Arch. Bas. App. Med.13 (2025) 54-60
www.archivesbamui.com
www.ojshostng.com/index.php/abam

Research Article

β-Caryophyllene Improves Sperm Quality and Protects Testicular Histology in Rats Exposed to 1,2-Dimethyl Hydrazine Via Reduction of Oxidative Stress

***Akinwumi K.A.[1], Eleyowo O.O. [1,2], Adegbola O.O.[1,3], Adebayo E.D.[1] and Nwabuenu J.C[1]**

[1]*Department of Chemical Sciences, Bells University of Technology, Ota, Nigeria*
[2]*Department of Chemical Sciences, Lagos State University of Science and Technology, Ikorodu, Nigeria*
[3]*Department of Science Laboratory Technology, Federal Polytechnic Ilaro, Ilaro, Ogun State Nigeria.*

Accepted: 15th February, 2025

Abstract

Male infertility is a growing problem worldwide. There are many risk factors, including cancers and cancer-causing agents. The 1,2-Dimethylhydrazine (DMH) is a cancer-causing agent that harms the reproductive system. This study examines the impact of β-caryophyllene (BCP), a molecule possessing antioxidant and anti-inflammatory characteristics in counteracting DMH-induced sperm quality alteration and testicular oxidative injury in a rat model. Thirty (30) Sprague Dawley rats were allocated into six (6) equal groups. They were administered distilled water, 20 mg/kg DMH, 5 mg/kg BCP, 10 mg/kg BCP, 5 mg/kg BCP + DMH, and 10 mg/kg BCP + DMH. The BCP was administered orally, while DMH was injected intraperitoneally throughout the 20 weeks of treatment. After treatment, sperm motility, viability, count, and total aberration were evaluated in test and control rats. The absolute and relative testis weights as well as testicular malondialdehyde (MDA), superoxide dismutase (SOD), and reduced glutathione (GSH) were determined. Histopathological evaluation of the testis was also carried out. Administration of DMH led to alteration of sperm quality parameters and oxidative stress as evidenced by elevation of MDA coupled with decline SOD activity and GSH level. Testicular germ cell depletion and atrophy was also seen in the DMH group. However, BCP restored the sperm quality parameters as well as MDA, SOD and GSH towards control values. Additionally, the testicular lesions were prevented by BCP. The data suggests that BCP mitigated DMH-induced sperm quality alteration and protected against testicular damage by suppressing oxidative stress.

Key Words: *1,2-Dimethylhydrazine, β-caryophyllene, testis, sperm quality parameters, oxidative stress, and antioxidant*

INTRODUCTION

Infertility is an escalating global issue, impacting 1 in 6 couples (Harris, 2023), with a notably greater incidence in certain locations, especially Africa (Cox *et al*., 2022). Infertility is the inability to attain a clinical pregnancy following a minimum of twelve months of consistent unprotected sex (WHO, 2019). The condition is not life-threatening, but it is frequently connected with physical, emotional, and psychological stress as well as socioeconomic burdens in affected couples (Thoma *et al*., 2021). In Africa, it is a major source of worry in affected couples mostly because of the adverse social impacts including, isolation, disinheritance, stigmatization, and even divorce in some cases (Roomaney, 2024). In many parts of the continent, it is erroneously believed that infertility issues are only attributable to the female. However, recent research from Africa and other parts of the world revealed that male factors contribute substantially to infertility (Igibah *et al*., 2023; Vander *et al*., 2018). Male factors encompass a variety of conditions including reproductive tract obstruction, endocrine disorders, testicular failure, and low sperm quality (Sharma *et al*., 2021). Various health challenges including infectious diseases, diabetes, and cancers are also known to increase the frequency of male infertility. In addition, exposure to some drugs and environmental agents negatively impacts male infertility through mechanisms such as endocrine disruption, and interference with the regulation of spermatogenesis (Walker *et al*., 2024; Wdowiak *et al*., 2024).

1,2-Dimethylhydrazine (DMH), a hydrazine derivative, is widely used in military, aviation, agriculture, and medical research sectors (Venkatachalam *et al*., 2020). It serves as a rocket propellant component. Human exposure primarily occurs occupationally through inhalation or dermal contact among aerospace and chemical industry workers (International Agency for Research on Cancer, 2001). It may be unintentionally encountered in contaminated food and environmental sources due to industrial activities. The DMH is used to induce colon cancer in experimental animals. Mice and rats that were injected with DMH developed tumors of the colon and rectum. In addition, rats with DMH-induced colon cancer exhibited perturbation of sperm quality and testicular damage (Freitas *et al*., 2013). Moreover, DMH-induced tissue damage is associated with oxidative stress

**Author for Correspondence:* Tel: +2348035639816

E-mail: *qaakinwumi@yahoo.co.uk*

(Hassan *et al*., 2023; Allal *et al*., 2023). Relieving oxidative stress may be a strategy for protecting the testes against DMH-induced toxicity. Dietary compounds with antioxidant values are currently being utilized as a measure against free radicals and oxidative stress-mediated male infertility in human and experimental models (Kaltsas, 2023).

