AMELIORATING EFFECT OF VITAMIN E ON PARACETAMOL-INDUCED TESTICULAR DAMAGE

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INTRODUCTION

Paracetamol, chemically named N-acetyl-p-aminophenol, is classified as a mild analgesic. It is commonly used for the relief of headaches and other minor aches and pains and is a major ingredient in numerous cold and flu remedies. In combination with opioid analgesics, paracetamol can also be used in the management of more severe pain such as post-surgical pain and providing palliative care in advanced cancer patients[1]. Though paracetamol is used to treat inflammatory pain, it is not generally classified as an NSAID because it exhibits only weak anti-inflammatory activity.

It is known that paracetamol can inhibit nitric oxide generation[2], which is essential for normal reproductive activity in the male rat[3]. In overdose, it can cause hepatic and different other organs damage, and this is invariably followed by recovery with the early administration of intravenous N-acetylcysteine or oral methionine (NAC) which is considered as antidote[4,5].

In paracetamol treated mice, shrinkage of the seminiferous tubules and disturbance of regular arrangement of the spermatogenic cells in the testis were observed i.e: the spermatogenic cells were not arranged in contact regular layer as in the case of the normal[5]. Also, a study in 1980 studied the long term effect of aspirin on the rat testis and found that aspirin caused significant histological damage and concluded that the disturbed spermatogenesis may be due to the inhibition by aspirin of prostaglandin synthesis that is necessary for the completion of spermatogenesis[6]. Paracetamol also has a mild inhibitory action on prostaglandin synthesis in peripheral tissues[7]. Aceptaminophen toxicity may result from a single toxic dose, from ingestion of large doses of acetaminophen (e.g., 7.5 – 10 gm daily for 1-2 day)[8,9]. In high doses (500-1000

mg/kg) impair fertility on male rats[10]. These observations raise the possibility that use of paracetamol may interrupt normal reproductive function due to its antigonadotrophic activity.

Antioxidants are molecules, which can safely interact with free radicals and terminate the chain reaction, before vital molecules are damaged. Although there are several enzymes systems within the body that scavenge free radicals, the principal micronutrient (vitamin) antioxidants are vitamins E, beta-carotene, and vitamin C. Vitamin E is synthesized by plants and is an antioxidant that protects all membranes and other fat-soluble parts of the body, such as low-density lipoprotein cholesterol, from damage. Some of the food sources of vitamin E include Alfalfa sproats, avocado, bee pollen, carrot, chickweed, cumfrey root, dadelion root, garlic, greens (leafy), lemon grass, marsh mallow and mushrooms. Others are seeds, sunflower seeds and sunlight. Vitamin E is absorbed from the intestine through lymph. It circulates through the body plasma in associations with Beta-lipoprotein.. Vitamin E has been used in connection with the following conditions like anemia, burns, epilepsy, immune function for elderly people, intermittent claudication, rheumatoid arthritis, tardive dyskinesia, Alzheimer's disease, Angina, atherosclerosis, bronchitis, cold sores, down's syndrome, dysmenorrhea, heart attack, leukoplakia, osteoarthritis, Parkinson's disease, preeclampsia, stroke, skin ulcers, infertility, age related cognitive decline etc[11] It has been recognized since the 1940s that Vitamin E (α-Tocopherol) is a powerful antioxidant that is absolutely vital for the maintenance of mammalian spermatogenesis. The effect of supplementation of Vitamin E on sperm characteristics, lipid peroxidation and seminal plasma enzymes of mature male rabbits has been analyzed and the results indicated that supplementation of Vitamin E reduced production of free radicals and can improve semen quality[12].

The effect of Paracetamol on reproductive males cannot be ignored and it is highly desirable to search for protective measures to minimize its harmful effects. Hence, this study is aimed at determining the possible ameliorating effects of α-tocopherol (vitamin E) on paracetamol-induced testicular damage in adult male wistar rats.

AIM AND OBJECTIVES OF THE STUDY

The study is designed to document the effect of administration of Paracetamol on the cytoarchitecture of the testes in adult male wistar rats and determine the possible ameliorating effect of Vitamin E on the Paracetamol-induced testicular function perturbation.

Specific objectives of the study is to;

- evaluate the body weights in the control group and experimental groups.
- elucidate histomorphological changes that may occur in the testes.
- examine the sperm parameters; sperm count, sperm motility, sperm morphology, life and death ratio, sperm progressivity.
- determine oxidative stress level by quantifying Superoxide Dismutase (SOD) and Malondialdehyde (MDA) activities.

The scope of this investigation extends from the general, histological studies using haematoxylin and eosin (H&E) staining technique, histochemical studies using Periodic acid – Schiff for glycogen and Feulgen's reaction for DNA as observable with the light microscope, to the hormonal (TT, LH and FSH) and oxidative marker (SOD and MDA) levels in serum as well as sperm parameters.

MATERIALS AND METHODS

The research work was conducted in conformity with the rules and guidelines of the Animal Ethics Committee of the University of Ilorin.

EXPERIMENTAL ANIMALS

Thirty (30) adult male wistar rats (*rattus novergicus*) with average weight of 200g were used for this study. The rats were procured and maintained in the animal house of the College of Health sciences, University of Ilorin. The animals were allowed to acclimatize for two weeks prior to treatment. They were housed in cages under light controlled conditions (12/12-hr light/dark cycle) and at a favourable room temperature/humidity. Food and water made available ad-libitum. The animals were divided into five (5) groups of six (6) rats each.

DRUGS

The Paracetamol dosage of 100 mg/kgBW is the therapeutic dose according to previous studies[13] while 100mg/kgBW is average supplementary dose according to study by Shen *et al*[14]. The paracetamol tablets were dissolved in distilled water; 500mg tablet was dissolved in 10ml of distilled water to get a concentration of 10mg/0.2ml, while Vitamin E capsules were diluted with Olive oil, as it is oil soluble; 100 IU (equivalent to100mg) capsule was diluted with 2ml olive oil to get a concentration of 10mg/0.2ml. the drugs were administered orally for two (2) weeks.

TABLE I: Grouping and Dosage of Drugs and Placebo

Groups(n=6)		Drug Administration
A	-	100 mg/kgBW PCM Only
B	-	100 mg/kgBW PCM + 100 mg/kgBW Vit. E
C	-	100 mg/kgBW Vit. E only
D	-	0.2 ml of Distilled water
E	-	0.2 ml of Olive oil

The animals were weighed prior to grouping, and the weighing was done once a week subsequently. Twenty four (24) hours after the 14^{th} day of treatment, the rats were sacrificed. The rats were at the time of sacrifice first weighed and then anaesthetized with 0.5ml ketamine injection. The abdominal cavity was open up through a midline abdominal incision to expose the heart. Blood was withdrawn from the apex of the heart and transferred into lithium-heparinized and EDTA bottles to prevent blood clotting. Serum was separated from the blood cells by centrifugation. Then, blood serum was immediately stored at -20 ˚C for biochemical assay. The testes of each rat was excised and immediately fixed. The caudal epididymides were transferred into plain bottles containing normal saline for semen analysis.

The testes were fixed in Bouin's fluid for 24 hours. Dehydration of the tissue is essential because the impregnating medium (paraffin wax) is immiscible with water. Ethanol was used and the tissues were passed through ascending concentrations of alcohol as follows; 50% alcohol for 1 hour, 70% alcohol for 1 hour, 90% alcohol for 1 hour, Absolute alcohol I for 1 hour, Absolute alcohol II for 1 hour. Clearing was done to remove the dehydrating fluid from the tissue, thereby paving the way for proper impregnation in paraffin wax. The tissues were passed through two changes of Xylene, each for a period of 1 hour.

Infiltration is done to remove clearing agent prior to embedding. Molten paraffin wax (melting point 56^0c) was used for infiltration. The wax was placed in an electrically heated, thermostatically regulated oven. The temperature of the oven was maintained at 60^0c. Infiltration was done twice for 1 hour each. Tissues were placed in embedding cassettes in an oriented position with the use of forceps. The cassettes were subsequently filled with molten wax and cooled to solidification in tap water at room temperature. Solidification of the embedding medium makes it firm enough to support the tissue for subsequent sectioning. The blocks of tissues were trimmed to a suitable size and shape with a knife leaving 1-2mm of embedding medium on the sides. Thereafter, the blocks were mounted on wooden blocks that can be held by the rotary microtome clamps during sectioning.

