ISSN No:-2456-2165

Effect of Citrullus Lunatus (Watermelon) on Semen and Testis Against Acetaminophen Induced Toxicity in Wistar Rats

Nnamani Onyinye Esther, Ukoha Ukoha and Ofoego U.C.
Department of Anatomy Faculty of Basic Medical Science, College of Health Sciences Okofia Unizik.

Abstract:- The use of polyphenols, as natural antioxidants, are gaining importance, due to their health benefits for humans, decreasing the risk of liver disease, inflammation, cardiovascular and degenerative diseases by reduction of oxidative stress and counteraction of macromolecular oxidation. Although acetaminophen is the most widely used painkilling and antipyretic, high intake or consistent use of paracetamol have been associated to a well-known contrary effect which includes nephrotoxicity, hepatotoxicity (Olaleye and Rocha, 2008). Experimental animals were randomly allocated into six groups; A, B, C, D, E, F of six animals per group (n=6). Group A was the control group and received only standard diet animal feeds and distilled water only. Group B received 4ml/kg body weight of fresh *Citrullus lanatus* juice only for 28days. Group C received feed and water for 28 days, and was treated only with 2g/ kg of acetaminophen on the 29th and 31th day. Group D received 4ml/kg body weight of *Citrullus lanatus* juice for 28 days, and was treated with single oral dose of 2g/ kg of acetaminophen, on the 29th day. Group E and F received 2ml/kg and 4ml/kg body weight of fresh *Citrullus lanatus* juice respectively for 28days, and was treated with 2g/ kg body weight of acetaminophen on the 29th and 31th day. At the end of the experiment, the Wistar was sacrificed and organs collected for analysis. The results of our study suggest that watermelon juice pretreatment boosted antioxidant status and offered some protection against high doses of acetaminophen induced injury in Wister rat's testes.

Keywords:- *Testis, Citrulline Lunatus, Acetaminophen And Watermelon Juice.*

I. INTRODUCTION

The use of polyphenols, as natural antioxidants, are gaining importance, due to their health benefits for humans in decreasing the risk of cardiovascular diseases, liver diseases, and degenerative diseases and inflammation by reduction of oxidative stress and counteraction of macromolecular oxidation.

Although acetaminophen is the most widely used pain reliever and antipyretic, high doses or prolonged use of acetaminophen have well-known adverse effects including nephrotoxicity, hepatotoxicity (Olaleye and Rocha, 2008), reduction in reproductive capacity (Ratnasooriya and Jayakody, 2000), change in testicular ultrastructure (Yano and Dolder, 2002) and seminal quality impairment (Luangpirom and Maynoi, 2007). Acetaminophen was suspected to cause oxidative stress in various organs including testes (Yano and Dolder, 2002), because the oxidative damage which developed in the testes and sperms were highly dependent on their oxidative defense status (Sikka, 2001). Drug metabolizing enzyme activity is also found in the testes, but the oxidative metabolite scavenging system in testes is low as compared to the liver (Seng *et al.*, 1991).

Phytochemicals such as lycopene and β carotene have been shown to have antioxidant, anti-inflammatory and hypotensive properties; therefore, their inclusion on diet results in positive effects on the human body.

Watermelon is a natural source of antioxidants such as beta carotene (Charoensiri *et al*.,2009), vitamin C (Altas *et al*., 2011), citrulline (Collins *et al*., 2007) and lycopene (Perkins-Veazie *et al*., 2001). The therapeutic effect of watermelon has been ascribed to its ability to scavenge free radicals (Leong and Shui 2002; Lewinsohn *et al*., 2005). Lycopene is an acyclic isomer of ß -carotene. It has been demonstrated to play a prominent role in the treatment and management of ailments such as cancer and cardiovascular diseases (Lewinsohn *et al*., 2005), also reported as having analgesic and anti-inflammatory effects (Rimando and Perkins-Veazie, 2005; Madhavi *et al*., 2012), with anti-ulcerative activity (Alok *et al*., 2012; Okunrobo *et al*., 2012), antimicrobial activity (Elsir and Hassan, 2011), laxative activity of the fruit and hepatoprotective (Rimando and Perkins-Veazie, 2005).

It is also a source of amino acid citrulline which is present in all parts of the fruit (Collins *et al*., 2007). Previous studies indicated that both citrulline and arginine were able to improve sexual function in patients with erectile dysfunction (Zorgniotti and Lizza, 1994; Melman, 1997; Cormio *et al*., 2011;). According to Phukphon *et al* (2014), watermelon flesh extract may be useful for the treatment of erectile dysfunction and for increasing potency in males.

Therefore, the present study is aimed to evaluate the potential of watermelon flesh extract on acetaminophen induced toxicity in testes and semen quality.

ISSN No:-2456-2165

II. MATERIALS AND METHODS

➢ *Procurement of Citrullus lanatus and preparation of crude extract fresh Citrullus lanatus juice*

Healthy watermelon fruits (green skin, red flesh) were purchased from Nkwo Nnewi market in Nnewi local government area of Anambra state, Nigeria. Watermelon rind and the seeds was removed. The mesocarp of the ripe fruit (pulp) was chopped into thin slices and crushed to juice with a blender without adding water, until a fine solution was obtained. This crude *Citrullus lanatus* juice was prepared fresh daily throughout the experiment.