β-caryophyllene (BCP), a sesquiterpene, is currently receiving attention because of its valuable medicinal properties, including antigenotoxic, anticancer, and antioxidant properties. The multi-faceted phytocannabinoid is found in large quantity in essential oils of many edible and nutritional plant including spices like cloves, black pepper, basil, hops, breakfast mint, thyme, oregano, rosemary, coriander, curry leaves and *Cannabis sativa* (Jha *et al*., 2021). The BCP received FDA approval as a food additive, after it was deemed generally safe (He *et al*., 2021). Previous studies have demonstrated the anti-inflammatory, anti-arthritic, anticancer, antibacterial, cardioprotective, and anticholinesterase effects in cultured cells and rodents (Klauke, 2014). Recent studies have demonstrated the modulatory and pharmacological effects of BCP on various organ pathologies, including the liver, kidney, brain, and reproductive organs (Amorati *et al*., 2023). In addition, it was recently shown that BCP protected against the toxicity of some environmental toxicants in reproductive organs (Espinosa-Ahedo *et al*., 2022). However, information is scarce on its effect on DMH-induced toxicity in the testes. Consequently, the current investigation sought to ascertain the protective influence of BCP against the detrimental effects of DMH on sperm quality indices, testicular oxidant-antioxidant balance and histology.

MATERIALS AND METHODS

Chemicals: The β-caryophyllene (BCP), 1,2-Dimethylhydrazine, 2-thiobarbituric acid, epinephrine, and reduced glutathione were purchased from Sigma-Aldrich (St. Louis, MO, USA). Other chemicals and reagents used for the experiments were of analytical grade.

Animal and Treatment: Thirty adult male Sprague Dawley rats that weighs ranging from100-130g were obtained from the Central Animal House at the Faculty of Basic Medical Sciences, University of Ibadan, Ibadan, Nigeria. The rats were kept and acclimatized at the vivarium of the Bells University of Technology for one week as documented by Obernier and Baldwin (2006) before the commencement of treatment. They were housed in well-ventilated polystyrene cages maintained at standard atmospheric temperature and humidity, with a 12-hour light/dark cycle. During the experimental period, the rats were given free access to water and a rat standard feed obtained from Hybrid Feeds Limited (Ibadan, Oyo State, Nigeria). All animal procedures were done following established guidelines (National Research Council, 2011), and ethical approval (FMCA/470/HREC/01/2023/12) was obtained from the Federal Medical Centre, Abeokuta before the commencement of the study.

After the period of acclimatization, all the experimental rats were healthy and selected for the experiment. They were randomly assigned into six equal groups such that the difference between the average weight of the groups did not exceed 0.5 g. The animal received the following treatments: distilled water (control group); 5 mg/kg BCP; 10 mg/kg BCP; 20 mg/kg DMH; 5 mg/kg BCP + DMH and 10 mg/kg BCP + DMH. The two doses of BCP were selected based on previous literature (Ceccarelli *et al*., 2020; Gertsch *et al*., 2008) and were orally administered once daily for twenty weeks. The DMH was dissolved and administered subcutaneously once a week at a dose of 20 mg/kg as earlier reported (Abdel-Rasol *et al*., 2022). A day following the last administration of test and control substances, rats were weighed before anesthesia with 45 mg/kg ketamine and 5mg/kg xylazine recently described (Akinwumi *et al*., 2024). Subsequently, the rats were sacrificed by cervical dislocation and the left and right epididymis and testes were removed following laparotomy.

Determination of Relative Testicular Weight: The fatty tissues around the testes of each rat were trimmed before the paired testes were weighed. Subsequently, the testicular-somatic index was evaluated using the formula:

RTW: TW/BW *100
Where RTW: relative testes weight
TW: paired testes' weight
BW: final body weight

Determination of Sperm Parameters: The right caudal epididymis was excised, placed in 1 ml normal saline, and incised to release spermatozoa. The suspension was incubated at 37 °C for fifteen to liquefy and used for assessing motility, viability, and morphology. Motility was evaluated within few minutes after liquefaction. Exactly 10 µL of the suspension was mixed with two drops of pre-warmed 2.9% sodium citrate on a slide, covered with a cover slip, and examined under a light microscope at ×40 magnification. Progressive motility was scored across four fields and averaged.

Sperm viability was evaluated using the eosin-nigrosin staining method (Moskovtsev and Librach, 2013). Fifty microlitres of liquefied semen was immediately mixed with an equal volume of eosin–nigrosin, incubated for 30 seconds, air-dried on slides and examined under ×100 magnification. Dead spermatozoa appeared pink or red, while live cells remained unstained. Viability was calculated from 200 spermatozoa per rat as previously reported (Akinwumi *et al*., 2021). The sperm count was assessed following WHO guidelines (2021). Liquefied sperm from the left epididymis was diluted 1:20 with a diluent (5% sodium hydrogen carbonate, 0.35% formaldehyde, 0.25% trypan blue). Ten microlitres of the mixture was loaded into an improved Neubauer hemocytometer, settled for 15 minutes, and counted at ×40 magnification. Morphological abnormalities were evaluated as described by Narayana *et al*. (2002) with minor modifications. The left epididymis was crushed in 1 ml saline, filtered through an 80 µm nylon mesh, stained with 1% Eosin-Y, and incubated for 40 minutes. A 10 µL aliquot was air-dried on a pre-labelled slide and examined at ×100 magnification (Akinwumi *et al*., 2021). Morphological defects were scored in 500 spermatozoa per animal.