Each block was clamped on the microtome chuk and was placed a few microns to the knife edge by manual control. The microtome was set at a thickness of 4µm while the knife was placed at an angle of 45^0 to the block of wax containing the tissue. Ice blocks were applied to the surface of the tissue blocks before and during sectioning to reduce the heat generated. Ribbons produced were carefully picked up allowed to flatten out in cold water bath and then transferred to a warm water bath to enhance the spreading out of sections. Glass slides were dipped vertically into warm water bath to lift out the sections at an angle to the plane of the water so that the sections can spread out flat on the slides. The slides were allowed to dry at room temperature and excess wax around the tissues was later removed by placing the slides on a hot plate for a short period of time. The slides were then stored in slide-racks before staining.

STAINING TECHNIQUES

HAEMATOXYLIN AND EOSIN . Ehrlich's haematoxylin (Alum haematoxylin) was prepared using following reagents; Haematoxylin 2 g, Absolute alcohol 100 cm^3, Glycerol 100 cm^3,

Distilled water 100 cm^3 Glacial acetic acid 10 cm^3, Aluminium potassium sulphate (potassium alum) in excess (12g)**.** The haematoxylin was dissolved in the alcohol before adding the other ingredients. The stain was ripened naturally by allowing standing in a large flask, using cotton wool as a loose stopper, in a warm place and exposed to sunlight for a few weeks. The flask was shaken frequently. When good staining is attained on the test slide, the solution was bottled and filtered before use[15]. The sections were examined microscopically at this stage to confirm a sufficient degree of staining to check if sufficient.

PERIODIC ACID – SCHIFF REACTION (PAS)

a) The PAS reaction is use to demonstrate the presence of carbohydrates in tissue using Periodic acid solution and Schiff's Reagent[16];

The histochemical technique for the demonstration of DNA in the testicular cells (Fuelgen Reaction) was done[17]. The stained slides were viewed with computerized digital microscope. Micrographs were taken at magnifications of 40x, 100x, 400x and 1000x. Coloured printing using HP coloured printer for improved tissue architectural differentiation. Serum for hormonal assay (Testosterone,)was aspirated from the centrifuged blood in the lithium heparinised bottles using Pasteur pipettes. Before proceeding with the assay, all reagents and serum were brought to room temperature. Testosterone was assayed for using Accu-Bind ELISA Kit[18]. Absorbance was read in each well at 450 nm in the microplate reader. The quantitative determination of LH concentration in the serum was done using Accu-Bind ELISA kit[19].

The quantitative determination of FSH concentration in the serum was done using Accu-Bind ELISA kit[20].

DETERMINATION OXIDATIVE STRESS MARKERS

Serum from the EDTA bottles was used for the enzyme assays.

For determination of serum superoxide dismutase level, a method originally described by Misra and Fridovic was employed[21]. This method involves inhibition of epinephrine autoxidation, in an alkaline medium at 480nm in an ultraviolet spectrum. For the determination of specific activity of SOD in 1ml of blood serum, the rate of autoxidation of epinephrine was noted at 30s intervals in all groups.

Malodialdehyde levels in serum were measured according to the method of Stock and Domandy[22]. Absorbance of tests and standards were read at 532 nm.

SEMINAL ANALYSIS

The epididymis were excised and transferred immediately to 2mls of normal saline in a sterile plain bottle.

SPERM CONCENTRATION. The epididymis was minced with scissors in 5ml of normal saline, placed in a rocker for 10minutes and allowed to incubate at room temperature for 2 minutes. After incubation, the supernatant was diluted at 1:100 with a solution containing sodium bicarbonate and 1ml formalin (35%). The new improved Neuber's counting chamber (hematocytometer) was used in counting the total number of spermatozoa[23]. About 10µl of the diluted sperm suspension was transferred to each counting chamber of the hematocytometer and observed under a binocular light microscope[24].

SPERM MOTILITY. The fluid from the caudal epididymis was diluted with Tris buffer solution[25] to 0.5ml. An aliquot of the solution was then observed under the light microscope. The mean motility estimation was reported as the final motility score for each sample.

SPERM MORPHOLOGY. The morphology of the spermatozoa was determined using the original dilution for motility, diluted 1:20 with 10% neutral buffered formalin. The sperm cells were categorized based on the presence of one or more abnormal features such as tail defects (short, irregular coiled or multiple tail), neck and middle piece defects (distended, irregular, bent or abnormally thin middle piece), and head defects (small, large, double or detached head). Findings were expressed as percentage of morphologically normal sperm[25].

Analysis of the result was done using SPSS version 20.0 software and presented as mean± standard error of mean (SEM) to show statistical significance between each group using the ANOVA table. Values of $P<0.05$ were considered to be statistically significant. The results are represented in tables and graphs.

RESULTS

BODY WEIGHT CHANGES

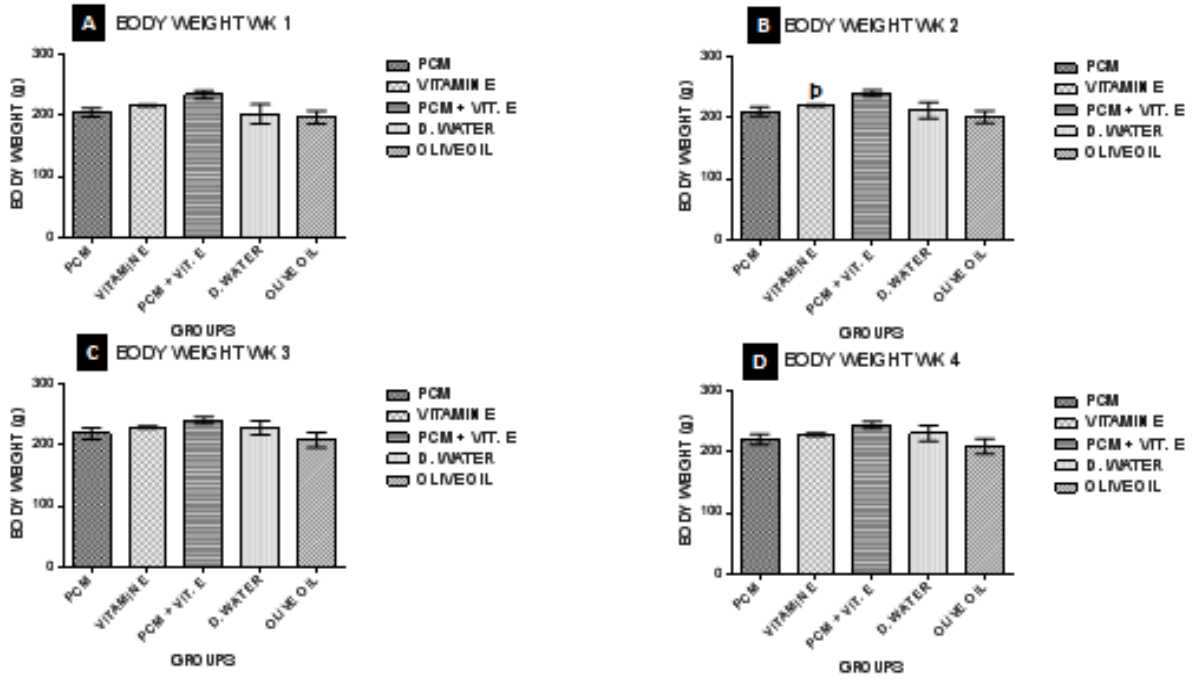
The weights of the animals were recorded on purchase (week 1), on commencement of administration (week 2), first week of administration (week 3) and at the end of administration (week 4). There was an average weight increase in all groups. However, there was a statistically significant increase in group B compared to group E ($p\leq0.05$) in week 2.