➢ *Animal*

Thirty-six adult male albino Wister rats weighting between 90-142g were procured from an animal farm in the College of Health Sciences, Nnamdi Azikiwe university, Nnewi, Anambra state, and conditioned by the investigator on the animal holding unit of the Anatomy Department, Nnamdi Azikiwe University (NAU) Nnewi, Nigeria. The animals were housed in well-ventilated stainless-steel cages and maintained under standard and good laboratory conditions of light (12hrs dark/12hrs light), temperature (25-28^{0}C), humidity and ventilation. They were fed standard rat diet (finisher) and distilled water (*ad libitum*) and were acclimatized for two weeks before the commencement of the experiment.

➢ *Procurement and reconstitution of acetaminophen*

Acetaminophen (Paracetamol) 500mg from Emzor pharmaceutical was purchased from inland medical and was dissolved in water to obtain the required dilution of 100mg/ml by dissolving 5000mg of acetaminophen in 50 ml of water at room temp.

➢ *Experimental protocol*

After acclimatization, animals were randomly allocated into six groups; A, B, C, D, E, F of six animals per group (n=6). Group A was the control group and received only standard diet animal feeds and distilled water only. Group B received 4ml/kg body weight of fresh *Citrullus lanatus* juice only for 28days. Group C received feed and water for 28 days, and was treated only with 2000mg/ kg of acetaminophen on the 29th and 31th day. Group D received 4ml/kg body weight of *Citrullus lanatus* juice for 28 days, and was treated with single oral dose of 2000mg/ kg of acetaminophen, on the 29th day. Group E and F received 2ml/kg and 4ml/kg body weight of fresh *Citrullus lanatus* juice respectively for 28days, and was treated with 2000mg/ kg body weight of acetaminophen on the 29th and 31th day.

At the end of the last administration of treatment (i.e. 24hours after last administration), animals were sacrificed and the testes were removed along with the epididymis. The caudal epididymis was separated from the testes. The testes were weighted, fixed in Bouin's reagent and then processed for paraffin sectioning. Sections of 5 mm thickness were stained with hematoxylin and eosin for microscopic observation

➢ *Testosterone determination*

This was done using the enzyme-linked immunosorbent assay (ELISA) technique using the Fortress kit.

➢ *Semen analysis*

- *Semen Collection*

The caudal epididymis separated from the testes were blotted with filter papers and lacerated to collect the semen.

- *Sperm motility*

Semen was squeezed from the caudal epididymis onto a pre-warmed microscope slide (30^{0}C) and two drops of warm 2.9% sodium citrate was added, the slide was then covered with a warm cover slip and examined under the microscope using X400 magnification. Ten fields of the microscope were randomly selected and the sperm motility of 10 sperms was assessed on each field. Sperms were labelled as motile, or immotile. The percentage of motile sperms was defined as the number of motile sperms divided by the total number of counted sperms (i.e., 100) (Mohammad-Reza et al., 2005).

- *Sperm morphology*

This was done by adding two drops of warm Walls and Ewas stain to the semen on a prewarmed slide, a uniform smear was then made and air-dried; the stained slide was immediately examined under the microscope using x400 magnification (Laing, 1979). Five fields of the microscope were randomly selected and the number of abnormal spermatozoa evaluated from the total number of spermatozoa in these fields. The number of abnormal spermatozoa were expressed as a percentage of the total number of spermatozoa.

- *Sperm count*

A graduated tube was filled to the 1ml mark with semen, and sodium bicarbonate-formalin diluting fluid was added to the 20ml mark and mixed. Using a Pasteur pipette, the improved Neubauer ruled chamber was filled with well mixed diluted semen and left for 3-5min for the spermatozoa to settle, and then examined using a microscope. The number of sperm counted in 1ml of fluid is multiplied by 10^6.

➢ *Statistical Analysis*

The mean and standard error of mean (S.E.M.) were calculated for all values. Comparisons between the control and the treated groups were done using the student's t-test. The differences were considered statistically significant at $p<0.05$.

III. RESULTS

Table 4.1. Effects of *Citrullus lanatus* pulp on testosterone level, total sperm count, and testicular weight following acetaminophen toxicity

Table 4.1 result revealed a significant decrease in the testosterone level in-group C compared to A (*p=0.00*), groups B, D, and F had significant increase (*p=0.00; p=0.00; p=0.01*), and group E had a non-significant increase (*p=0.13*) compared to group C. The total sperm count result indicated a significant decline (*p=0.01*) in-group C compared to A, groups B and D had significant rise (*p=0.00; p=0.02*), and groups E and F had a non-significant (*p=0.34; p=0.11*) increase compared to group C. The absolute testicular weight results revealed significant increase (*p=0.01*) in all groups compared to C.