Determination of Malondialdehyde and Endogenous Antioxidants: The right testis of each rat was immediately homogenized in 10X its volume in a cold Tris-HCl (pH=7.4)

buffer using a Teflon pestle fitted to a homogenizer. Homogenates were spun and preserved at -20ºC as in our previous report (Akinwumi *et al*., 2021). The supernatant from each testis was separated and utilized for the spectrophotometric measurement of oxidative stress indices. The level of testicular MDA was determined in the supernatant according to the method previously established by Estabeur and Cheeseman (1990), while SOD was measured following the method Sun and Zigma (1978). GSH was determined using Elliman's reagent as earlier described by Beutler *et al*. (1963).

Testicular Histological Evaluation: Testicular histology was performed as earlier described (Akinwumi *et al*., 2021) with minor modification. Briefly, the left testis was lightly nicked before being lightly fixed in Bouin's reagent. Following fixation, testes underwent ethanol serial dehydration before being cleared with xylene. The tissues were paraffin-embedded and cut into 3-4 µm sections. Sections were mounted on slides and stained with Hematoxylin and Eosin. A qualified pathologist who was blinded to the treatment used light microscopy to analyze and score the stained tissues.

Statistical Analysis: Data were represented as the mean ± standard error of the mean. The differences between means were tested for statistical significance using one-way analysis of variance (ANOVA) in the 17th version of SPSS, while the difference between test and control groups was evaluated using the Duncan Multiple Range Test. The p-value was deemed significant $p < 0.05$.

RESULTS

Absolute and Relative Testis Weight: The result of the testis weight (TWT) and relative testis weight (RTW) is shown in Figure 1. The values of TWT and RTW were markedly ($p< 0.05$) reduced in the group injected with DMH alone compared to the control. In contrast, BCP increased TWT and RTW towards control values. The groups treated with either of the two doses of BCP alone had similar TWT and RTW values with the control.

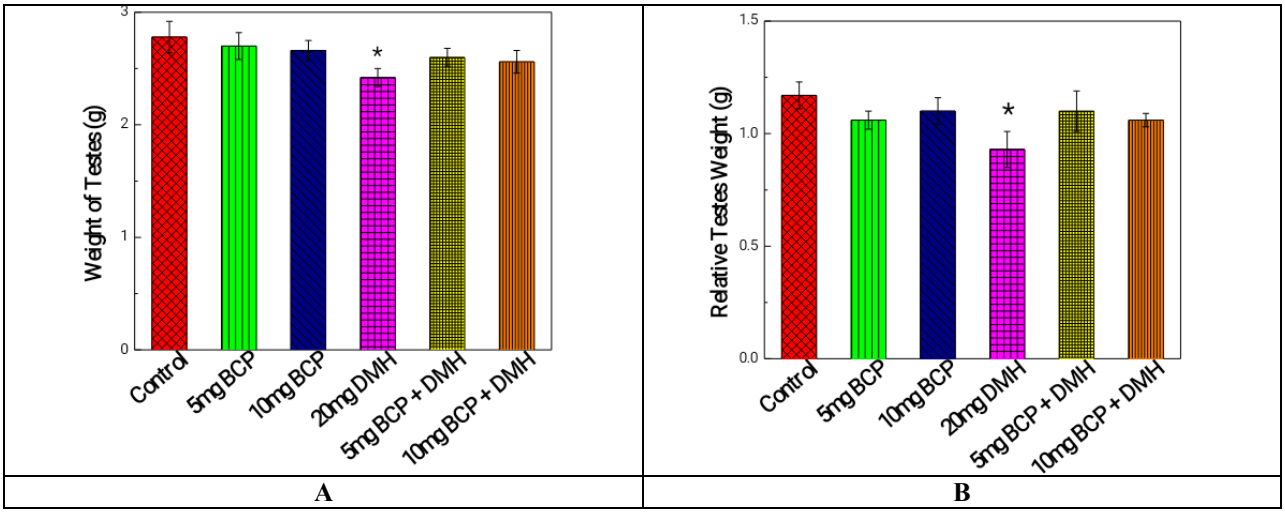

Figure 1:
The effect of BCP on DMH-induced reduction of absolute and relative testicular weight. BCP: β-caryophyllene, DMH: 1,2 dimethyl hydrazine. *Significantly different from control.

Sperm Quality Indices: The effect of BCP on sperm quality measures in rats administered DMH is presented in Figure 2. Administration of DMH markedly ($p <0.05$) decreased sperm motility and count compared to values obtained for the control. It also significantly increased the morphological aberration in comparison with the control. Supplementation with BCP reversed the trends in sperm quality parameters by increasing motility and counts, while morphological aberration was significantly ($p<0.05$) decreased. The three parameters were similar to control values in the rats treated with either of the two doses of BCP alone. Viability values were identical across all the groups.

Lipid Peroxidation and Endogenous Antioxidants: The effects of DMH on testicular MDA, SOD, and GSH with or without the two doses of BCP are shown in Fig. 3. The DMH alone group showed a significant ($p < 0.05$) 2.4-fold increase in MDA levels in comparison with the control. Conversely, the testicular MDA was significantly ($p<0.05$) reduced dose-dependently by simultaneous administration of BCP. The MDA levels in the groups that received only one of the two BCP dosages were comparable to the control. Testicular SOD and GSH activities were significantly ($p <0.05$) diminished following DMH administration compared to the control value. Simultaneous exposure to either of the two doses of BCP enhanced SOD activity and GSH levels in the testes and returned them to near-control values. When either of the two doses of BCP were administered simultaneously with DMH, testicular SOD and GSH increased and approached control values. The SOD activity in the rats that were treated with BCP doses alone was comparable to the control. A similar trend was also observed for GSH.