TABLE I: Average Body Weight Per Week in Animals

GROUP	WEEK 1	WEEK 2	WEEK 3	WEEK 4
A (PCM)	204.40±7.36	209.60±7.83	218.40±9.45	220.40±8.54
B (VIT. E)	216.00±1.27	219.60±1.50 Þ	228.60±1.80	228.40±2.48
C (PCM + VIT. E)	233.20±6.89	239.60±5.03	240.00±5.49	243.80±5.33

D (DIST. WATER)	202.00±15.83	211.60±13.28	227.40±11.60	230.20±13.04
E (OLIVE OIL)	196.80±10.35	201.00±10.15	207.60±12.16	208.80±12.56

Þ: p<0.05; significant increase compared to Olive Oil .



Þ: p<0.05; significant increase compared to Olive Oil

CHART I: Average Body Weight of Animals in each group for (a) week 1, (b) week 2, (c) week 3, (d) week 4.

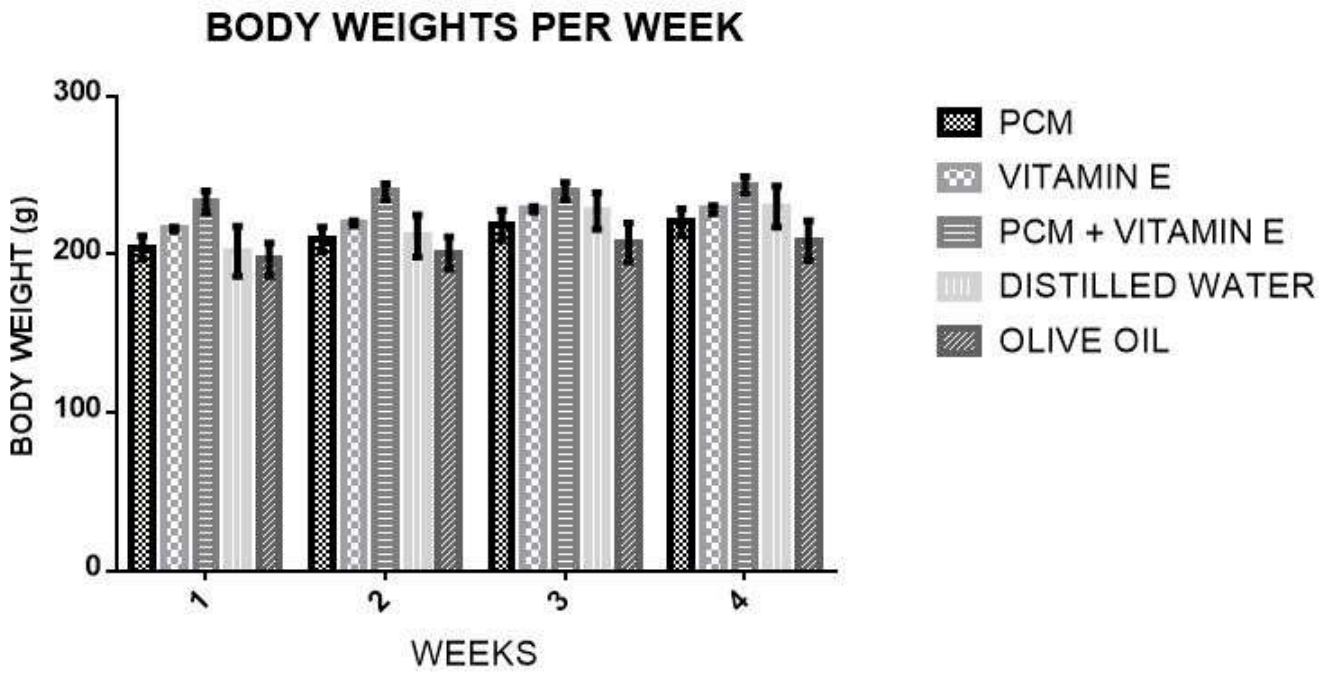


CHART II: Average Body Weight per week of Animals in all groups.

TABLE II: Effect of Vitamin E and/or Paracetamol on Serum LH, FSH and TT Levels.

GROUP	LH (mIU/ml)	FSH(mIU/ml)	TT(ng/ml)
A	4.65±1.03	8.61±3.26	4.80±1.09
B	7.90±0.99	3.54±1.55	4.11±1.77 **Þ***
C	8.68±1.74	6.60±2.45	6.81±3.05
D	11.06±3.78	7.43±1.71	12.16±0.02^
E	6.44±2.25	1.63±0.53	12.22±0.06^

***: p<0.05; significant diff. compared to control (D. water)**

^: p<0.05; significant diff compared to Vit. E

Þ: p<0.05; signigicant diff. compared to Olive Oil

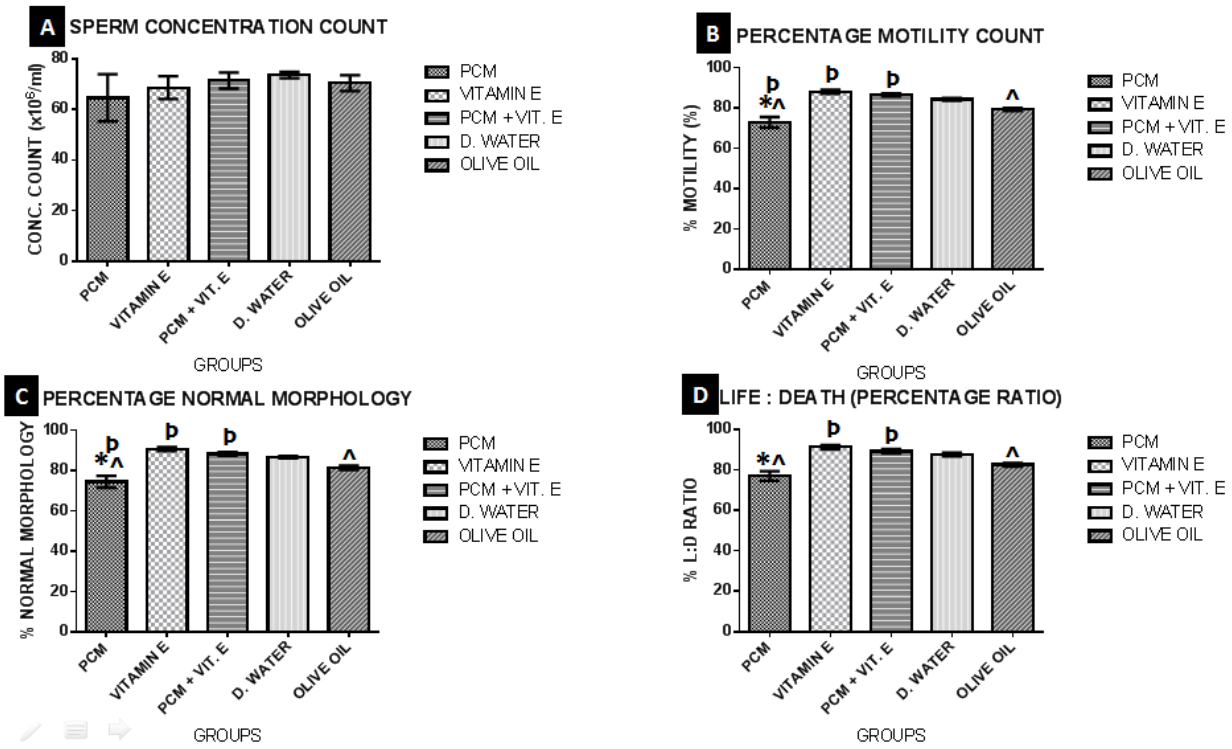
SPERM PARAMETERS

The sperm concentration count of group D was higher than all other groups, group A being the lowest (64.60±9.30 $x10^6$/ml), this difference was not statistically significant. The percentage sperm motility and normal morphology of group A was statistically significantly lower compared to group D and E while groups B and C were statistically significantly higher than group E. There was a statistically significant decrease in groups A and E compared to group B. Also, the sperm viability in group A was statistically significantly lower compared to group D while groups B and C were statistically significantly higher than group E. There was a statistically significant decrease in groups A and E compared to group B.

