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EFFECT OF ALOE VERA AND CHLOROQUINE ON TESTES, SPERM MORPHOLOGY AND SERUM LEVELS OF REPRODUCTIVE HORMONES IN ALBINO RAT

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Abstract

Many herbal plants have medicinal property and used throughout the world as safe source of medicine. *Aloe vera* is a succulent herb used for healing various ailments. Further it has an anti-androgenic property. Chloroquine is the most commonly used drug in the treatment of malaria worldwide as well as in the treatment of discoid lupus, extra intestinal amoebiasis and rheumatoid arthritis and can cause reduction in sperm motility, fertilizing ability of sperms. In the present study the effect of *Aloe vera* and chloroquine on testes of male albino rats have been investigated. In this study 18 male albino rats weighing between 180-240 gm and aged 3-4 months were randomly divided into 3 groups of 6 animals each. The group I served as control provided normal saline, group II and III are experimental, treated with 25 mg/kgbw of *Aloe vera* gel and 2mg/kg bw chloroquine respectively for 45 days daily orally. Histopathological studies showed atrophic seminiferous tubules, germ cell debris, vacuolization of sertoli cells and interstitial cells, disrupted basement membrane, empty lumen and increased intercellular spaces filled with interstitial fluid. The sperm morphological abnormalities found to be increased while serum FSH, LH and testosterone level decreased in *Aloe vera* and chloroquine treated groups as compared to control. Thus it is concluded that *Aloe vera* and chloroquine cause toxic effect on the testes, sperm morphology and serum hormonal level of albino rats and may have antifertility potential.

Key words: Aloe vera, chloroquine, testes, sperm morphology, FSH, LH, testosterone

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Introduction

Aloe is a cactus-like perennial, herbaceous and succulent plant belongs to a family-Liliaceae and collectively called *Aloe vera* (AV) The main constituents of the AV plant are anthraquinones (Aloin, Aloe Amodine, and Coumaric Acid), polysaccharides, glycoproteins, prostaglandins, phytoestrogens such as beta-cytosterol, cholesterol, and fatty acids like camposterol (Braun, 2005, Baby *et al*., 2010 and Estakhr *et al*., 2011). A recent study on the effect of this plant on testosterone and gonadotropin hormones in adult male rats showed that, this plant has an anti-androgenic property and reduce androgen-dependent parameters including secretion of gonadotropins and probably cause oligospermia (Shariati *et al*., 2009). Different compositions of AV plant including aloe amodin, and phytoestrogens such as beta-cytosterol, could affect sex hormones (Poorfarid *et al*., 2013).

Chloroquine is the most commonly used drug in the treatment of malaria worldwide as well as in the treatment of discoid lupus, extra intestinal amoebiasis and rheumatoid arthritis. Generally chloroquine is given for a short period of time, but in malaria endemic region of tropical Africa, treatment is often repeated in a period as short as 2 weeks due to repeated attack of the sickness (Asuquo O.R *et al*., 2009). Chloroquine had been reported to cause reduction in sperm motility, fertilizing ability of epididymal sperms (Adeeko *et al*., 1998) and fertility in male rats (Vawva *et al.*, 1987). In vivo study in Sprague Dawley rats also reported that the injection of rats with chloroquine for 16 weeks eliminated all leydig cells; due to this the testosterone and other leydig cells may also eliminate which are required for spermatogenesis (Ejebe *et al*., 2008). In the present study it is therefore, considered the toxicity of AV and chloroquine on testicular histopathology, reproductive hormones and sperm morphology in male albino rats.