Testicular Histopathology
The impact of BCP and DMH on the testicular architecture in rats is presented in Plate 1 (A-F). The testes of the control rats displayed normal testicular structure (A). A similar observation was made in the BCP groups (B and C). The group treated with 20 mg/kg DMH however, showed germ cell depletion (red arrow) and atrophy of tubules (blue arrow) (D). In contrast, rats in the combined exposure groups (E and F) showed preserved testicular architecture.

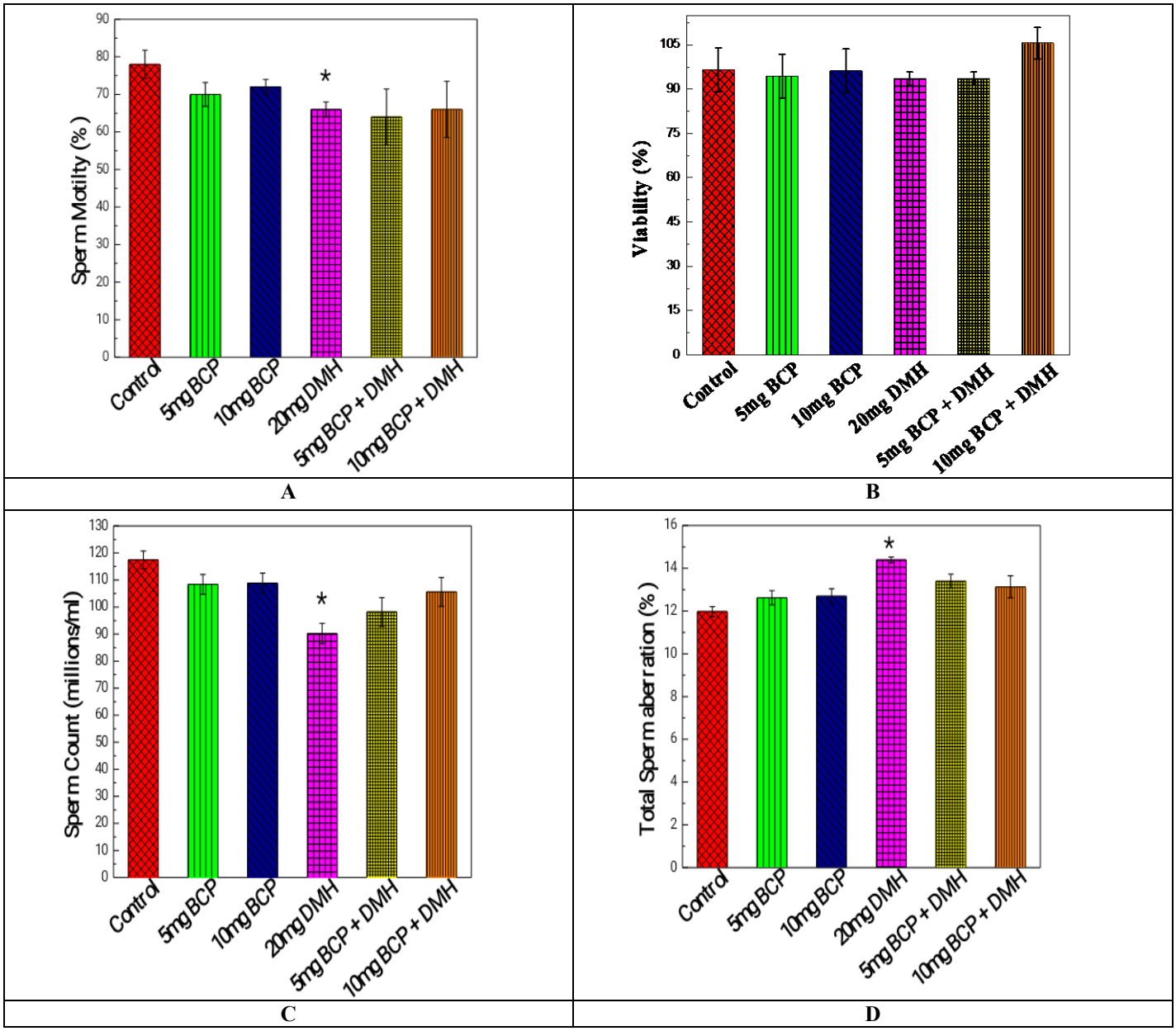

Figure 2:
The impact of BCP on DMH-induced induced alteration of sperm quality indices in rats. BCP: β-caryophyllene, DMH: 1,2 dimethyl hydrazine. *Significantly different from control