TABLE 4.3: Effect of Vitamin E and/or Paracetamol on Spermiogram

GROUP	CONC. (10^6/ml)	MOTILITY (%)	MORPH.(%normal)	VIABILITY(%)
A	64.60±9.30	73.00±2.64Þ^*	74.60±2.99Þ^*	77.00±2.34*^
B	68.60±4.51	88.20±0.91Þ	90.80±0.91Þ	91.20±0.97Þ
C	71.40±3.17	86.60±0.67 Þ	88.40±0.92 Þ	89.20±1.07Þ
D	73.60±1.21	84.40±0.68	86.80±0.49	87.60±0.87
E	70.40±3.12	79.40±0.81^	81.60±1.03^	82.60±0.87^

***: p<0.05; significant diff. compared to control (D. water)**
^: p<0.05; significant diff compared to Vit. E
Þ: p<0.05; signigicant diff. compared to Olive Oil



***: p<0.05; significant diff. compared to control (D. water)**
^: p<0.05; significant diff compared to Vit. E
Þ: p<0.05; signigicant diff. compared to Olive Oil

CHART IV: Effect of Vitamin E and/or Paracetamol on sperm (a) concentration, (b)motility, (c)morphology and (d)viability.

OXIDATIVE STRESS MARKERS

There is no statistically significant difference in the SOD and MDA levels in all the groups. It was observed that there was a slight increase in the SOD level in groups B and E compared to group D. This might be as a result of anti-oxidant content in these substances. In group A, a slight reduction was observed in the SOD level compared to control. MDA level was highest in animals in group D, though insignificant compared to other groups.

TABLE IV: Effect of Vitamin E and/or Paracetamol on Serum SOD and MDA levels.

GROUP	SOD(µ/ml)	MDA(µmol/ml)

A	332.00±2.52	2.433±0.12
B	354.00±7.37	2.633±0.18
C	336.33±2.60	2.433±0.06
D	336.67±8.82	3.000±0.32
E	346.33±12.60	2.633±0.26

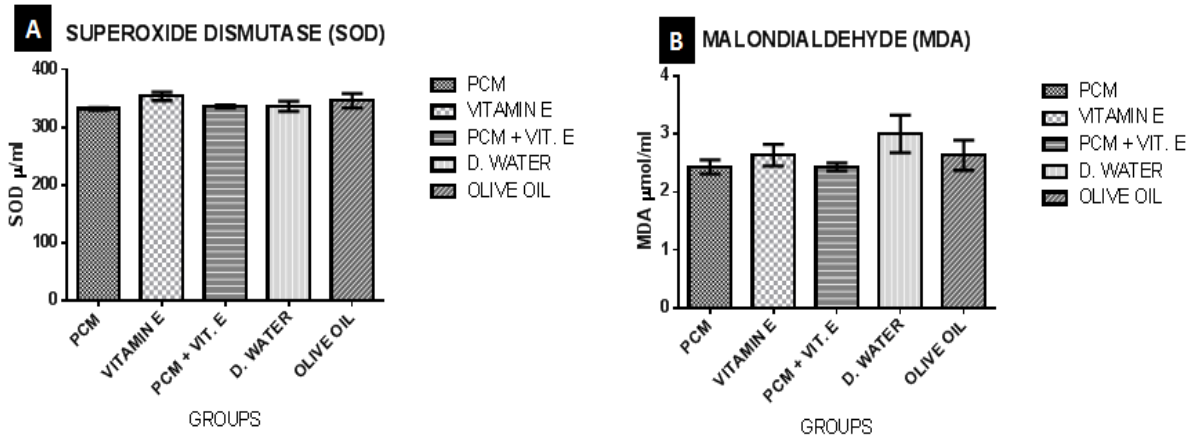


CHART V: Effect of Vitamin E and/or Paracetamol on serum (a) SOD and (b) MDA levels.

HISTOLOGICAL ANALYSIS OF THE TESTES

Haematoxylin and Eosin Sections

Histological examination of the testes of rats given 100mg/kg BW Vit. E (group B) Distilled Water (group D) and Olive Oil (group E) are similar and show seminiferous tubules which contain spermatogenic cells in various stages of spermatogenesis, including spermatogonia, spermatocytes and spermatids. The tubule also contains non-spermatogenic cells called Sertoli cells which support and nourish the developing spermatozoa. The basal layer of germinal cells is supported by a basement membrane and the lumen is clearly seen, containing spermatozoa. The interstitium contains Leydig cells (Figure IV(ii), IV(iv) and IV(v).

The testis of rats given 100 mg/kg BW of PCM + 100mg/kg BW of Vit. E (group C) shows seminiferous tubules with mild tubular deformity, with vacoulations in the interstitium (Figure 4.3). However, the cells of various stages of spermatogenesis and spermiogenesis, the spermatogenic series and non-spermatogenic cells are relatively well preserved compared with the rats that received 100mg/kg BW PCM only(group A), where severe cellular degeneration and tubular deformity is seen. The tubules showed a major reduction in the thickness of the spermatogenic cells layer and did not show any ordered progression of spermatogenic cells (Figure IV).

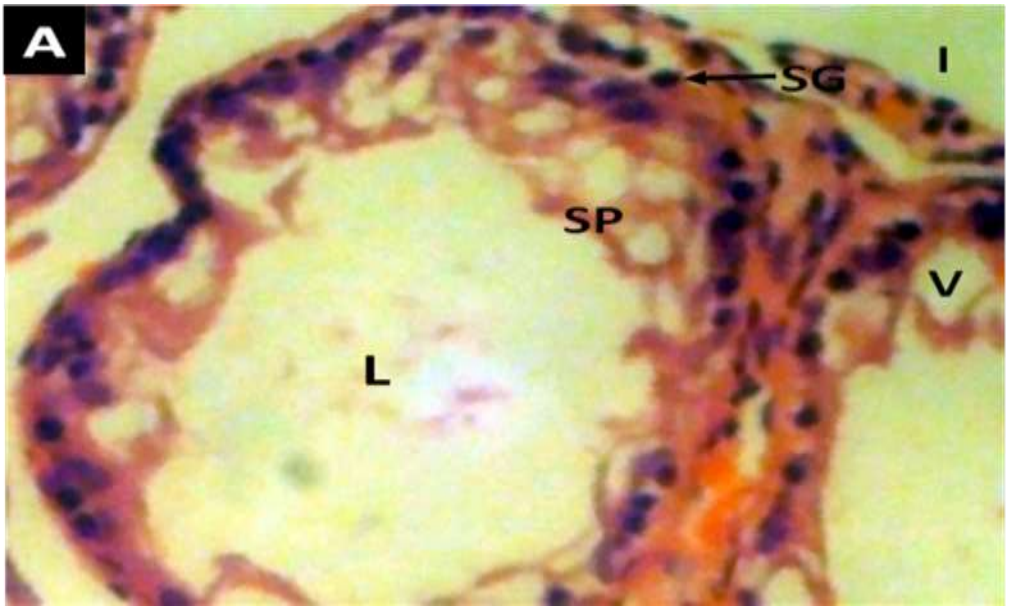


Figure IV(i): Cross Section of seminiferous tubules of PCM treated rat (group A) showing cellular degeneration and tubular deformity. Stain: H&E; Mag.X400 I- interstitium, SG- spermatogonia, SP- spermatid, L- lumen, V- vacuolation.

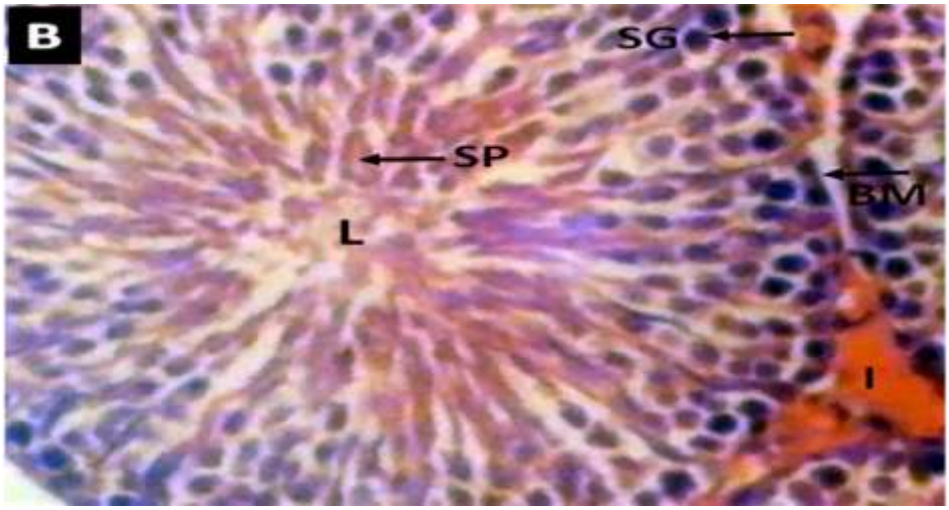


Figure IV(ii) Cross Section of seminiferous tubules of VIT. E treated rat (group B), showing a ordered progression of spermatogenic cells with lumen containing spermatozoa. Stain: H&E; Mag.X400. I- interstitium, BM- basement membrane, SG- spermatogonia , SP- spermatid, L- lumen.

