Novelty Journals

ISSN 2394-966X

International Journal of Novel Research in Life Sciences
Vol. 11, Issue 2, pp: (31-37), Month: March - April 2024, Available at: www.noveltyjournals.com

CURATIVE EFFECT OF CUCURBITA MAXIMA ON PARACETAMOL-INDUCED TESTICULAR INJURY IN ADULT WISTAR RATS

Ifeanacho Ezeteonu Abireh[1], Chiadikobi Lawrence Ozoemena [1], Christian Ejuiwa Mba[1]

[1] Department of Anatomy, Faculty of Basic Medical Sciences, Enugu State University of Science and Technology, Enugu, Nigeria.

[1] Department of Anatomy, Faculty of Basic Medical Sciences, Enugu State University of Science and Technology

AUTHOR OF CORRESPONDENCE: Mba, Christian Ejuiwa

DOI: https://doi.org/10.5281/zenodo.11091487

Published Date: 30-April-2024

Abstract: **Curcubita Maxima plant is widely cultivated from the warm temperate zone to the tropics, mainly for its edible fruit but also for its leaves and seed plus its various medicinal uses and the seeds are diuretic, tonic and vermifuge. This study investigated the curative effect of Curcubita maxima leaf extract on Paracetamol-induced testicular injury. Twenty-four male Wistar rats were grouped into 6 groups with 4 rats in each group. Group 1 received oral normal saline 0.5ml daily throughout the period of the experiment. Groups 2, 3, 4, 5, and 6 received oral Paracetamol 300mg/kg daily from day 1-14. Then from day 15-21, groups 3, 4 and 5 received oral Cucurbita maxima leaf extract 25mg/kg, 50mg/kg and 75mg/kg, respectively, while group 6 received oral Vitamin E 500iu/kg. The Wistar rats were sacrificed following intraperitoneal injection of Thiopentone 50mg/kg. The testes were stained with Haematoxylin and Eosin stain and analyzed using light microscope (X100). The study observed that the testis of group 2 which received Paracetamol showed necrotic interstitial cells and shrunken seminiferous tubules with few luminal spermatozoa in the lumen, while groups 3, 4 and 5 that received Cucurbita maxima leaf extract at different doses showed moderate restoration of the histoarchitexture of interstitial cells and the seminiferous tubule. This study concludes that Cucurbita maxima leaf has the potential to restore the histoarchitexture of the testis following testicular injury caused by Paracetamol overdose.**

Keywords: **Paracetamol, Cucurbita maxima, Testicular injury, Testis, plant, histoarchitecture.**

1. INTRODUCTION

Paracetamol is an analgesic and antipyretic medication which can be procured as an over the counter drug, requiring no prescription by the physician. It is used to treat pain that is not severe, such as headache and sprain, and can also be used to treat patients with fever.[1]

The exact mechanism of action of paracetamol is not completely understood. It was thought to cause the inhibition of Cyclo-oxygenase-2,[2] but current evidence available shows that Paracetamol which is also known as Acetominophen (N-acetyl-p-

aminophenol) acts through several mechanisms within the central nervous system, such as the serotonergic, nitric oxide and the endocannabinoid pathways. P-aminophenol which is the product of deacetylation of Paracetamol passes into the brain by crossing the blood brain barrier and subsequently converted to N-arachidonoylphenolamine by action of Amide hydrolase. N-arachidonoylphenolamine then targets the calcium channels, cannabinoid receptors and TRPV1 receptors and other receptors through which it induces the analgesic and antipyretic effects. The activation of suprasinal TRPV1 receptors and the serotonergic receptors by N-arachidonoylphenolamine leads to antinociception and pain relief.[3]

Paracetamol is safe when taken at prescribed dosage and duration, but tends to cause adverse effect on several organs and systems of the body when overdose is taken or when duration is exceeded. Such side effects include hypertension, Reye's syndrome, asthma, gastrointestinal bleeding, hepatotoxicity, acute kidney injury, analgesic nephropathy, neurodevelopmental disorders, cryptorchidism, testicular dysgenesis syndrome with accompanying low sperm count and testicular germ cell cancers and reduced plasma testosterone level.[2]

The abuse of over the counter analgesic is common in our environment due to the fact that the majority of the people are of the low socioeconomic group. They are mostly illiterates and are involved in menial jobs, with the need to relieve pain always after such jobs. This abuse may lead to overdose and severe adverse effects.[4] The adverse effect of paracetamol on the testis such as reduced plasma testosterone level and low sperm count can result from the destruction of the seminiferous tubule of the testis, and the destruction of the seminiferous tubules is associated with testicular atrophy and infertility.[5]

The Cucurbita maxima is rich in flavonoids, tocopherols and other compounds such as Omega 3 and Omega 6, and has shown antioxidant and antimicrobial effects.[6] It also has antitumour and antidepressant effects, and has also been used to treat diseases such as benign prostatic hyperplasia.[7]

The aim of this study was to investigate the possible potential of the Cucurbita maxima leaf extract to cure the testicular injury caused by ingestion of paracetamol in Wistar rat.