Materials and methods

Experimental animal:

For this study 18 male albino rats weighing between 180-240 gms and aged 3-4 months were obtained from Shree animal farms, Nimgao, dist. Bhandara, Maharashtra, India. The animals were allowed to acclimatize to the laboratory condition for 7 days prior to start of the experiment. The experimental protocol was approved by Institutional Animal Ethics Committee (Registration number 478/01/a CPCSEA) of the RTM Nagpur University, Nagpur. The rats were caged in a polycarbonate cages with stainless steel lids under hygienic laboratory condition, maintaining 12 hours light/dark cycle of photoperiod with temperature 25±40C and relative humidity. They were fed with standard diet pellets and water ad libitum throughout the experimental period.

Preparation of *Aloe vera* pellets

The fresh AV leaf cut down with the help of sharp sterilized knife. Washed with water and cut transversely into slices. The gel extracted by squeezing the thick epidermis and collected in a small petriplate. Immediately 25mg Aloe vera gel weighed and mix with 2gm flour to make small pellets by using little distill water.

Preparation of chloroquine pellets

2mg chloroquine phosphate powder was weighed and mixed with 2gm of flour to make small pellets by using little distill water. The pellets of AV and C are then given orally daily for 45 days.

Histopathological study

After the completion of 45 days treatment, on 46th day the rats were sacrificed under ether anesthesia and dissected. The testes were excised immediately, weighed and fixed in Bouin's fixative for 24 hours, washed and transferred to 70% alcohol and dehydrated by passing through descending grades of alcohol, cleared in xylene and embedded in paraffin wax. The tissues were cut in serial sections at 5µm. The sections then stained with hematoxylin and eosin (HE) and examined under light microscope for histopathological study. The photomicrographs were taken with the help of digital camera Nikon COOLPIX 8400 attached to the light microscope (Nikon Eclipse E200) and magnified to the required size.

Sperm morphological analysis

The semen sample collected by lacerating the cauda epididymis mixed with normal saline solution and a drop of 5% aqueous eosin (WHO, 2010). The sample was observed under microscope at 100x magnification for assessment of sperm morphological changes. From each rat total 250 spermatozoa were assessed for sperm morphological changes.

Hormone analysis

2ml blood was drawn by cardiac puncture with the help of disposable syringe and collected in a non EDTA tubes. The blood samples send for hormone analysis to Dhruv Pathology Laboratory Nagpur, Maharashtra (India). Serum level of follicle stimulating hormone (FSH), luteinizing hormone (LH) and testosterone (T) were assessed by Chemiluminescent Microparticle Immunoassay (CMIA) Architect Abbott method.

Statistical Analysis

Data from treated and control groups are expressed as mean ± standard error (SEM) and analyzed using student t-test to compare values from experimental and control groups at individual time periods with the help of Graphpad calculator. (Graphpad, 2000). Differences between groups were considered significant at $P<0.001$and $P<0.0001$.

Results

Histopathological study of testes in group I for 45 days duration appeared to be normal showing uniformly arranged seminiferous tubules (ST) bounded by compact basement membrane (BM) separated from one another by interstitial connective tissue (ICT) composed of rounded or polygonal shaped Leydig cells (LC) with central nucleus (N). Each seminiferous tubule is lined by stratified germinal epithelium (GE) followed by spermatogonia (SG) consisting of small and dark nuclei and two stages of spermatocytes (S): primary spermatocytes (PS) present just below the spermatogonial cells, followed by secondary spermatocytes (SS). Two stages of spermatids (S) round and elongated, the lumen (L) of each seminiferous tubule occupied with a whorl like

arrangement of spermatozoa (SZ) and sertoli cells (SC) were observed. (Fig.1. and 1a). Group II showed prominent alterations in testes such as seminiferous tubules (ST) with empty lumen (EL), and cell debris (CD) increased intratubular spaces (ITS), degenerated spermatogonial cells (SG) and vacuolated spermatocytes (VS) were observed as compared to group I (Fig.2. and 2a). Group III for 45 days resulted in atrophic seminiferous tubules (AT), degeneration of interstitial connective tissue (ICT) and Leydig cells (LC). Accumulation of interstitial fluid (IF) between the seminiferous tubules (ST) was prominently observed. It was significantly noticed that due to gradual degeneration of cellular matter inside the seminiferous tubule (spermatogonia, spermatocytes, sertoli cells and spermatids) there was significant increased in intratubular spaces (IS) and the central lumen (L) was found to be occupied with cell debris (CD) as compared to group I (Fig.3. and 3a).