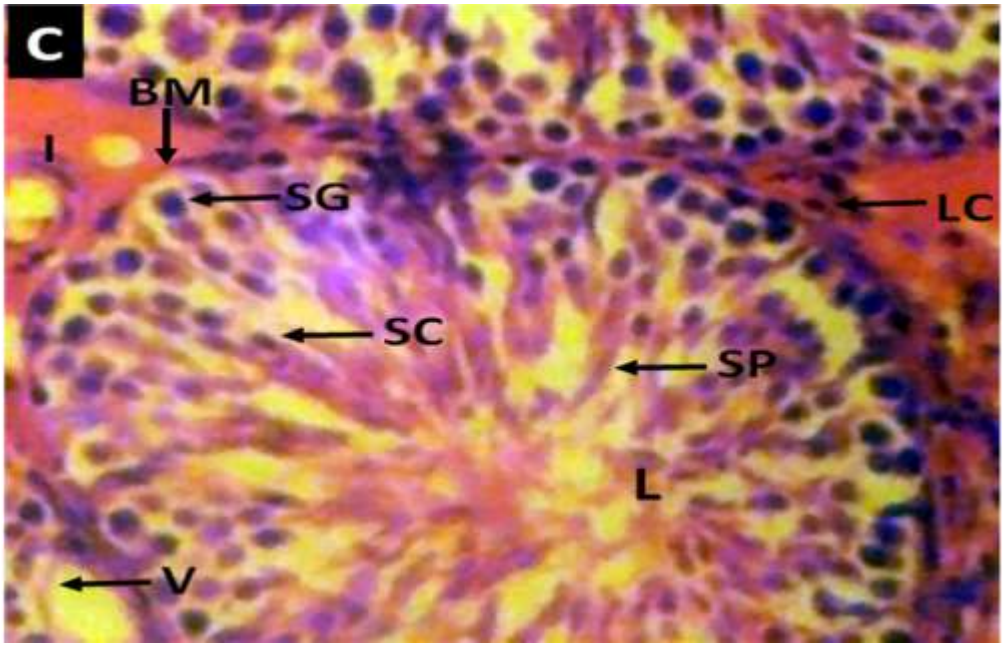


Figure IV(iii): Cross Section of seminiferous tubules of PCM + VIT. E treated rat (group C), showing mild tubular deformity and vacoulation in the interstium. Stain: H&E; Mag.X400. I- interstitium, , BM- basement membrane, SG- spermatogonia SP- spermatid, L- lumen, LC- leydig cell, V- vacuolation, StC- sertoli cell, SC- spermatocytes.

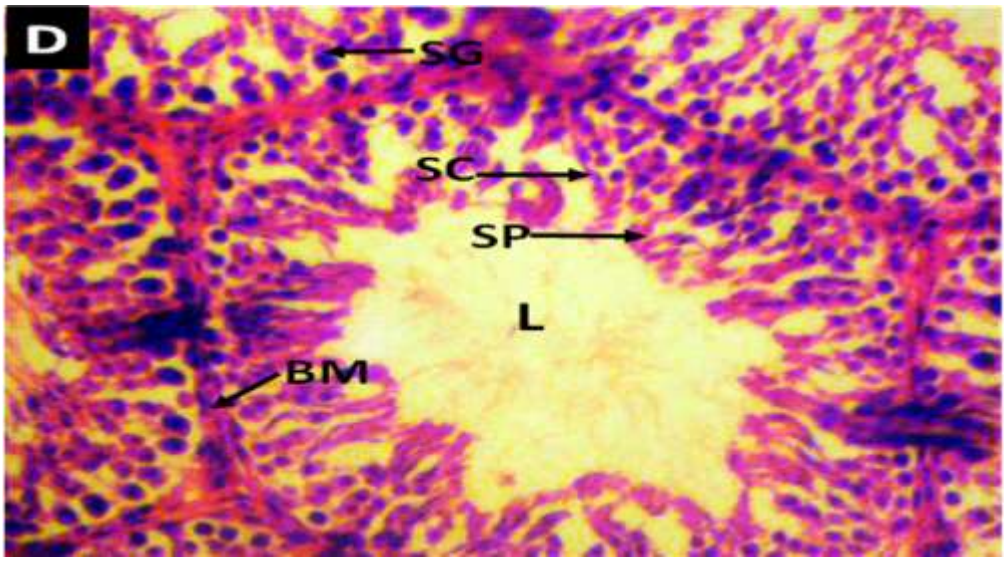


Figure IV(iv) Cross Section of seminiferous tubules of Distilled Water treated rat (group D) showing a well outlined tubular structure. Stain: H&E; Mag.X400. I- interstitium, BM- basement membrane, SG- spermatogonia, SP- spermatid, L- lumen, LC- leydig cell, SC- spermatocyte

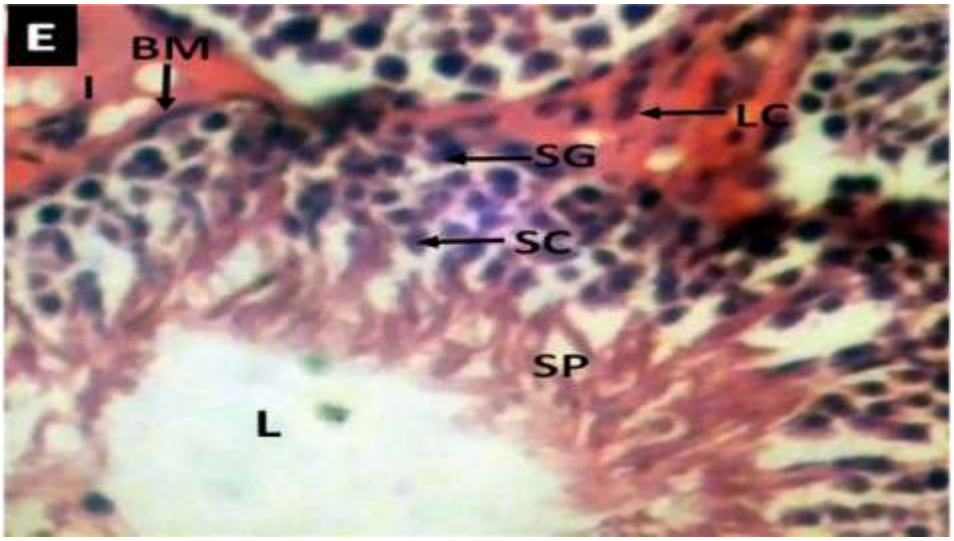


Figure IV(v): Cross Section of seminiferous tubules of Olive Oil treated rat (group E) showing a well outlined tubular structure with leydig cells in interstitium. Stain: H&E; Mag.X400. I- interstitium, BM- basement membrane, SG- spermatogonia, SP- spermatid, L- lumen, LC- leydig cell, SC- spermatocytes.

Periodic Acid-Schiff Reaction

The spermatogenic cells in the seminiferous tubules sections of rats in groups B, D and E show rapidly dividing cells, undergoing spermiogenesis. These cells are markedly magenta-stained and hence PAS positive (Figure IV(vii), IV(ix) and IV(x)), though those of group B (Figure IV(vii) were less magenta stained compared to those of groups D and E(Figure IV(ix) and IV(x).

The sections of groups A and C show less dividing cells in spermiogenesis (Figure IV(vi) and IV(viii). The cells are PAS – positive, though less magenta stained compared to those of groups B, D and E (Figure IV(vii), IV(ix) and IV(x).

