricata: A review of Its Traditional Uses, Isolated AcetogeninsAnd Biological Activities. Int. J. of Mol. Sci., 16, 15625-15658; doi:10.339/ijms160715625

[15] Aslam, M. S., Ahmad, M. S., Mamat, A. S., Ahmad, M. Z., & Salam, F. (2016). An update review on polyherbal formulation: A global perspective. *Systematic Reviews in Pharmacy*, *7*(1), 35-41.

[16] Fofana, S., Keita, A., Balde, S., Ziyaev, R., and Aripova, S. (2012). Alkaloids from leaves of Annona muricata. Chem. *Nat. Comp. 48*, 714–714. doi: 10.1007/s10600-012-0363-5

[17] Steyn FJ, Xie TY, Huang L, Ngo ST, Veldhuis JD, Waters MJ, Chen C. (2013). Increased adiposity and insulin correlates with the progressive suppression of pulsatile GH secretion during weight gain. Journal of Endocrinology. 218(2):233-244

[18] Misra HP, Fridovich I. he Role of Superoxide Anion in the Autoxidation of Epinephrine and a Simple Assay for Superoxide Dismutase. Journal of Biological Chemistry. 1972; 247: 3170-3175.

[19] Shah JK., Walker's AM. (1989). Quantitive determination of MDA. Biochem. Biophys. Acta,; 11: 207-11

[20] Chavan, P. A. (2016). Evaluation of antimicrobial activity of various medicinal plants extracts of Latur Zone against pathogens. *International Journal of Life Science and Scientific Research*, *2*(5), 612-618.

[21] Coria-Téllez, A. V., Montalvo-Gónzalez, E., Yahia, E. M., and Obledo-Vázquez, E. N. (2016). Annona muricata: a comprehensive review on its traditional medicinal uses, phytochemicals, pharmacological activities, mechanisms of action and toxicity. *Arabian J. Chem.*doi: 10.1016/j.arabjc.2016.01.004

[22] Dey, A., Mukherjee, A., and Chaudhury, M. (2017). "Alkaloids from apocynaceae: origin, pharmacotherapeutic properties, and structure-activity studies," in Studies in Natural Products Chemistry ed Atta-ur-Rahman (Amsterdam: Elsevier), 373–488.

[23] Hajdu, Z., and Hohmann, J. (2012). An ethnopharmacological survey of the traditional medicine utilized in the community of Porvenir, Bajo Paraguá Indian Reservation, Bolivia. J. Ethnopharmacol. 139, 838–857. doi: 10.1016/j.jep.2011.12.029.

[24] Kumar A, Iavarasan RI, Jayachandran T, Decaraman M, Aravindhan P, Padmanabhan N, Krishnan MRV (2009). Investigation on a tropical plant Syzygiumcumini from Kattuppalayam, Erode District, Tamil Nadu, South India. Pakistan Journal Nutrition,; 8: 83-85.

[25] Yakubu, M.T. Akanji,.M, AndOladeji A.T (2007). Male sexual dysfunction and methods used in accessing medicinal plants with Aphrodisiac potentials. *Pharmacy*, rev. 1:1.

[26] FasuyiAO.(2006). Nutritional potentials of some tropical vegetable leaf meals. Chemical characterization and functional properties. African Journal Biotechnology, 5: 49–53.

[27] Kaur, P., Kaur, G., and Bansal, M. P. (2006) Tertiary-butyl hydroperoxide induced oxidative stress and male reproductive activity in mice: Role of transcription factor NF-kappaB and testicular antioxidant enzymes*. Reprod Toxicol*.22:479–484.

[28] Pitteloud, N., Dwyer, A. A., DeCruz, S., Lee, H., Boepple, P. A, Crowley, W. F. and Hayes, F. J. (2008). Inhibition of luteinizing hormone secretion by testosterone in men requires aromatization for its pituitary but not its hypothalamic effects: evidence from the tandem study of normal and gonadotropin-releasing hormone-deficient men. *The Journal of Clinical Endocrinology and Metabolism*. 93 (3): 784–91.

[29] Onaolapo A. Y., Oladipo B. P, Onaolapo O. J. (2017). Cyclophosphamide-induced male subfertility in mice: An assessment of the potential benefits of Maca supplement. Andrologia.;12911.1-10

[30] Arhoghro E. M., John T. R., Eboh A. S., Proph T. P. and Angalabiri-owei B. (2016). Biochemical parameters and sperm characteristics in male rats given plantain stem juice in cyclophosphamide-induced reproductive toxicity. *World J. of Pharmaceutical Research. 5(6):* 278-298.