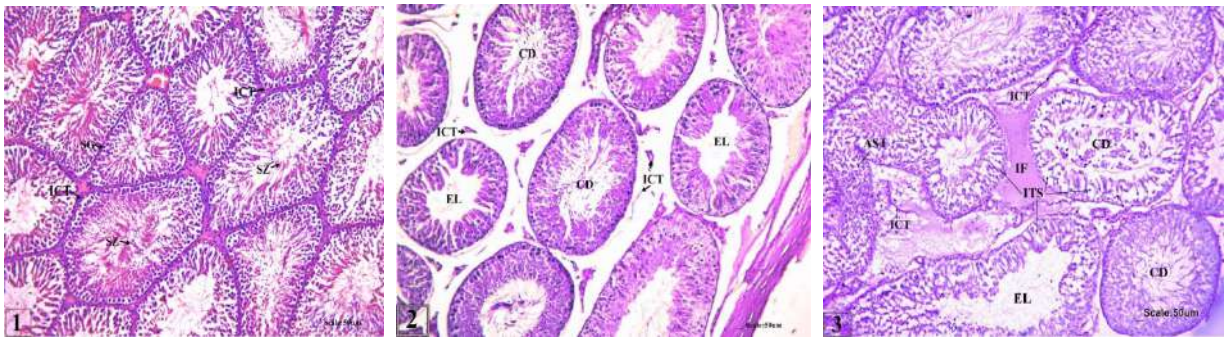

Fig1. Transverse section of testis of group I showing normal histoarchitechture of testes- with uniform seminiferous tubules (ST), spermatogonia (SG), spermatocyte (SCY), spermatozoa (SZ), spermatid (S) and Interstitial cells (IC). Fig 2. Transverse section of testis of group II showing histopathological changes-cell debris (CD), empty lumen (EL), detached basement membrane (BM), and degenerating spermatogonia (SG). Fig 3. Transverse section of testis of group III showing atrophic tubules (AT), accumulated interstitial fluid (IF) and cell debris (CD) (Stained with H&E, Scale bar =50µm).

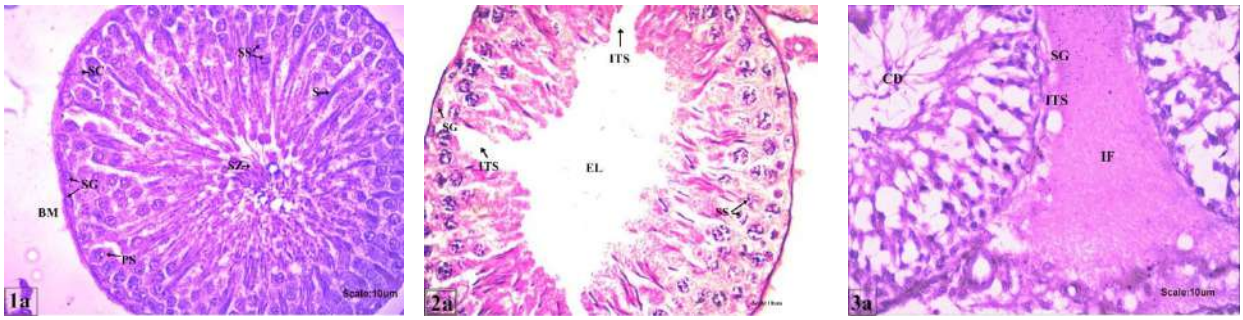

Fig.1a, 2a, and 3a indicates magnified images of fig. 1, 2 and 3 respectively (Stained with H&E, Scale bar = 10µm).