Parameters	Group A (control)	Group B (C. lanatus)	Group C (Acetaminophen)	Group D (4ml of *C. lanatus* + 2000mg of Acetaminophen)	Group E (2ml of *C. lanatus* + 2000mg/kg of Acetaminophen)	Group F (4ml of *C. lanatus* + 2000mg/kg of Acetaminophen)
Testosterone level (ng/ml)	2.06±0.12[b]	2.38±0.12[b]	0.31±0.11	1.26±0.10 [b]	0.63±0.21 [a]	1.13±0.12 [b]
Total sperm count (X 10^6/mls)	69.76±5.72[b]	73.66±2.96[b]	52.46±1.79	69.46±4.51 [b]	58.36±6.34 [a]	62.83±1.12 [a]
Testicular weight (g/%)	2.32±0.43 [b]	2.93±0.12 [b]	1.98±0.46	2.60±0.04 [b]	2.35±0.12 [b]	2.74±0.13 [b]
Each value represents the mean ± SD of six animals. The comparisons made were as follows: a group A vs groups B, C; group C vs group D, E, F; **[a] (not significant), and [b] (significant)**						

Table 4.2 effect of *Citrullus lanatus* pulp on sperm motility following acetaminophen toxicity

Table 4.2 result demonstrated a significant (*p=0.04*) decrease in sperm motility of animals in group C compared to A, groups B and D had significant higher (*p=0.00; p=0.01*) active motility, and groups E and F had a non-significant increase (*p=0.34; p=0.07*) in the active motility compared to group C. The non-motile sperm result showed a significant (*p=0.04*) increase in-group C compared to A, groups B and D had significant decrease (*p=0.00; p=0.01*), and groups E and F had a non-significant decrease (*p=0.34; p=0.07*) in the number of non-motile sperm compared to group C.

Parameters	Group A (control)	Group B (*C. lanatus*)	Group C (Acetaminophen)	Group D (4ml of *C. lanatus* + 2000mg of Acetaminophen)	Group E (2ml of *C. lanatus* + 2000mg/kg of Acetaminophen)	Group F (4ml of *C. lanatus* + 2000mg/kg of Acetaminophen)
Actively motile (%)	86.67±3.33[b]	95.33±0.33 [b]	75.00±2.88	85.00±2.88 [b]	78.33±1.67 [a]	81.67±1.67 [a]
Non-motile sperm (%)	13.33±3.33[b]	4.66±0.33 [b]	25.00±2.88	15.00±2.88 [b]	21.67±1.67 [a]	18.33±1.67 [a]

Table 4.3 effect of *Citrullus lanatus* pulp on sperm morphology following acetaminophen toxicity

Table 4.3 result revealed a non-significant (*p=0.18*) decrease in-group C compared to A, groups B, D, E, and F had a non-significant (*p=0.18; p=0.18; p=0.46; 0.18*) increase compared to group C in the normal sperm cells. However, the abnormal sperm cells result demonstrated a non-significant (*p=0.14*) increase in-group C compared to A, groups B, D, E, and F had a non-significant (*p=0.14; p=0.14; p=0.39; p=0.14*) decrease compared to group C in the normal sperm cells.

Parameters	Group A (control)	Group B (*C. lanatus*)	Group C (Acetaminophen)	Group D (4ml of *C. lanatus* + 2000mg of Acetaminophen)	Group E (2ml of *C. lanatus* + 2000mg/kg of Acetaminophen)	Group F (4ml of *C. lanatus* + 2000mg/kg of Acetaminophen)
Normal sperm cells (%)	85.00±2.88 [a]	85.00±2.88 [a]	63.33±24.21	85.00±5.77 [a]	75.00±2.88 [a]	85.00±7.64 [a]
Abnormal sperm cells (%)	15.00±2.88 [a]	15.00±2.88 [a]	38.33±23.51	15.00±5.77 [a]	25.00±2.88 [a]	15.00±7.64 [a]