[31] Patti F, Lo Fermo S. (2011). Lights and shadows of cyclophosphamide in the treatment of multiple sclerosis. Autoimmune Dis.; 961702.

[32] Altaylý E., Malkoc E., Alp B. F., Korkmaz. (2012). A. Prevention and treatment of cyclophosphamide andifosfamide-induced hemorrhagic cystitis. Journal of Molecular Pathophysiology, 1: 53-62.

[33] Liza O., Peter S., and David M. K. (2017). Endocrinology of the Male Reproductive System and Spermatogenesis. National Library of Medicine, 3(7):45-49

[34] Çeribaşi AO, Türk G, Sönmez M, Sakin F, and Ateşşahin A. Toxiceffect of cyclophosphamide on sperm morphology, testicular histologyand blood oxidant-antioxidant balance, and protective roles of lycopene and ellagic acid. Basic & Clinical Pharmacology & Toxicology. 2010; 107: 730–736

[35] Ayoka, O. A., and Oladele, A. A. (2016). Neuro-endocrine effects of aqueous extract of Amaranthus viridis (Linn.) leaf in male Wistar rat model of cyclophosphamide-induced reproductive toxicity.(3), pp. 608-619

[36] Nawwar, M., Ayoub, N., Hussein, S., Hashim, A., El-Sharawy, R., and Wende, K. (2012). Flavonoltriglycoside and investigation of the antioxidant and cell stimulating activities of Annona muricata Linn. *Arch. Pharm. Res. 35,* 761–767. doi: 10.1007/s12272-012-0501-4

[37] Higuchi, H., Nakaoka, M., and Kawamura, S. (2001). Application of computer-assisted sperm analysis system to elucidate lack of effects of cyclophosphamide on rat epididymal sperm motion. J ToxicolSci.,; 26: 75 – 83

[38] Mishra S., Kumor N., and Sharma B.k. (2013). Annona muricata (the cancer killer); A review. Glob. J. pharm. 2, 1613-1618.

[39] Hutheyfa A. A., Nabeel M. A., Saif S. A., and Ali A. A. (2020). The Pathological Features of CyclophosphamideInduced Multi-Organs Toxicity in Male Wister Rats. Systematic Review Pharmacy.11(6): 24-28.

[40] Liu, N., Yang, H. L., Wang, P., Lu, Y. C., Yang, Y. J., Wang, L., et al. (2016). Functional proteomic analysis revels that the ethanol extract of Annona muricata L. induces liver cancer cell apoptosis through endoplasmic reticulum stress pathway. *J. Ethnopharmacol. 189,* 210–217. doi: 10.1016/j.jep.2016.05.045

[41] Aroona, C., Shokrzadeh, M., Farshad, N., Salehi, F., and Amirhossein, A. (2015). Melatonin ameliorates oxidative stress and reproductive toxicity induced by cyclophosphamide in male mice.hum. amp Exp. Toxicol., 33(2), pp. 185-195

[42] Cao Y, Wang X, Li S, Wang H, Yu L, and Wang P. (2017).The effects of L-carnitine against cyclophosphamide-induced injuries in mousetestis. Basic Clinical Pharmacology and Toxicology.; 120(2): 152-158.

[43] Comish P.B., Drumond A.L., Kinnell H L., Anderson R.A., Matina, Meistrich M L, and Shetty G. (2014;). Fetal cyclophosphamide exposureinduces testicular cancer and reduced spermatogenesis andovarian follicle numbers in mice. PLoS One. 9(4): e93311

[44] Akram Hosseini., SamadZare., Zahra Borzouel., and Firouz Ghaderi Pakdel. (2018). Cyclophosphamide-induced testicular toxicity ameliorate by American ginseng treatment: Int J Reprod BioMed. 16(11): 711-718

[45] Akomolafe S.F, and Aluko B.T. (2020). Protective effect of curcumin on fertility in cyclophosphamide exposed rats: involvement of multiple pathways. *J Food Biochem*.;44:e13095. doi: 10.1111/jfbc.13095

[46] Shalizar, J. shapour, H. and Hassan, M. (2011). Chemoprotective effct of *Crataegus monogyna*aqueous extract against cyclophosphamide induce reproductive toxicity. *Verterinary Research Forum. 2*(4). 266-273.