2. MATERIALS AND METHODS

Preparation of extract

The extract of Cucurbita maxima leaves was prepared by removing the stalks, washed with distilled water and then dried at room temperature. The dried leaves were then grinded to powder form using grinding machine. The grinded leaves powder was then sieved to removed ungrounded fibers. The sieved powdered form was then extracted, 1000g at a time. The aqueous extract was then filtered using Whatman No. 2 filter paper and evaporated to concentrate form using an evaporator that was set at 40°C.[8]

Procurement of materials

Paracetamol was procured from the Pharmacy section of Enugu State University Teaching Hospital.

The Wistar rats were procured from the Animal House, College of Medicine, Enugu State University of Science and Technology.

Animal Handling and grouping

Twenty-four (24) male Wistar rats with average weight of 140g were procured for the experiment. The rats were then grouped into six (6) groups of four (4) rats each and housed in netted iron cages. They were allowed 12-hour light and dark cycles, with temperature of 25°C and humidity of 60-70% throughout the period of the experiment, according to the protocol of the Committee for the purpose of control and supervision of experiments on Animals.[9] The rats, in their separate cages, were allowed two (2) weeks for acclimatization before the commencement of administration of agents. The rats had free access to commercial rat chow and water throughout the experiment period which lasted for twenty-one (21) days.

Administration of agents

From day 1-14, the rats in group 1 were administered with oral normal saline 0.5ml daily, while the rats in groups 2, 3, 4, 5 and 6 were administered with oral Paracetamol 300mg/kg daily. From day 15-21, group 1 continued to receive oral normal

saline 0.5ml daily, while groups 3, 4, and 5 received oral Cucurbita maxima leaf extract 25mg/kg, 50mg/kg, and 75mg/kg daily, respectively. Group 6 was administered with oral Vitamin E 500iu/kg from day 15-21. The rats in Group 2 did not receive Paracetamol nor Cucurbita maxima leaf extract from day 15-21, as shown in table 1. All the rats had free access to commercial rat chow throughout the period of the experiment.

Table 1: administration of agents to the various groups

Group	Day1-14	Day 15-21
1	normal saline 0.5ml daily	normal saline 0.5ml daily
2	Paracetamol 300mg/Kg dly	normal saline 0.5ml daily
3	Paracetamol 300mg/Kg dly	C. maxima 25mg/kg dly
4	Paracetamol 300mg/Kg dly	C. maxima 50mg/kg dly
5	Paracetamol 300mg/Kg dly	C. maxima 75mg/kg dly
6	Paracetamol 300mg/Kg dly	Vit. E. 500iu/kg dly

Animal sacrifice and sample collection

On day 22, the experimental rats were anaesthetized using intraperitoneal injection of thiopentone at dose of 50mg/kg.[10] The rats were subsequently sacrificed and the testis excised and fixed immediately in Bouin solution before histological analysis of the histoarchitecture of the testis.

Sample Analysis

The histological slides of the testis were prepared using the standard histological techniques with Haematoxylin and Eosin stain. The slides were then analyzed using a light microscope at magnification of X100.

3. RESULTS

The testis in group 1 which received normal saline only throughout the period of the experiment showed normal findings of the testis with the histology showing numerous and densely packed seminiferous tubules embedded with the interstitial connective tissue of the testis and with good spermatogenic activity and matured spermatozoa, as shown figure 1. The testis in group 2, which received paracetamol only throughout the period of the experiment showed shrunken seminiferous tubules and necrotic interstitial cells and very few spermatozoa in the lumen of the seminiferous tubule as shown in figure 2. The testis of the rats in group 3 that received 25mg/kg of Cucurbita maxima in addition to the oral Paracetamol 300mg/kg shows degeneration of the germinal epithelium of the seminiferous tubules and mild loss of mature spermatozoa in the lumen of the seminiferous tubule (figure 3). The testes of the rats in group 4, which were treated with 50mg/kg of Cucurbita maxima in addition to the Paracetamol showed focal necrosis of germinal cells, while the other structures appear normal as shown in the figure 4. The rats in group 5 were treated with oral Cucurbita maxima at 75mg/kg in addition to the Paracetamol, and the testis showed mild tissue degeneration and mild loss of mature spermatozoa in the lumen of the Seminiferous tubules, while the group 6 that received oral Vitamin 500iu/kg in addition to Paracetamol 300mg/kg shows mild tissue degeneration and mild loss of mature spermatozoa in the lumen of the Seminiferous tubules as shown in figure 6.