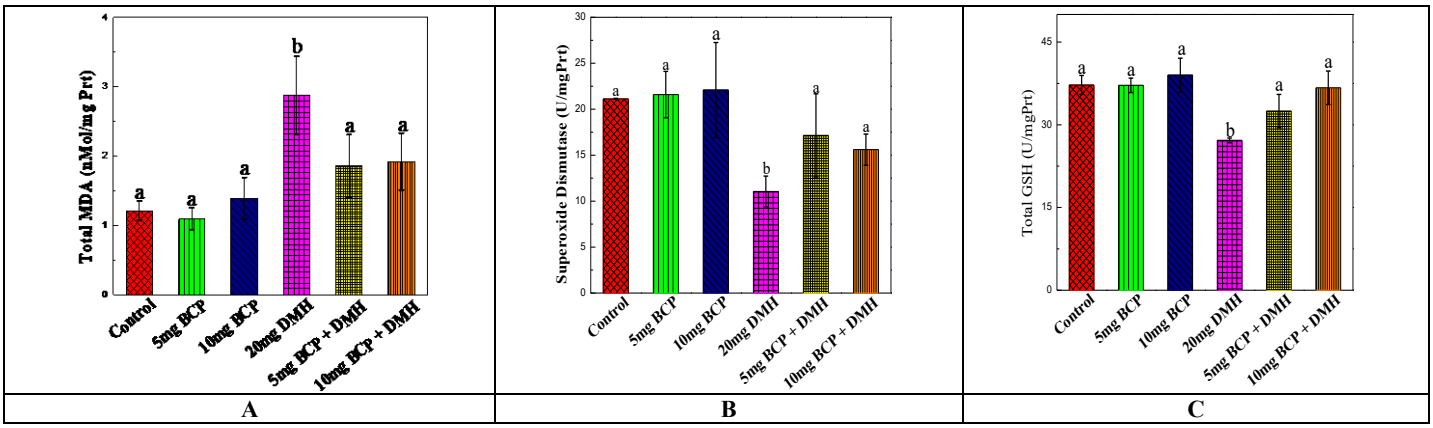

Figure 3:
The effect of BCP on DMH-induced disturbance of some oxidative stress parameters in rats. BCP: β-caryophyllene, DMH: 1,2 dimethyl hydrazine. Values with the same alphabet are similar

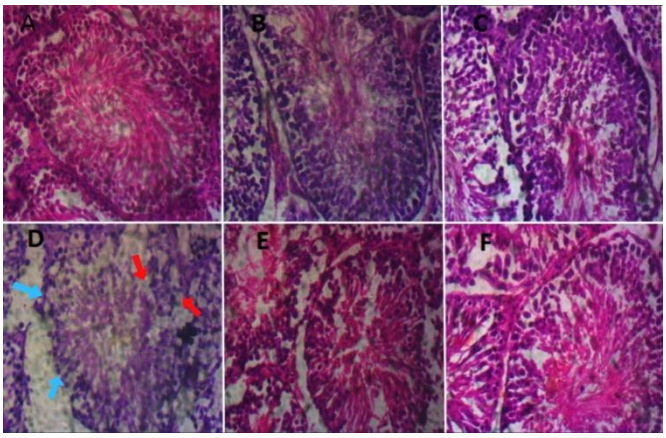

Plate 1:
Prevention of DMH-induced testicular degeneration and atrophy by BCP in rats. (A) Control: No visible Lesion; (B) 5 mg/kg BCP No visible lesion; (C) 10 mg/kg BCP: No visible Lesion; (D) DMH: germ cell depletion (red arrow) and tubular atrophy (blue arrow); (E) 5 mg/kg BCP + DMH: No visible Lesion.; (F) 10 mg/kg BCP + DMH: No visible Lesion

DISCUSSION

The BCP has various beneficial pharmacological effects including, anticancer, anti-inflammatory, and antioxidant properties. The capacity of BCP to prevent DMH-induced alteration in sperm quality and its deleterious effect on testicular histology was investigated in this study. The results showed that BCP improved sperm quality and protected the testes against damage caused by DMH exposure.

The reduction in actual and relative testicular weight in the DMH-treated group is suggestive of testicular toxicity. A comparable drop in testes weight was reported in a previous study (Freitas *et al*., 2013). It has been documented that DMH reduction of testis weight might cause a reduction in testicular parenchyma weight (Freitas *et al*., 2013). Alteration of testicular parenchyma is a vital marker of testicular function in testosterone production and spermatogenesis (Spaggiari *et al*., 2020). In addition, the decreased testis weight after DMH administration is suggestive of germinal depletion and cellular constriction. This would reduce secretory activities in the testes and could have an adverse impact on the quality of sperm and male fertility. In the current study, DMH- induced perturbation of sperm quality was confirmed by a reduction of motility and sperm count together with increased sperm morphological alteration. Rats injected with DMH exhibited reduced seminiferous tubular volume and length (Freitas *et al*., 2013), which can negatively impact spermatogenesis and could have contributed to the reduction of semen quality observed in the DMH group.