Sperm morphological abnormalities

In the present investigation cauda epididymal sperms of group I (control) for 45 days duration appears normal (97%) showing hook or sickle shaped sperm head containing a dense nucleus with less dense tip, the acrosome. The slender mid piece and the tail region is straight,

uniform and thinner than the mid piece (Fig.4.). The sperm morphological abnormalities observed in group II and III for 45 days durations of treatment are deformed head, detached head, bifurcated head, banana shaped head, hook less head, bent neck, curved mid piece, coiled mid piece along with tail, bent tail, headless tail, looped tail (Fig.5-15).

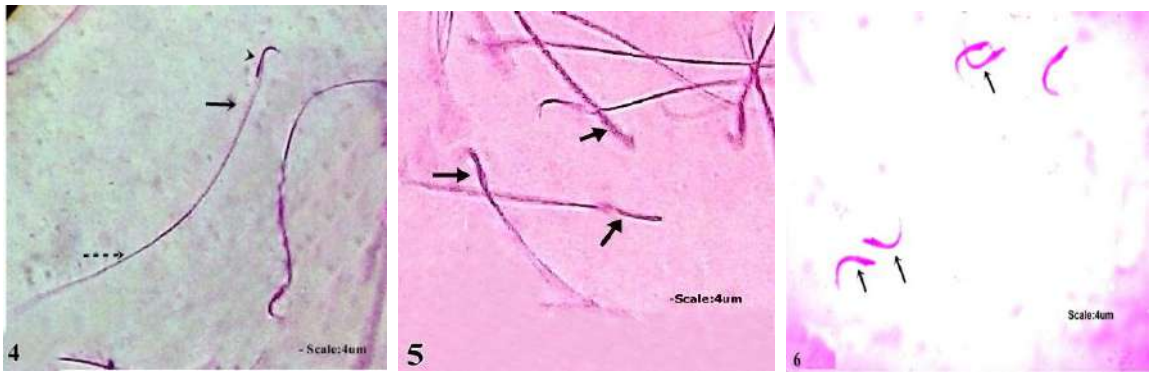

Fig. 4. Sperm showing normal morphology, fig. 5 deformed head, fig. 6 detached head (indicated by arrow).