Groups 3, 4, and 5 that received additional 25mg/kg, 50mg/kg and 75mg/kg of Cucurbita maxima, respectively, after administration of Paracetamol 300mg/kg showed testicular architecture with less testicular histoarchitextural distortion when compared with testis in group 2 (Paracetamol only). However, group 4 has the least distortion of the testicular histoarchitexture of these groups when compared with group 2 (group that received Paracetamol only).

Group 6 which received oral Vitamin E 500iu/kg in addition to Paracetamol showed less testicular histoarchitecture distortion when compared with group 2. However, when group 6 was compared with group 4 (50mg/kg of Cucurbita maxima), it showed that the histoarchitexure of the testis of group 4 has lesser testicular histoarchitextural distortion.

Novelty Journals

ISSN 2394-966X

International Journal of Novel Research in Life Sciences
Vol. 11, Issue 2, pp: (31-37), Month: March - April 2024, Available at: www.noveltyjournals.com

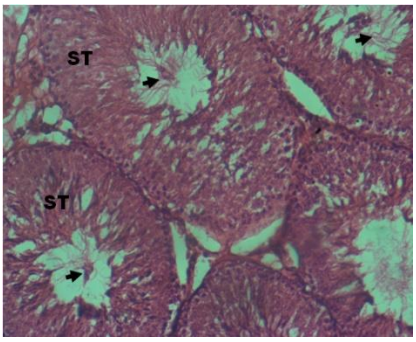

Figure 1: testis of group 1 (H&E x100) showing normal seminiferous tubules (ST) with complete spermatogenic and mature spermatozoa (Arrow) in the lumen.

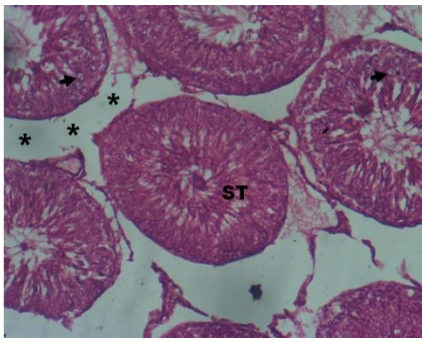

Figure 2: testis in group 2 (H&E X100) showing necrotic interstitial cells and shrunken seminiferous tubules with few luminal spermatozoa

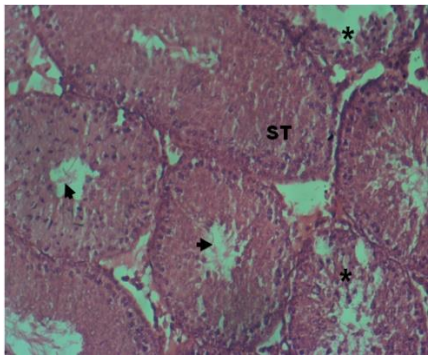

Figure 3: testis in group 3 (H&E X100) showing degeneration of the germinal epithelium (*) of the seminiferous tubules (ST) and mild loss of mature spermatozoa in the lumen of the seminiferous tubule.

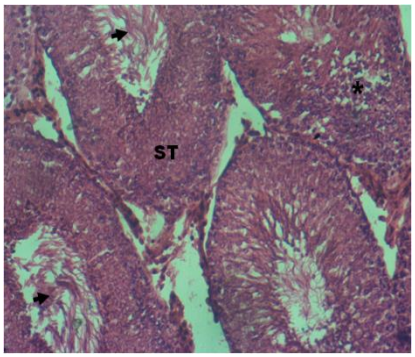

Figure 4: testis in group 4 (H&E X100) showing focal necrosis of germinal cells (*), while other structures appears normal.

Novelty Journals

ISSN 2394-966X

International Journal of Novel Research in Life Sciences
Vol. 11, Issue 2, pp: (31-37), Month: March - April 2024, Available at: www.noveltyjournals.com

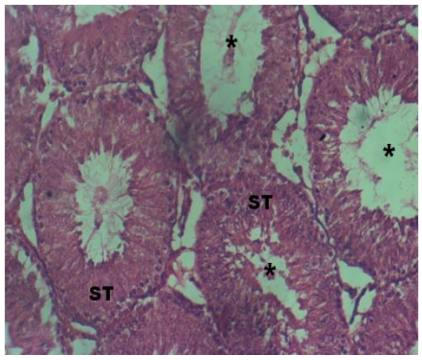

Figure 5: testis in group 5 (H&E X100) showing mild tissue degeneration and mild loss of mature spermatozoa (*) in the lumen of the Seminiferous tubules (ST).