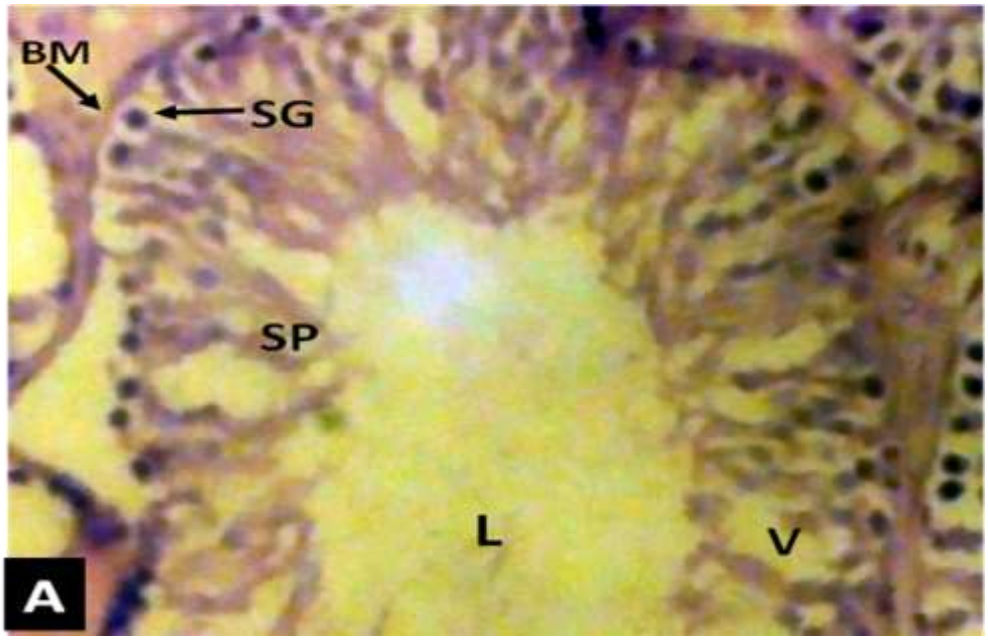


Figure IV(vi): Cross Section of seminiferous tubules of PCM treated rat (group A) showing less dividing cells in spermiogenesis. Stain: PAS; Mag.X400. I- interstitium, BM- basement membrane, SG- spermatogonia, SP- spermatid, L- lumen, V- vacoulation.

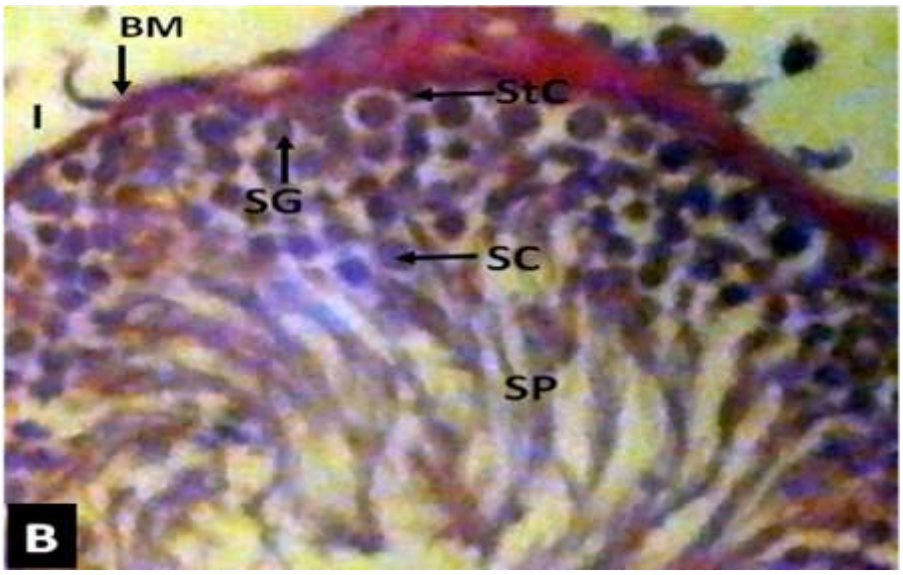


Figure IV(vii): Cross Section of seminiferous tubules of Vit. E treated rat (group B) showing dividing cells undergoing spermatogenesis. Stain: PAS; Mag.X400. I- interstitium, BM- basement membrane, SG- spermatogonia, SP- spermatid, SC- spermatocytes, StC- sertoli cells.

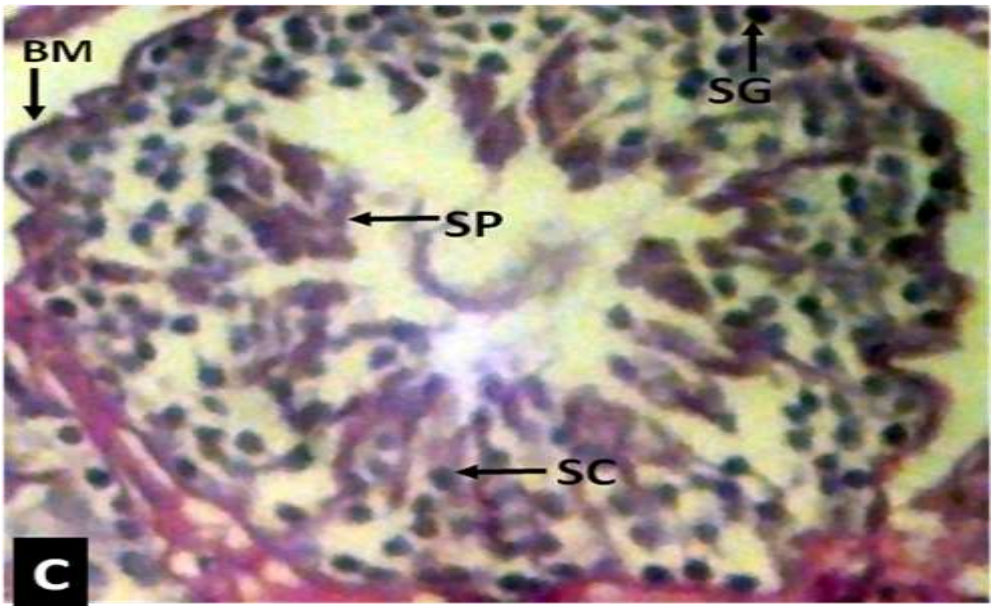


Figure (viii): Cross Section of seminiferous tubules of PCM + Vit. E treated rat (group C) showing less dividing cells compared to group D. Stain: PAS; Mag.X400. I- interstitium, SG- spermatogonia, SP- spermatid, SC- spermatocytes.

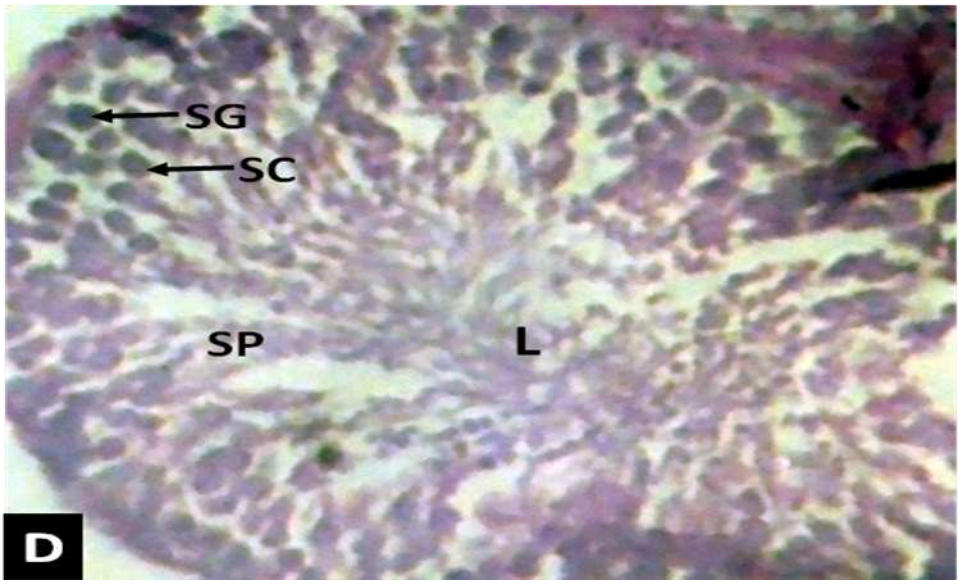


Figure IV(ix): Cross Section of seminiferous tubules of rats given Distilled water (group D) showing rapidly dividing cells undergoing spermatogenesis. Stain: PAS; Mag.X400. SG- spermatogonia, SP- spermatid, SC- spermatocytes, L- lumen.