ISSN No:-2456-2165

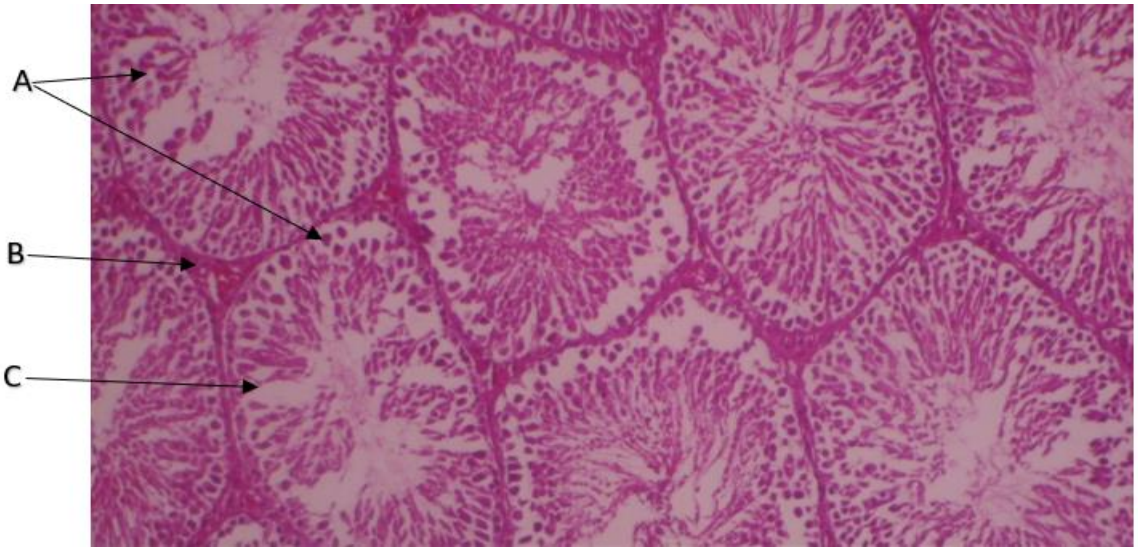

Plate 1. Control. Rat testis composed of A, tubules lined by spermatogenic series, B, interstitial cells of leydig and C, sertoli cells (H&E x 100)

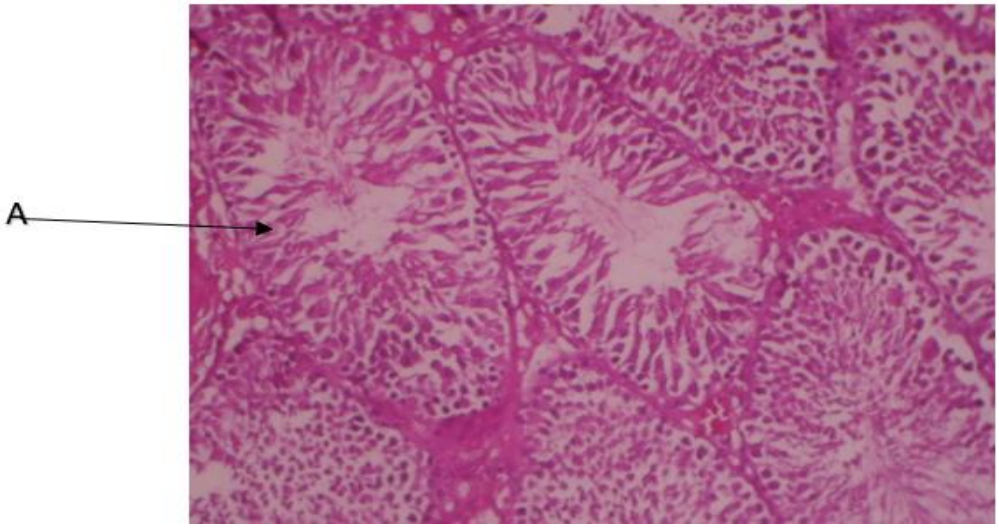

Plate 2. Rat testis given Extract only showing A: spermatocytes in normal sequential maturation (H&E x 100)

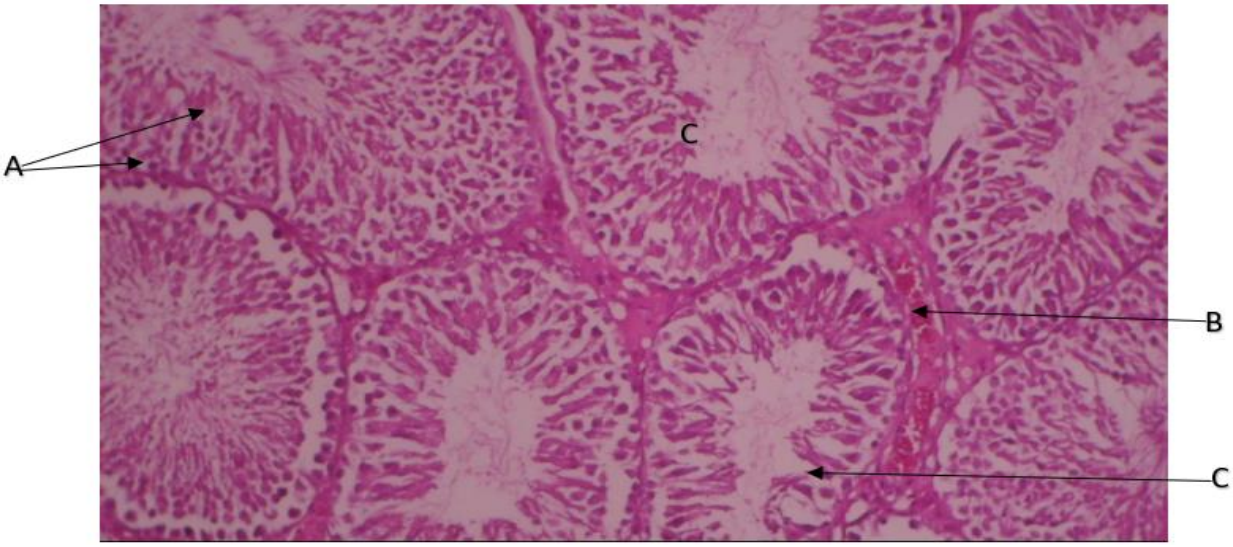

Plate 3. Testis of rat given Acetaminophen only showing: A, tubules lined by spermatocytes in normal sequential maturation and B, active interstitial congestion as well as C: maturation arrest (H&E x 100)