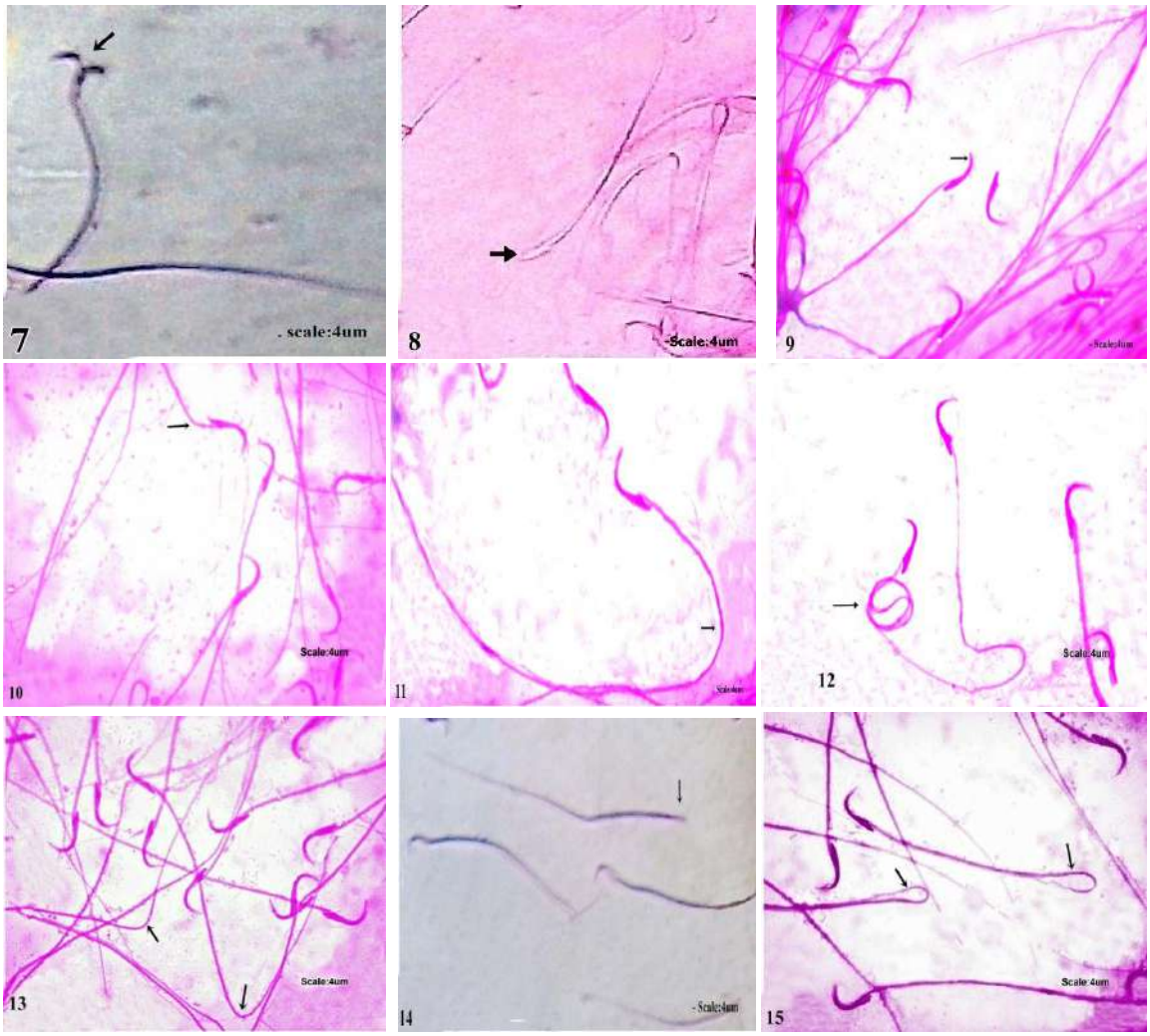

Fig. 7 bifurcated head, fig. 8 banana shaped head, fig. 9 hook less head, fig. 10 bent neck, fig. 11 curved mid piece, fig. 12 coiled mid piece along with tail, fig. 13 bent tail, fig. 14 headless tail, fig. 15 looped tail (indicated by arrow).

Serum hormonal level

The estimated serum FSH and LH level in group I was found to be (0.168 ± 0.007), (0.188 ± 0.008) mIU/ml respectively. After 45 days treatment of AV, significant ($P \leq 0.001$) decline in serum FSH (0.091± 0.005) and LH (0.110 ± 0.008) levels were observed in group II whereas group III treated with C showed extremely significant ($P \leq 0.0001$) decline in serum FSH (0.075± 0.003) and LH (0.091± 0.007) levels as compared to group I (Fig. 16).

The estimated serum T level in group I was found to be (42.96 ± 0.98) ng/ml. After 45 days treatment of AV, significant ($P \leq 0.001$) decline in serum T (35.26 ±1. 0007) levels were observed in group II whereas group III treated with C showed extremely significant ($P \leq 0.0001$) decline in serum T (32.18 ± 1.006) levels as compared to group I (Fig. 17).