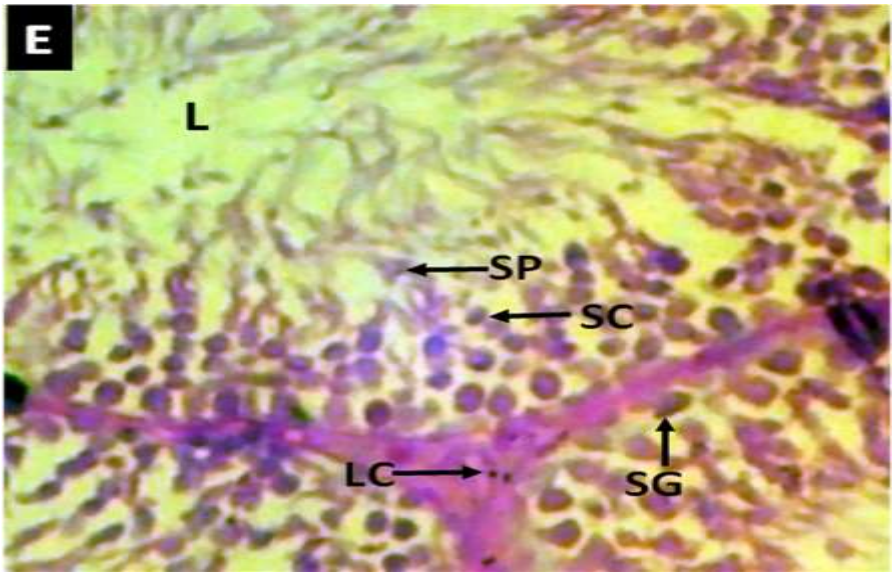


Figure IV(x): Cross Section of seminiferous tubules of rats given olive oil (group E). Stain: PAS; Mag.X400. SG- spermatogonia, SP- spermatid, SC – spermatocytes, L- lumen, LC- leydig cells.

Feulgen's Reaction

Sections from groups B, D, and E stained positive for Feulgen's reaction and showed regular seminiferous tubules with thin basal lamina. The angular interstitial space stained positive; the intense staining of the spermatogenic cells were clearly demonstrated within the seminiferous tubules (Figure IV(xii), IV(xiv) and IV(xv).

Sections from groups A and C also stained positive for Feulgen's reaction but with less intensity, showing irregular seminiferous tubule structure and disrupted connective tissues. The spermatogenic cells and the interstitial spaces were not clearly outlined (Figure IV(ii) and IV(xiii).

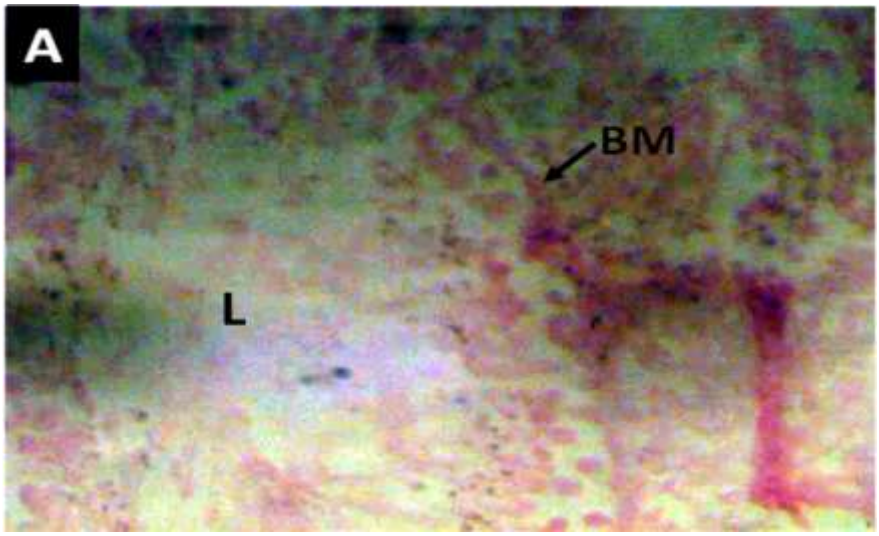


Figure IV(xi) Cross Section of seminiferous tubules of PCM treated rat (group A) showing little staining intensity compared to group D. Stain: Feulgen; Mag.X400. BM- Basement Membrane.

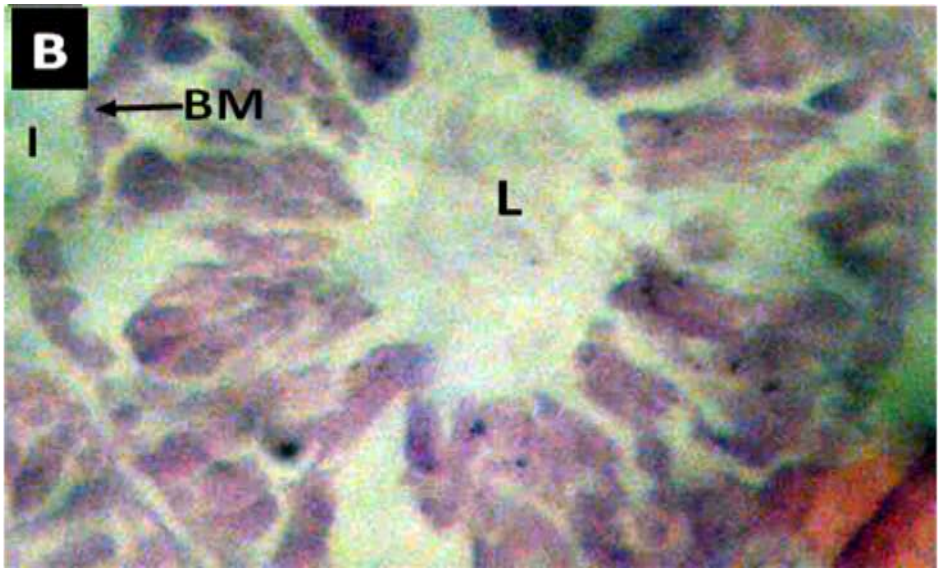


Figure IV(xii). Cross Section of seminiferous tubules of Vit. E treated rat (group B) showing intensely stained tubular structure. Stain:Feulgen; Mag.X400. I- interstitium, BM- basement membrane,L- lumen.

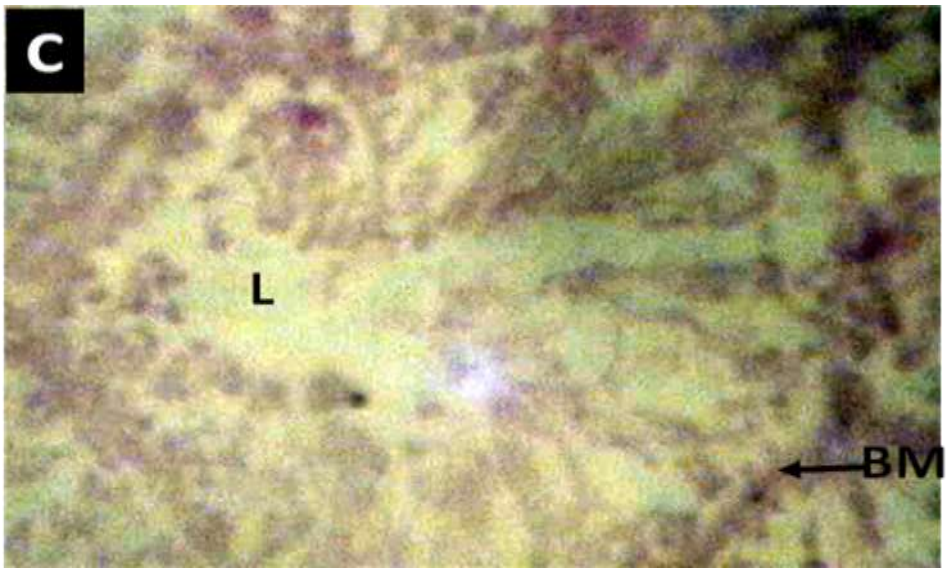


Figure IV(xiii): Cross Section of seminiferous tubules of PCM + Vit. E treated rat (group C) poorly stained deformed tubular structure. Stain: PAS; Mag.X400. BM- basement membrane, L- lumen.