ISSN No:-2456-2165

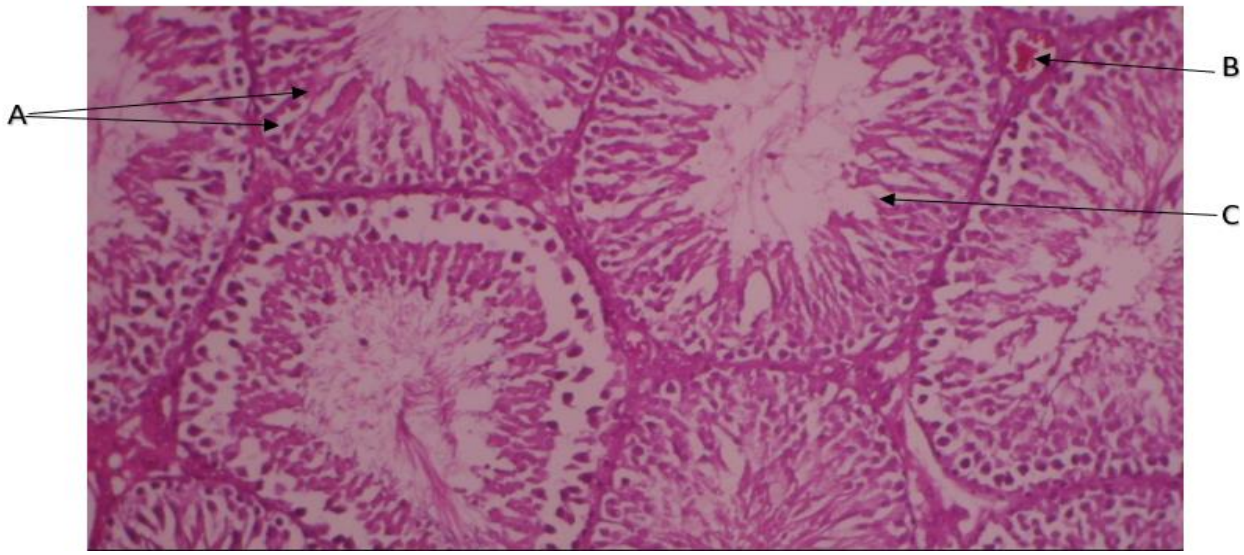

Plate 4. Rat testis given Extract + high dose Acetaminophen showing: A, spermatocytes in normal sequential maturation and B, interstitial congestion as well as C: maturation arrest (H&E x 100)

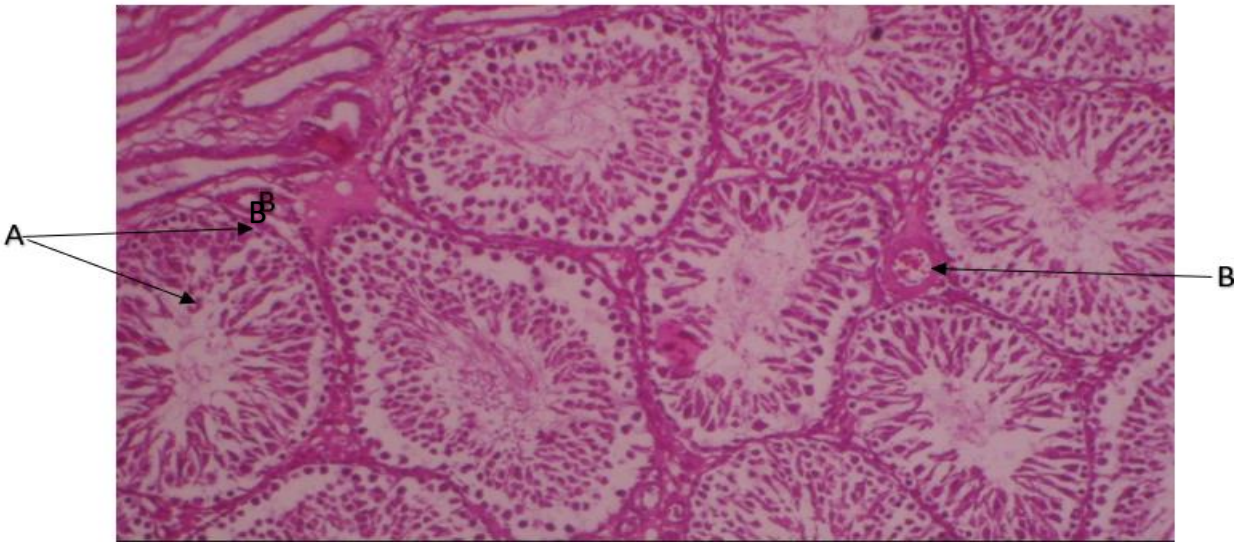

Plate 5. Rat testis given Extract + low dose Acetaminophen showing: A, spermatocytes in normal sequential maturation and B: normal testicular artery (H&E x 100)