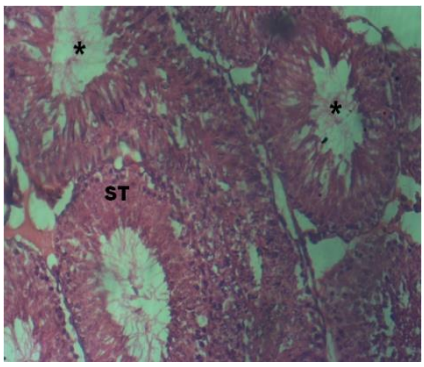

Figure 6: testis in group 6 (H&E X100) showing mild tissue degeneration and mild loss of mature spermatozoa in the lumen of the Seminiferous tubules (ST).

4. DISCUSSION

Paracetamol is a common over the counter analgesic and antipyretic that is used as painkiller for headache, pain and fever. The side effects of this medication include, but not limited to testicular injury, kidney injury, gastrointestinal bleeding and hypertension. Cucurbita maxima has shown some effectiveness as antioxidant, antimicrobial, [6] antitumour and antidepressant.[7]

This study showed that Cucurbita maxima has a curative effect when used in the treatment of paracetamol-induced testicular injury. This may be due to its high antioxidant property as studied by Jahan and colleagues who found that the free radical scavenging properties of Cucurbita maxima is high. Also that the Half Inhibitory Concentration (IC50) value is higher in the leaves than the seeds and this indicates higher DPPH free radical scavenging activities.[11,12] The antioxidant property of Cucurbita maxima has been observed to be the reason for its protective effect on the brain, liver and kidney. Mice that were pretreated with Cucurbita maxima leaf extract and then administered with intra-peritoneal formaldehyde were observed to have the histology of their brain, liver and kidney preserved, while those that did not receive the Cucurbita maxima had altered histology of the brain, liver and kidney.[13] Also, Akang and colleagues administered Cucurbita maxima seed oil to Sprague-Dawley rats at 400mg/kg and 800mg/kg for 56 days. They observed an improved sperm count and histology of the testis in the group administered with Cucurbita maxima when compared with the control.[14] However, they did not induce any testicular injury prior to administration of the Cucurbita maxima.

The findings of this study is also similar to that of Fawzy and colleagues who administered male mice with oral Bisphenol A 50mg/kg daily for 28 days, then treated the mice with oral Cucurbita maxima seed oil 1ml/kg daily for 28 days. They observed that Bisphenol A induced DNA damage and histoarchitextural alterations in the testes of the mice. These alterations in the histoarchitecture of the testis improved towards that of the control following administration of Cucurbita maxima seed oil.[15] However, their study differs from the current study due to the fact that they used Bisphenol-A for induction of testicular injury while this study used Paracetamol. And they also used Cucurbita maxima seed oil while this study used Cucurbita maxima leaf extract.

The study by Abarikwu and colleagues used Busulphan to induce testicular injury and oxidative stress in adult mice. They administered the mice with intraperitoneal Busulphan 15mg/kg at day 1 and day 7. Then the mice were treated with ethanolic extract of Cucurbita maxima seed at 200mg/kg for 40 days. They observed that the Busulphan inflicted extensive seminiferous tubule and interstitial tissue damage. And that the concomitant administration of Cucurbita maxima seed showed an improvement in the histoarchitexture of the testis with proliferation of the cells of the seminiferous tubules and the interstitial cells.[16] However, they used Busulphan to induce testicular toxicity and administered Cucurbita maxima seed concomitantly with the Busulphan, while this current study used Paracetamol to induce testicular injury and administered Cucurbita maxima leaf extract after administration of the Busulphan.

Literature search for studies that may have observed any contrary action and effect of Cucurbita maxima leaf extract on histoarchitexture of testis treated with Paracetamol did not reveal any such study.

5. CONCLUSION

Paracetamol is a commonly consumed analgesic and antipyretic which may cause testicular injury as side effect. Cucurbita maxima leaf extract which has antioxidant effects on the testis may be used to cure testicular injury caused by ingestion or administration of paracetamol.

ETHICAL CLEARANCE

Ethical clearance was obtained from The Ethical Committee, Faculty of Basic Medical Sciences, Enugu State University of Science and Technology

COMPETING INTEREST

The authors have declared no competing interest.