Although the mechanism by which DMH induces toxicity in the testes and sperm quality is poorly understood, its metabolism results in DNA methylation, and induction of oxidative stress in tissues (Spencer and Kisby, 2021). Moreover, the mammalian testis is oxidant-sensitive and susceptible to oxidative damage. In the present investigation, DMH increased testicular lipid peroxidation was demonstrated by elevated testicular malondialdehyde. Raised lipid peroxidation modifies membrane fluidity and permeability, perhaps resulting in diminished sperm movement and male infertility (Mannucci *et al*., 2022). Additionally, the raised lipid peroxidation was accompanied by a reduction of GSH and SOD in the DMH-treated group. Reduced levels of GSH and SOD observed herein were consistent with earlier findings in other tissue of experimental models (Afzal *et al*., 2021; Caetano *et al*., 2020). The SOD is a crucial antioxidant involved in the direct removal of superoxide anion, while GSH is a sulfhydryl donor and cellular reductant that is used in neutralizing many oxidants including ROS, and detoxifying several toxicants. Reduction of both antioxidants could therefore further expose the testes to the damaging effect of free radicals and lipid peroxidation. The reduction of both antioxidants could be due to their expenditure in counteracting free and/ or suppressing excessive free radical production. The increment in lipid peroxidation and reduction of SOD and GSH is indicative of oxidative stress in the testes of DMH-treated rats, which could negatively impact its histoarchitecture and output. In the current study, the disruption was manifested by the degeneration of germinal cells and seminiferous tubular atrophy in the DMH-treated. A similar observation was noted in an earlier study (Freitas *et al*., 2013). The viability of germ cells is critically dependent on the synchronized operation of diverse cellular components and processes within the testicular environment. Any disruption to this delicate ecosystem typically leads to apoptosis of germ cells, and their subsequent expulsion into the seminiferous tubule lumen (Mao *et al*., 2018). Moreover, oxidation of the membrane lipid matrix of spermatozoa reduces sperm quality (Alahmar *et al*., 2019) as also obtained in this study.

However, the BCP seemed to counter the adverse effects of DMH on testes and sperm quality by improving the testicular weight and relative testicular weight as well as sperm quality parameters toward the value of the control. The augmentation of testicular weight and relative testicular weight may indicate a reduction of testicular cell loss and improvement of testicular secretory activities. Moreover, simultaneous administration of BCP remarkably relieved DMH-induced testicular oxidative stress by reducing malondialdehyde levels and augmenting SOD activity as well as GSH concentration in the testes. Thus, suggesting the alleviation of oxidative stress and restoration of redox balance are important mechanisms underlying the protection offered by BCP against DMH in the testes. Similar antioxidant action was displayed by BCP against other toxicants in recent studies (Espinosa-Ahedo *et al*., 2023; Refaat and El-Boshy, 2022; Da Silveira *et al*., 2021). The potent scavenging action of BCP against free radicals (Yovas *et al*., 2023) may be responsible for the observed action. The BCP has double bonds in its structure that permit the incorporation of free radicals that could prevent the breakdown of the prooxidant/antioxidant balance in tissues (Villalobos-Gutierrez *et al*., 2022). Apart from its antioxidant properties, BCP exhibits selective CB2R activity and rat's testis is rich in CB2R (Brown *et al*., 2002). Activation of CB2R by BCP in the testis could promote germ cell differentiation and compensate for cellular disruption encountered during DMH intoxication. Furthermore, the CB2R controls Sertoli cell survival and exerts protection against apoptosis with a favorable effect on the spermatogenic output (Maccarrone, 2008). Moreover, the absence of testicular lesions in the co-exposed rats further corroborates the beneficial effects of

BCP in countering the deleterious effects of DMH in the testes.

CONCLUSION

Overall, our research showed that 1,2-dimethyl hydrazine deteriorated sperm quality and induced oxidative damage in the testes. However, these adverse effects were reversed by concurrently administration BCP through its antioxidant action.

REFERENCES

Abdel-Rasol, M.A., N.M. El-Beih, S.S. Yahya, and W.M. El-Sayed. 2022. The antitumor activity of ginger against colorectal cancer induced by dimethylhydrazine in rats. *Anti-Cancer Agents in Medicinal Chemistry*. 22(8):1601-1610.

Afzal, S.M., A. Vafa, S. Rashid, P. Barnwal, A. Shahid, A. Shree, J. Islam, N. Ali, and S. Sultana. 2021. Protective effect of hesperidin against N,N′-dimethylhydrazine induced oxidative stress, inflammation, and apoptotic response in the colon of Wistar rats. *Environmental Toxicology.* 36(4):642-653.

Akinwumi, K.A., O.O. Eleyowo, A.A. Adesina, T.A. Jarikre, E.D. Adebayo, and Z.A. Oyekola. 2024. Safety evaluation of Avicenna germinans leaf extracts in rats. *Phytomedicine Plus.* 4(4):100629.

Akinwumi, K.A., O.O. Osifeso, A.J. Jubril, J. Omirinde, and J.A. Aboyewa. 2021. Alteration of hematological profile and nephro-testicular damage in rats exposed to coatings of mobile charge cards. *Scientific African*. 13:e0095.

Alahmar, A.T. 2019. Role of oxidative stress in male infertility: an updated review. *Journal of Human Reproductive Sciences.* 12(1):4-18.

Allal, B.E., A. Bounaama, D. Silva, *et al*. 2023. Long-term 1,2-dimethylhydrazine triggers pathological remodeling of colon mucosa through repression of sestrin2, nuclear factor (erythroid-derived 2)-like 2, and sirtuin4 stimulating mitochondrial stress and metabolic reprogramming. *Naunyn-Schmiedeberg's Archives of Pharmacology.* 396:1291-1307. https://doi.org/10.1007/s00210-023-02403-x

Amorati, R., D.P. de Sousa, R.O.S. Damasceno, H.A. Elshabrawy, R.D. de Castro, D.P. Bezerra, V.R.V. Nunes, R.C. Gomes, and T.C. Lima. 2023. Essential oils: Chemistry and pharmacological activities. *Biomolecule*s. 13(7). https://doi.org/10.3390/biom13071144

Beers, R.F., Jr., and I.W. Sizer. 1995. Sulfide inhibition of catalase. *Science* (New York, N.Y.) 120(3105):32-33.