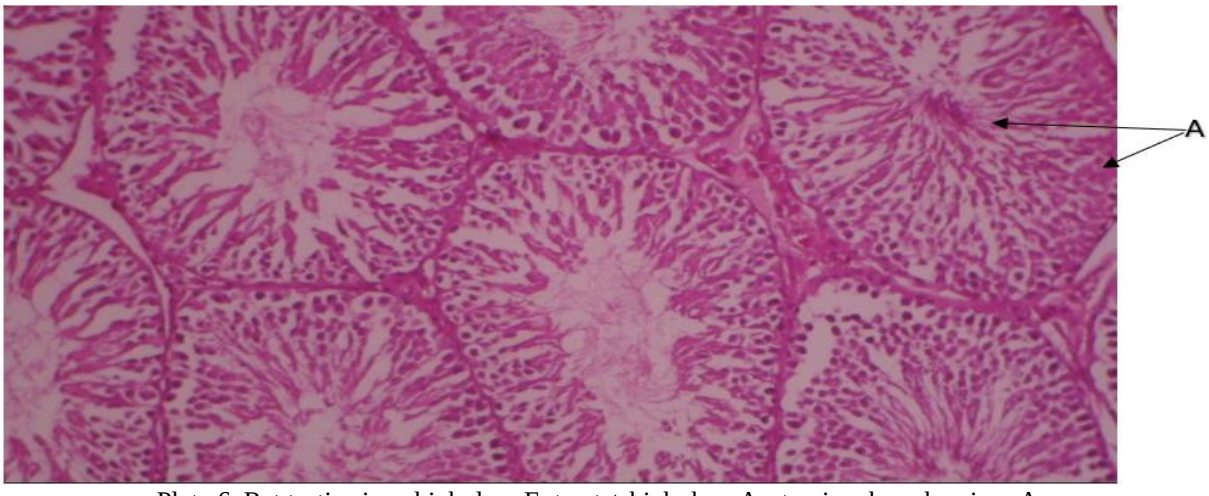

Plate 6. Rat testis given high dose Extract + high dose Acetaminophen showing: A, spermatocytes in normal sequential maturation (H&E x 100)

IV. DISCUSSION

Watermelon contains numerous antioxidants such as lycopene, beta- carotene, etc. and some specific amino acids (e.g arginine, citrulline). Consumption of fresh watermelon is considered a healthy addition to diet owing to the presence of lycopene (Kamil *et al.*, 2011). The antioxidants can interfere with the oxidation process by reacting with free radicals, chelating catalytic metals, acting as oxygen scavengers (Shahidi and Wanasundara, 1992; Sánchez-Moreno *et al.*, 1999) and preventing lipid autooxidation (Bondet *et al*., 1997; Brand-Williams *et al.*, 1997). In this study, morphology of Testis of rat given only Acetaminophen (Plate 3) showed tubules lined by active interstitial congestion as well as maturation arrest of early spermatids. Also, the total sperm count (Table 4.1) and active motility (Table 4.2) results indicated a significant decline in group C compared to the control and with a highest number of abnormal sperms compared to all other groups (Table 4.3). These are in line with previous authors who reported that high dose of acetaminophen caused an inhibition of DNA replication that was followed by reduced spermatogenesis (Wiger *et al.*, 1995), testicular atrophy, oligospermia (Boyd, 1970; Ratanassoriya and Jayakody, 2000), activation of the apoptosis of spermatocytes and early spermatids and reduction in testicular weight (Yano and Dolder, 2002). Experimental group which received only watermelon flesh extract (*citrullus lanatus*) showed increased sperm motility, and concentration in comparison to control group. This increase could be due to citrulline which is known to boost testosterone level, improve blood flow and levels of nitric necessary for spermatogenesis, maturation and capacitation of sperm (Yuxin *et al.*, 2021), and helps in erectile dysfunction (Cormio *et al*., 2011; Phukphon *et al.*, 2014). For groups pretreated with watermelon juice extract before acetaminophen administration, group D which received 4ml of *C. lanatus* extract and a single dose of 2000mg/kg body weight of acetaminophen had a significant higher active motility and significant decrease in number of non-motile sperms compared to group C, while animals in group E and F which received acetaminophen twice showed a non-significant increase in count and active motility and a non-significant decrease in number of non-motile sperms compared to C. These protective effects could be attributed to the antioxidant constituents such as flavonoids and carotenoids present in *C. lanatus* due to their anti-inflammatory effects and their free radical scavenging ability (Aderiye *et al*., 2020).

Testis morphology of groups D, E, and F (plate 4,5,6) rats pretreated with *Citrullus lanatus* extract before acetaminophen administration showed spermatocytes in normal sequential maturation and normal testicular artery. This suggest the antioxidant potential of watermelon juice and indicate that watermelon juice may be helpful for alleviating the pathological changes and also the protective effect of *citrullus lanatus* flesh extract administration due to its phenolics and carotenoid constituents (shahidi, 2009). Antioxidants protect DNA and other important molecules from oxidation and can improve sperm quality and consequently increase fertility rate in men (Yang et al., 2006).