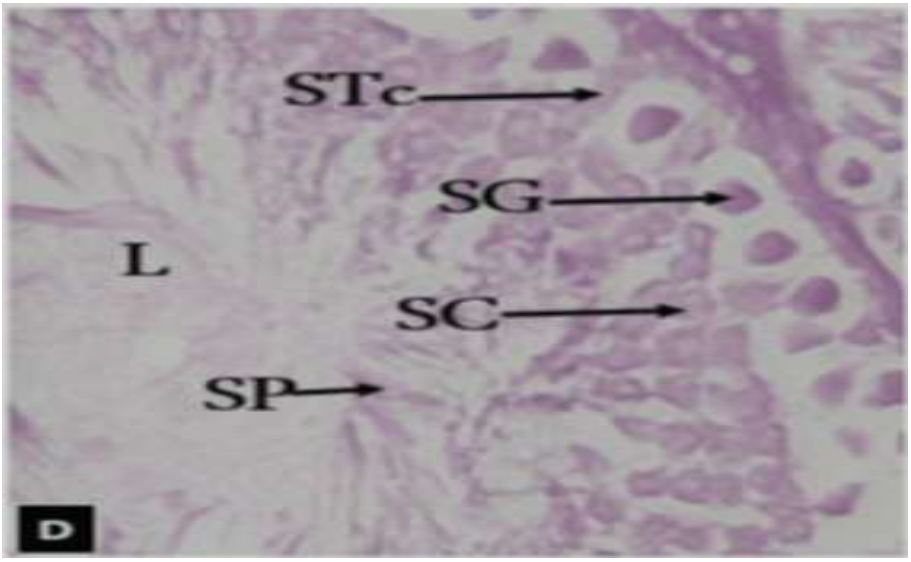


Figure (xiv) Cross Section of seminiferous tubules of rats given Distilled water (group D). Stain: feulgen; Mag.X400. SG- spermatogonia, SP- spermatid, SC – spermatocytes, StC- Sertolli Cell L- lumen.

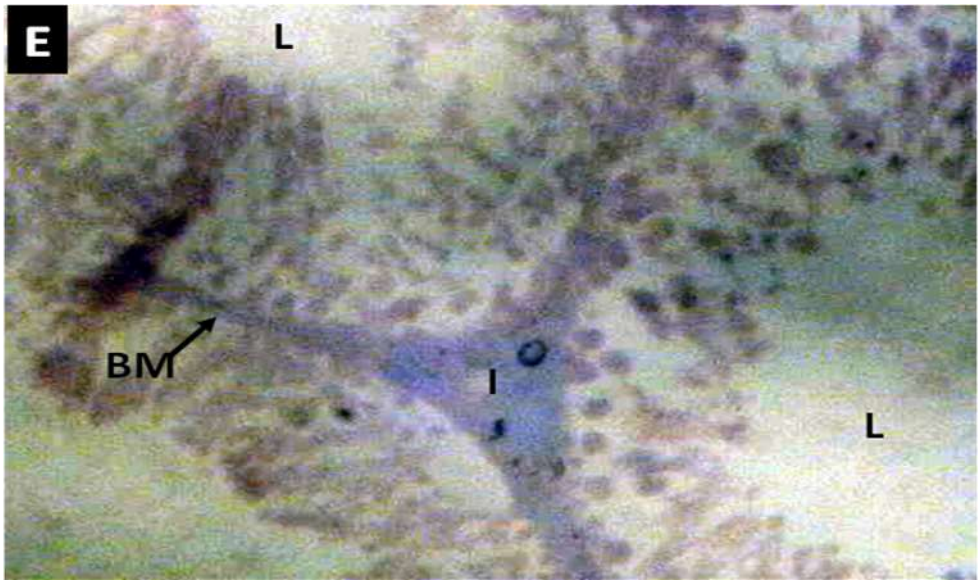


Figure IV(xv): Cross Section of seminiferous tubules of rats given olive oil (group E) clearly outlined tubular structure. Stain: Feulgen; Mag.X400. BM- Basement Membrane, I- Interstitium, L- lumen.

DISCUSSION

Paracetamol is principally metabolized in the liver through two major hepatic routes: glucouronidation and sulphation, the latter route can be rapidly saturated at doses above therapeutic doses, then elimination by dose – dependent formation of glucouro- and sulpho-conjugate derivatives (Goodman and Gilman, 2001; Bethesda and McEvoy, 2007), and tntrast, the administration of Vitamin E causes a significant increase in the sperm number, in spite of short exposure period to the drug, this may be explained on the bases of the activity of oil components on the enzyme of oxidative phosphorylation (Azzarito *et al.*, 1996).

Deficiency of vitamin E leads to a state of oxidative stress in the testes that disrupts both spermatogenesis and the production of testosterone. Conversely, administration of Vitamin E has been shown to suppress lipid peroxidation in testicular microsomes and mitochondria and to reverse the detrimental effects of oxidative stress on testicular function mediated by exposure to NSAIDs (Lucoseli and Fraga, 1999). Nevertheless, there was no statistically significant difference between the SOD and MDA levels of all the groups.

The morphology of the cells and tissues of the testes in sections of groups B, D and E animals were well preserved. In the H&E sections, the testes showed large number of dividing cells, more in group B and then groups D and E compared to the groups A and C. The dividing cells are less in the group that received 100 mg/kg BW PCM + 100 mg/kg BW VIT.E compared to the group that received 100 mg/kg BW of PCM. This observation is in agreement with the work of Hassan *et al., (*1999), who reported that the seminiferous tubules were affected following paracetamol administration. Similarly, Rihab Ghalib (2012), reported that the seminiferous tubules were distorted and atrophied as a result of the loss of their structure following paracetamol administration. Also, there was widening of the interstitial spaces due to testicular

edema. This was mainly observed in groups A and C. The spermatogenic series cells were also reduced in size and number. This could lead to low sperm quantity and quality.

The periodic acid Schiff's reaction shows positive reaction (magenta staining) in all the groups of animals. The intensity of the stain was greater groups B, D, and E compared to groups A and C. Clermont and Leblond (1955) described how human spermatids develop through a series of 12 steps. The first 7 steps are characterized by subtle changes in acrosome morphology, in that the nucleus remains spherical. The consequence of this is that the carbohydrate made available to the spermatogenic cells for energy production was reduced in the groups with less staining intesity. This energy is required for spermiogenesis and hence spermatogenesis.

The presence of DNA was well demonstrated in the spermatogenic cells by positive Feulgen reaction. The intensity of the magenta stain was lesser in the groups A and C compared to the other groups. The magenta stain was more in the group A compared to group C. The higher intensity of the stain or positivity of the DNA Feulgen reaction, indicates the presence of more DNA and hence rapid cell division of the spermatogenic cells occurs.

5.2: CONCLUSION

The current study revealed that the consumption of the paracetamol over a period as short as 14 days, had deleterious effect on the quality and the quantity of the spermatogenic series characteristic of the adult male Wistar rats. This was supported by the decreased level of the serum testosterone, sperm motility, morphology and vitality. However there was no statistically significant difference in the SOD and MDA levels, and sperm concentration count.

The morphology of the tissues was not well preserved in the treated groups A and C. There was distortion in the cytoarchitecture of the testes of the treated groups and less dividing cells undergoing spermatogenesis were seen compared to the control groups D and E as well as the Vitamin E treated group B. Conversely, groups A and C sections were less positive for periodic

acid - Schiff's, and DNA Feulgen reaction compared to the groups B, D and E, thereby indicating impaired spermatogenesis.

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