[47] Jong-Choon, K., Sung-Hwan, K., Hyung-Seon, B., and Sung-Ho, K. (2016). Protective effect of diallyl disulfide on cyclophosphamide-induced testicular toxicity in rats. *Laboratory Animal Research, 29(*4): 204-211.

[48] Moghadamtousi, S. Z., Fadaeinasab, M., Nikzad, S., Mohan, G., Ali, H. M., and Kadir, H. A. (2015). Annona muricata (Annonaceae): a review of its traditional uses, isolated acetogenins and biological activities. *Int. J. Mol. Sci. 16,* 15625–15658. doi: 10.3390/ijms160715625

[49] Iqubal A., Sharma S, and Ansari M. A. (2019). Nerolidol attenuates cyclophosphamide-induced cardiac inflammation, apoptosis and fibrosis in Swiss Albino mice. *Eur J Pharmacol.* ;863:172666. doi: 10.1016/j.ejphar.2019.172666

[50] Kern J.C, and Kehrer J.P. Acrolein-induced cell death: a caspase-influenced decision between apoptosis and oncosis/necrosis. Chem Biol Interact 2002; 139: 79–9

[51] Yinhua, W., Zhaoling Z., Amit, J., and Opeyemi, J.O.(2021). Unraveling the protective effects of Emodin against cyclophosphamide induced gonadotoxicity in male Wistar rats. Drug Des DevelTher. 15; pp 4404-4411

[52] Nayak G, Rao A, and MullickP(2020). Ethanolic extract of Moringa oleifera leaves alleviate cyclophosphamide-induced testicular toxicity by improving endocrine function and modulating cell specific gene expression in mouse testis. *J Ethnopharmacol*.;259:112922. doi: 10.1016/j.jep.2020.112922

[53] Ekeleme-Egedigwe C.A., Famurewa A.C., David E.E, Eleazu C.O., and Egedigwe U.O. (2019). Antioxidant potential of garlic oil supplementation prevents cyclophosphamide-induced oxidative testicular damage and endocrine depletion in rats. J NutrIntermed Metab.;18:100109. doi: 10.1016/j.jnim.2020.100109

[54] Ghobadi E, Moloudizargari M, Asghari M.H, and AbdollahiM.(2017). The mechanisms of cyclophosphamide-induced testicular toxicity and the protective agents. *Expert Opin Drug Metab Toxicol*.;13:525–536. doi: 10.1080/17425255.2017.1277205

[55] Ozkok A, Kaymaz S, Elcioglu O, Bakan A, and Odabas A. (2012)Cyclophosphamide induced early-onset interstitial lung disease. CEN Case Reports, 1: 128 129.

[56] Shokrzadeh M, Chabra A, Ahmadi A, Naghshvar F, Habibi E, and Salehi F.(2015). Hepatoprotective effects of zatariamultifloraethanolic extract on liver toxicity induced by cyclophosphamide in mice. Drug Research, 65: 169-175.

[57] Khorwal G, Chauhan R, and Nagar M. (2017). Effect of cyclophosphamide on liver in albino rats: a comparative dose dependent histomorphological study. International Journal of Biomedical and Advance Research, 8(3):102–107.

[58] Motawi TM, Sadik NA, and Refaat A. (2010). Cytoprotective effectsof DL alpha-lipoic acid or squalene oncyclophosphamide-induced oxidative injury: anexperimental study on rat myocardium, testicles andurinary bladder. Food ChemToxicol, 48(8-9): 2326-2336.

[59] Rezvanfar MA, Sadrkhanlou RA, Ahmadi A, Shojaei-Sadee H, Rezvanfar MA, Mohammadirad A, Salehnia A, and Abdollahi M. (2008).Protection of cyclophosphamide-induced toxicity in reproductive tract histology, sperm characteristics, and DNA damage by an herbal source; evidence for role of free-radical toxic stress. Human & Experimental Toxicology. 27: 901–910.

[60] Turk G, Ceribasi AO, Sakin F, Sonmez M, and Atessahin A (2010). Antiperoxidative and anti-apoptotic effects of lycopene and ellagic acid on cyclophosphamide induced testicular lipid peroxidation and apoptosis. Reproduction, Fertility and Development, 22(4): 587-596.