In this study, the total sperm count and active motility result indicated a significant decline in-group C compared to Control group and with a highest number of non-motile sperms compared to all other groups. This is in accordance with previous studies which reported that high dose of acetaminophen caused an inhibition of DNA replication that was followed by a reduction of spermatogenesis (Wiger *et al.*, 1995), oligospermia and decreased male reproductive competence (Boyd, 1970; Ratanassoriya and Jayakody, 2000), triggered apoptosis of spermatocytes and early spermatids and reduction of testicular weight (Yano and Dolder, 2002). Groups D and E had a non-significant increase in the active motility and a non-significant decrease in number of non-motile sperm compared to group C. Also group B had significant higher active motility and least number of non-motile sperm compared to control group.

Testosterone levels of group B animals was significantly increased when compared to group A animals. In general, the authors had credited this hormonal increase to the antioxidant activity of the components of the fruit, as the flavonoids and the minerals that would have acted in the testicles stimulating the testosterone production. The *Citrullus lanatus* crude extracts of the mesocarp also significantly protected against testosterone decrease in group D and F, and insignificantly protected against testosterone decrease in group E, when compared to animals in group C. Although the mechanism by which *C. lanatus* crude extracts protected against testosterone decrease is not known, we attributed these protective effects to be due to citrulline and phenolics which had acted independently or together to boost nitric oxide level and activated the immunity system (Wijnands *et al.*, 2015; Lee *et al*., 2018).

It was demonstrated by El-Aday and Taha, (2001) that C. lanatus extract contains nutritive components such as magnesium, calcium, potassium, iron, phosphorus and zinc which could progressively lead to increase in weight. The testicular weight of revealed significant increase ($p=0.01$) in all groups compared to C.

V. CONCLUSION

The results of our study suggest that watermelon juice pretreatment boosted antioxidant status and offered some protection against high doses of acetaminophen induced injury in Wister rat's testes.

COMPETING INTERESTS Authors have declared that no competing interests exist.

REFERENCES

[1]. Aderiye BI, David OM, Fagbohun ED, Faleye J, Olajide OM (2020). Immunomodulatory and phytomedicinal properties of watermelon juice and pulp (Citrullus lanatus Linn): A review: *GSC Biological and Pharmaceutical Sciences*. 11(02), 153–165 e DOI: https://doi.org/10.30574/gscbps.2020.11.2.0079