REFERENCES

[1] Banihani SA. Effect of paracetamol on semen quality. Andrologia. 2017; e12874

[2] McCrae JC, Morrison EE, MacIntyre IM, Dear JW, Webb DJ. Long-term adverse effects of paracetamol -a review. British Journal of Clinical Pharmacology. 2018; 84: 2218–2230

[3] Mallet C, Desmeules J, Pegahi R, Eschalier A. An Updated Review on the Metabolite (AM404)-Mediated Central Mechanism of Action of Paracetamol (Acetaminophen): Experimental Evidence and Potential Clinical Impact. Journal of Pain Research. 2023; 16: 1081–1094

[4] Hussain A, Kausar T, Din A, Murtaza A, Jamil MA, Noreen S, Iqbal MA. Antioxidant and Antimicrobial Properties of Pumpkin (Cucurbita maxima) Peel, Flesh and Seeds Powders. Journal of Biology, Agriculture and Healthcare. 2021; 11 (6): 42-51

[5] Mohammed HO, Sabry RM. The Possible Role of Curcumin against Changes Caused by Paracetamol in Testis of Adult Albino Rat (Histological, Immunohistochemical and Biochemical Study). The Egyptian Journal of Histology. 2020; 43(3): 819-834

[6] Elhardallou SB, Elawad AM, Khairi NA, Gobouri AA, Dhahawi HO. A review on Omega-3 and omega-6 Essential fatty Acids: uses, benefits and their availability in Pumpkin (Cucurbita maxima) seeds and desert dates (Balanites aegyptiaca) seed kernel oil. Pakistan Journal of Biological Sciences. 2014; 17(12): 1195-1208

[7] El-Maddawy KZ, El-Sayed YS. Comparative analysis of the protective effects of curcumin and N-acetyl cysteine against paracetamol-induced hepatic, renal, and testicular toxicity in Wistar rats. Environmental Science and Pollution Research. 2017; 4: 12-24

[8] Abireh IE, Ozioko OM, Ozor II, Finbarrs-Bello E, Ozioko US, Egbo F. Azadirachtaindica (Neem) leaf extract effect as an option of treatment of ibuprofen-induced nephrotoxicity. Journal of Advances in Medicine and Medical Research. 2020; 32(11):56-62.

[9] Abireh IE, Anyanwu GE. Synergistic Effect of Docetaxel Plus Saponin Fraction of Vitex doniana on Prostate Specific Antigen and p53 in Nitrsobis (2-oxopropyl) Amine-induced Prostate Toxicity. Journal of Applied Life Sciences International. 2021; 24(8): 17-22

[10] Abireh IE, Anyanwu GE. Suppression of Ki-67 immunomarker and prostate specific antigen by saponin fraction of Vitex doniana leaf in nitrosobis (2-Oxopropyl) amine-induced prostate toxicity. International Journal of Botany Studies. 2021; 6(5): 1542-1545

[11] Saha P, Mazumder UK, Haldar PK. In Vitro Antioxidant Activity of Cucurbita Maxima Aerial Parts. Free Radicals and Antioxidants, 2011, 1(1): 42-48

[12] Jahan F, Islam MB, Moulick SP, Bashera M, Hasan S, Tasnim N, Saha T, Boby F, Waliullah M, Saha AK, Hossain A, Ferdousi L, Rahman MM, Saha BK, Bhuiyan MNH. Nutritional characterization and antioxidant properties of various edible portions of Cucurbita maxima: A potential source of nutraceuticals. Heliyon, 2023, 9: e16628

[13] Paul M, Sohag MSU, Khan A, Barman RK, Wahed MI, Khan MRI. Pumpkin (Cucurbita maxima) seeds protect against formaldehyde-induced major organ damages. Heliyon, 2020, e04587

[14] Akang EN, Oremosu AA, Dosumu OO, Noronha CC, Okanlawon AO. The effect of fluted pumpkin (Telferia occidentalis) seed oil (FPSO) on testis and semen parameters. Agriculture and Biology Journal of North America. 2010, 1(4): 697-703

[15] Fawzy EI, Makawy AI, El-Bamby MM, Elhamalawy HO. Improved effect of pumpkin seed oil against the bisphenol-A adverse effect in male mice. Toxicology Reports. 2018, 5: 857-863

[16] Abarikwu SO, Mgbudom-Okah CJ, Onuah CL, Ogunlaja A. Fluted pumpkin seeds protect against busulfan-induced oxidative stress and testicular injuries in adult mice. Drug and Chemical Toxicology. 2022; 45(1): 22-32