Beutler, E., O. Duron, and B.M. Kelly. 1963. An improved method for the detection of blood glutathione. *Journal of Laboratory and Clinical Medicine*. 61:882-888.

Brown, S.M., J. Wager-Miller, and K. Mackie. 2002. Cloning and molecular characterization of the rat CB2 cannabinoid receptor. *Biochimica et Biophysica Acta*. 1576:255-264. https://doi.org/10.1016/s0167-4781(02)00341-x.

Caetano, B.F., M.B. Tablas, G.R. Romualdo, M.A. Rodrigues, and L.F. Barbisan. 2020. Early molecular events associated with liver and colon sub-acute responses to 1,2-dimethylhydrazine: Potential implications on preneoplastic and neoplastic lesion development. *Toxicology Letters*. 329:67-79.

Ceccarelli, I., P. Paolo, F. Pessina, J. Pinassi, M. Aglianò, V. Miragliotta, and A.M. Aloisi. 2020. The CB2 agonist β-caryophyllene in male and female rats exposed to a model of persistent inflammatory pain. *Frontiers in Neuroscience*. 14:850. doi: 10.3389/FNINS.2020.00850

Cox, C.M., M.E. Thoma, N. Tchangalova, G. Mburu, M.J. Bornstein, C.L. Johnson, and J. Kiarie. 2022. Infertility prevalence and the methods of estimation from 1990 to 2021: a systematic review and meta-analysis. *Human Reproduction* Open. 2022(4):051. https://doi.org/10.1093/hropen/hoac051.

Da Silveira, A.R., É.V. Rosa, M.H. Sari, T.B. Sampaio, J.T. Dos Santos, N.S. Jardim, S.G. Müller, M.S. Oliveira, C.W. Nogueira, and A.F. Furian. 2021. Therapeutic potential of beta-caryophyllene against aflatoxin B1-induced liver toxicity: *Biochemical and molecular insights in rats. Chemico-Biological Interactions*. 348:109635. https://doi.org/10.1016/j.cbi.2021.109635.

Espinosa-Ahedo, B.A., E. Madrigal-Bujaidar, M. Sánchez-Gutiérrez, J.A. Izquierdo-Vega, J.A. Morales-González, E.O. Madrigal-Santillán, and I. Álvarez-González. 2022. Potential protective effect of beta-caryophyllene against cadmium chloride-induced damage to the male reproductive system in mouse. *Reproductive Toxicology.* (Elmsford, N.Y.) 110:19-30. https://doi.org/10.1016/j.reprotox.2022.03.008

Esterbauer, H., Cheeseman, K.H. 1990. Determination of aldehydic lipid peroxidation products, malondialdehyde and 4-hydroxynonenal. *Method of Enzymology*. 186: 407-421

Freitas, K.M. de, A.J. Natali, W. Lunz, K.L.C. Costa, R.P. Leite, H. Dolder, and S.L.P. da Matta. 2013. Colon cancer and swimming exercise: Effect on Wistar rat testes. *Brazilian Archives of Biology and Technology*. 56(4):557-566.

Gertsch, J., M. Leonti, S. Raduner, I. Racz, J.Z. Chen, X.Q. Xie, K.H. Altmann, M. Karsak, and A. Zimmer. 2008. Beta-caryophyllene is a dietary cannabinoid. Proceedings of the *National Academy of Sciences of the United States of America.* 105:9099-9104.

Harris, E. 2023. Infertility affects 1 in 6 people globally. *Journal of the American Medical Association*. 329(17):1443.

Hassan, A.K., A.M. El-Kalaawy, A. El-Twab, M. Sanaa, M.A. Alblihed, and O.M. Ahmed. 2023. Hesperetin and capecitabine abate 1,2-dimethylhydrazine-induced colon carcinogenesis in Wistar rats via suppressing oxidative stress and enhancing antioxidant, anti-inflammatory, and apoptotic actions. *Life*. 13(4):984.

He, X.H., E. Galaj, G.H. Bi, Y. He, B. Hempel, Y.L. Wang, E.L. Gardner, and Z.X. Xi. 2021. β-Caryophyllene, an FDA-approved food additive, inhibits methamphetamine-taking and methamphetamine-seeking behaviors possibly via CB2 and non-CB2 receptor mechanisms. *Frontiers in Pharmacology*. 12:722476.

IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Volume 71. (2001). Hydrazine and Hydrazine Salts. Lyon, France.

Igibah, C.O., J. Okoeguale, S.C. Amadi, T.N. Ilenbodiaye, F. Erah, A.N. Okocha, M. Abbey, P.L. Kua, C.N. Ononuju, and O. Adewale. 2023. Abnormalities of semen parameters among male partners of infertile couples in a sub-urban tertiary hospital in Nigeria. *International Journal of Reproduction, Contraception, Obstetrics and Gynecology*. 12(10):2900-2905.

Jha, N.K., C. Sharma, H.M. Hashiesh, S. Arunachalam, M.N. Meeran, H. Javed, C.R. Patil, S.N. Goyal, and S. Ojha.

2021. β-Caryophyllene, a natural dietary CB2 receptor selective cannabinoid, can be a candidate to target the trinity of infection, immunity, and inflammation in COVID-19. *Frontiers in Pharmacology*. 12:590201.