[2]. Alok B, Rajeev K, Vivek D and Niyaz A. (2012). Evaluation of anti-ulcer activity of Citrullus Lanatus seed extract in wistar albino rats. *International Journal of Pharmacy and Pharmaceutical Sciences*, 4(5), 135-139.
[3]. Altas S, Kızıl G, Kızıl M, Ketani A, Hari P.I (2011). Protective effect of Diyarbakır watermelon juice on carbon tetrachloride-induced toxicity in rats, *Food and Chemical Toxicology*. 49 (9) 2433–2438.
[4]. Bondet V, Brad-William W, Berset C. Kinetics and mechanisms of anti-oxidant activity using DPPH free radical method. *LWT Food Science and Technology*. 1997; 31: 609-615.
[5]. Boyd EM (1970). Testicular atrophy from analgesic drugs. *Journal of Clinical Pharmacology., 10: 222-227.*
[6]. Brand-Williams W, Cuvelier ME and Berset C. Use of a free radical method to evaluate antioxidant activity. *LWT Food Science and Technology*. 1997; 28: 25-30.
[7]. Charoensiri R., Kongkachuichai R., Suknicom S., Sungpuag P. (2009). Beta-carotene, lycopene, and alpha-tocopherol contents of selected Thai fruits. *Food Chemistry*. 113 (1) 202–207. Doi:10.1016/j.
[8]. Collins J.K, G. Wu, Perkins-Veazie P, Spears K, Claypool P.L, Baker R.A, Clevidence B.A. (2007). Watermelon consumption increases plasma arginine concentrations in adults, *Nutrition* 23 (3) 261–266.
[9]. Cormio, L., M. De Siati, F. Lorusso, O. Selvaggio, L. Mirabella, F. Sanguedolce, and G. Carrieri. 2011. Oral L-citrulline supplementation improves erection hardness in men with mild erectile dysfunction. *Urology* 77: 119-122. DOI: 10.1016/j.urology.2010.08.028
[10]. Elsir L and Hassan A. (2011). In vitro antimicrobial activities of chloroformic, hexane and ethanolic extracts of *Citrullus lanatus* var. citroides. *Journal of Medicinal Plants Research*, 5(8), 1338-1344.
[11]. Jigna P, Sumitra C. Antibacterial and phytochemical studies on twelve species of Indian Medicinal plants. *African Journal of Biomedical Research*. 2007; 10:175-181.
[12]. Kamil MM, Gamal MF, Shaheen MS (2011). Fourier Transformer Infrared Spectroscopy for Quality Assurance of Tomato Products. *Journal of American Science*.; 7: 558-572.
[13]. Lee T. C, Su Y. T, Liu T. Y, Tsai C. M, Cheng C. H, Yu H. R (2018). L-arginine and L-citrulline Supplementation Have Different Programing Effect on Regulatory T-Cells Function of Infantile Rats. *Frontiers in immunology*, 9, 2911. http://doi.org/10.3389/fimmu.2018.02911
[14]. Leong LP and Shui G. (2002). An investigation of antioxidant capacity of fruits in Singapore markets. *Food Chemistry*, 76, 69.
[15]. Lewinsohn E, Sitrit Y, Bar E, Azulay Y, Ibdah M, Meir A, Yosef E, Zamir D and Tadnor Y. (2005). Not just colors carotenoid degradation as a link between pigmentation and aroma on tomatoes and watermelon fruits. Trends. *Food Science and Technology*, 16, 407-415.
[16]. Luangpirom A, Maynoi P (2007). Protective effect of green tea against paracetamol induced hepatotoxicity, testes and quality of seminal fluid impairment. *Khon Kaen University Science Journal*, 35(4): 222-230
[17]. Madhavi P, Rao M, Vakati K, Rahman H and Eswaraiah MC. (2012a). Evaluation of antiinflammatory activity of Citrullus lanatus seed oil by in vivo and in vitro models. *International Research Journal of Pharmaceutical and Applied Science*, 2(4), 104-108.
[18]. Okunrobo O, Uwaya L, Imafidon JE, Osarumwense K, Omorodion PE and Jude E. (2012). Quantitative determination, metal analysis and antiulcer evaluation of methanol seeds extract of Citrullus lanatus Thunb (Cucurbitaceae) in rats. *Asian Pacific Journal of Tropical Disease, 261-265.*
[19]. Olaleye MT, Rocha BTJ (2008). Acetaminophen induced liver damage in mice: Effect of some medicinal plants on the oxidative defense system. *Exprimental Toxicology and Phathology.*, 59: 319-327
[20]. Perkins-Veazie P., Collins J.K., Pair S.D., Roberts W. (2001). Lycopene content differs among red-fleshed watermelon cultivars, *Journal of Sciences, Food and Agriculture.* 81 (10) 983–987.
[21]. Phukphon Munglue1, Sajeera Kupittayanant and Pakanit Kupittayanant. (2014). Effect of Watermelon (Citrullus lanatus) Flesh Extract on Sexual Behavior of Male Rats. *Food and Applied Bioscience*. Vol.13(1) Doi: 10.12982/cmujns.2014.0054
[22]. Ratnasooriya W, Jayakody J (2000) Long-term administration of large doses of paracetamol impairs the reproductive competence of male rats. *Asian Journal of Andrology* 2:247–255.
[23]. Rimando, A.M., and P.M. Perkins-Veazie. 2005. Determination of citrulline in watermelon rind. *Journal of Chromatography* A 1078: 196-200. DOI: 10.1016/j.chroma.2005.05.009
[24]. Sánchez-Moreno C, Larrauri J, Saura-Calixto F (1999). Free radical scavenging capacity and inhibition of lipid oxidation of wines, grape juices and related polyphenolic constituents. *Food Research International.* 32(6), 407-412.
[25]. Seng J, Leakey JE, Ariotto MP, Paskinson A, Gandy J (1991). Cellular localization of cytochrome P450 II A in testes of mature Sprague Dawley rats. *Biology and Reproduction.*, 45: 876-882.
[26]. Shahidi, F. and Wanasundara P.K.J.P.D (1992). Phenolic antioxidant. *Critical Review in Food Science and Nutrition; 32: 67-103.*
[27]. Shahidi, F. Nutraceuticals and functional foods (2009). Whole versus processed foods. *Trends in Food Science and Technology*. 20: 376-387.
[28]. Sikka SC (2001). Relative impact of oxidative stress on male reproductive function. *Current Medicinal Chemistry*. 8: 851-862.
[29]. Singh, I 2010. Textbook of human histology. (6th ed). Jaypee publishing. Pp. 274-281.
[30]. Wiger R, Hongslo JK, Evenson DP, De-Angeles P, Schwarze PE, Holme JA (1995). Effects of acetaminophen and hydroxyurea on spermatogenesis and sperm chromatin structure in laboratory mice. *Reproduction and Toxicology*. 9(1): 21-33.

[31]. Wijnands K. A. Castermans T. M, Hommen M. P, Meesters D. M, Poeze M. (2015). Arginine and citrulline and the immune response in sepsis. *Nutrients*, 7(3)1426-1463. http://doi.org/10.3390/nu7031426
[32]. Yang HS, Han DK, Kim JR, Sim JC (2006). Effects of alpha-tocopherol on cadmium-induced toxicity in rat testis and spermatogenesis. *Journal of Korean Medical Science*. 21:445-451.
[33]. Yano CL, Dolder H (2002). Rat testicular structure and ultrastructure after paracetamol treatment. *Contraception.* 66: 463-467.
[34]. Yuxin Luo, Yabin Zhu, Wangdui Basing, Xin Wang, Chunjin Li, Xu Zhou (2021). Role of nitric oxide in regulation of reproduction: A review. *Front. Endocrinology*.12: 752410. Doi: 10.3389/fendo.2021.752410
[35]. Zorgniotti, A.W., and Lizza E.F (1994). Effect of large doses of the nitric oxide precursor, L-arginine, on erectile dysfunction. *International Journal of Impotence Research* 6(1): 33-35.