Kaltsas, A. 2023. Oxidative stress and male infertility: The protective role of antioxidants. *Medicina.* 59:1769. https://doi.org/10.3390/medicina59101769

Klauke, A.L., I. Racz, B. Pradier, A. Markert, A.M. Zimmer, J. Gertsch, and A. Zimmer. 2014. The cannabinoid CB2 receptor-selective phytocannabinoid beta-caryophyllene exerts analgesic effects in mouse models of inflammatory and neuropathic pain. *European Neuropsychopharmacology*. 24(4):608-620.

Maccarrone, M. 2008. CB2 receptors in reproduction. *Br. J. Pharmacol*. 153(2):189-198.

Mannucci, A., F.R. Argento, E. Fini, M.E. Coccia, N. Taddei, M. Becatti, and C. Fiorillo. 2022. The impact of oxidative stress in male infertility. *Frontiers in Molecular Biosciences*.8:799294.https://doi.org/10.3389/fmolb.2021. 799294.

Mao, B.P., L. Li, M. Yan, Q. Lian, R. Ge, and C.Y. Cheng. 2018. Environmental toxicants and cell polarity in the testis. *Reproductive Toxicology*. 81:253-258.

Moskovtsev, S.I., and C.L. Librach. 2013. Methods of sperm vitality assessment. In: Spermatogenesis: *Methods and Protocols*. 13-19.

Narayana, K., U.J. D'Souza, and K.S. Rao. 2002. Ribavirin-induced sperm shape abnormalities in Wistar rat. *Mutation Research / Genetic Toxicology and Environmental Mutagenesis*. 513(1-2):193-196.

National Research Council. 2011. Guide for the care and use of laboratory animals, 8th ed. Washington, USA: National Academies Press.

Obernier, J. A., and Baldwin, R. L. 2006. Establishing an appropriate period of acclimatization following transportation of laboratory animals. *Institute for Laboratory Animal Research Journal.* 47(4), 364-369.

Refaat, B., and M. El-Boshy. 2022. Protective antioxidative and anti-inflammatory actions of β-caryophyllene against sulfasalazine-induced nephrotoxicity in rats. *Experimental Biology and Medicine.* 247(8):691-699.

Roomaney, R., M. Salie, D. Jenkins, C. Eder, M.J. Mutumba-Nakalembe, C. Volks, N. Holland, and K. Silingile. 2024. A scoping review of the psychosocial aspects of infertility in African countries. *Reproductive Health*. 21(1):123.

Sharma, A., S. Minhas, W.S. Dhillo, and C.N. Jayasena. 2021. Male infertility due to testicular disorders. *The Journal of Clinical Endocrinology & Metabolism.* 106(2):442-459.

Spaggiari, G., M. Granata, A.R. Santi. 2020. Testicular ultrasound inhomogeneity is an informative parameter for fertility evaluation. *Asian Journal of Andrology*. 22(3):302-308.

Spencer, P.S., and G.E. Kisby. 2021. Role of hydrazine-related chemicals in cancer and neurodegenerative disease. *Chemical Research in Toxicology*. 34(9):1953-1969.

Sun, M., and S. Zigma. 1978. An improved spectrophotometric assay of superoxide dismutase based on epinephrine antioxidation. *Analytical Biochemistry*. 90:81-89.

Thoma, M., J. Fledderjohann, C. Cox, and R. Kantum Adageba. 2021. Biological and social aspects of human infertility: A global perspective. Oxford Research Encyclopedia of Global Public Health. Oxford University Press.

Vander, B.M., and C. Wyns. 2018. Fertility and infertility: Definition and epidemiology. *Clinical Biochemistry*. 62:2-10.

Villalobos-Gutierrez, P.T., M. de Jesus Romero-Hernandez, and O.G. Coronado. 2022. Evaluation of the effect of β-caryophyllene on oxidative stress in BALB/c mice with streptozotocin-induced diabetes. Metabolism. 128.

Venkatachalam, K.., Vinayagam, R., Arokia Vijaya Anand, M., Isa, N. M., and Ponnaiyan, R. 2020. Biochemical and molecular aspects of 1, 2-dimethylhydrazine (DMH)-induced colon carcinogenesis: a review. *Toxicological Research.* 9(1): 2-18.

Walker, T. 2024. Progress in the study of toxic effects of drugs on the male reproductive system. *Asia Pacific Journal of Pharmacotherapy and Toxicology.* 4:76-84. https://doi.org/10.32948/ajpt.2024.11.0

Wdowiak, N., K. Wójtowicz, A. Wdowiak-Filip, W. Pucek, A. Wróbel, J. Wróbel, and A. Wdowiak. 2024. Environmental factors as the main hormonal disruptors of male fertility. *Journal of Clinical Medicine*. 13(7):1986

World Health Organization. 2019. International Statistical Classification of Diseases and Related Health Problems 10th Revision, Geneva: *World Health Organization*. https://icd.who.int/browse10/2019/en#/N97 (last accessed December 4, 2024).

World Health Organization. 2021. WHO laboratory manual for the examination and processing of human semen. World Health Organization.

Yovas, A., S.P. Stanely, R. Issac, and S.M. Ponnian. 2023. β-caryophyllene blocks reactive oxygen species-mediated hyperlipidemia in isoproterenol-induced myocardial infarcted rats. *European Journal of Pharmacology*. 960:176102.