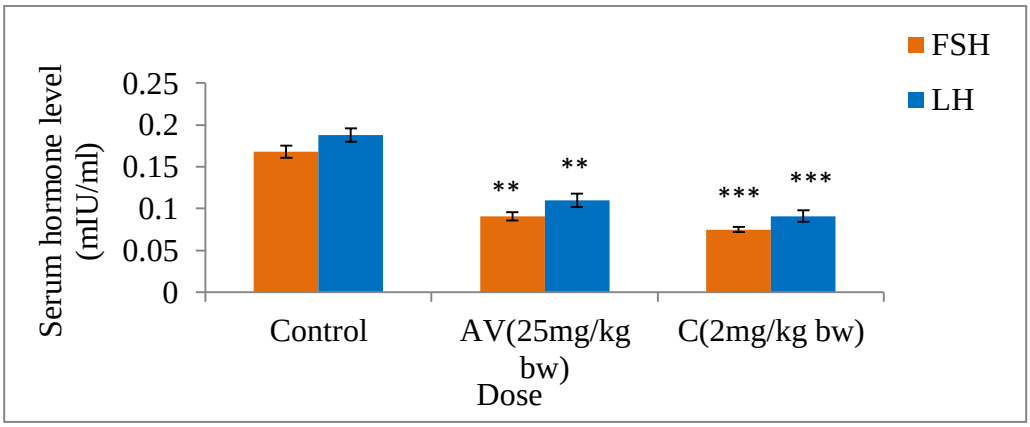

Fig. 16. Serum FSH and LH level of control, AV and C treated albino rats for 45 days duration. Data are expressed as mean ± standard error (SEM)
** P≤0.001, *** P≤ 0.0001.

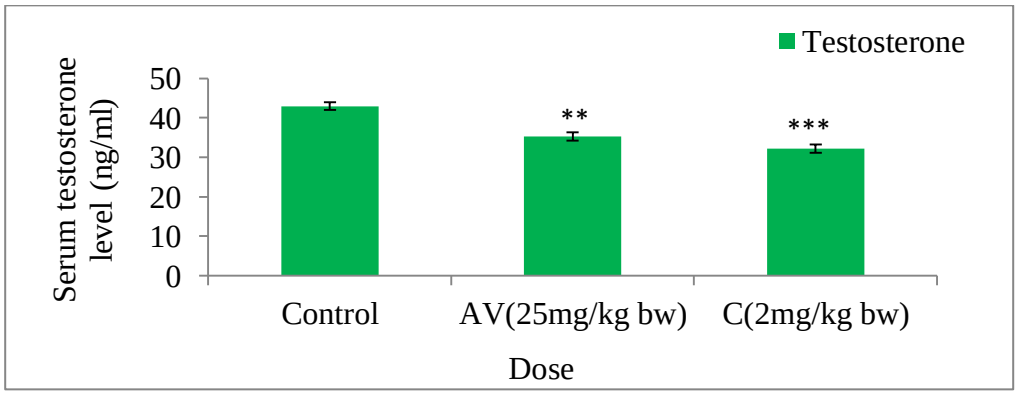

Fig.17. Serum testosterone level of control, AV and C treated albino rats for 45 days duration. Data are expressed as mean ± standard error (SEM)
** P≤0.001, *** P≤ 0.0001.

Discussion

The histopathological results of the present study revealed increased interstitial spaces, vacuolation of Leydig cells, increased intratubular spaces between the spermatogonia, detached basement membrane and central lumen with germinal cell debris in group II. These results are in agreement with AV (Ahbab *et al*., 2014; Owoyemi *et al*., 2015: Jiwantare *et al*, 2017: Dhurvey *et al*., 2020), *Chromolaen aodoratum* (Yakubu *et al*., 2007) and *Azadirachta indica* (Aladakatti *et al*., 2011). In the present investigation the prolonged treatment results in vacuolation in the spermatogonia and spermatocytes, depletion of germinal epithelium, empty lumen, fused seminiferous tubules due to depletion of germinal epithelial layer, thickening of perivascular wall of blood vessel with cellular exudate. These results are concomitent with Yinusa *et al*., (2005); Zheng *et al*., (2006); Sharaf *et al*., (2012); Thakur *et al*., (2014); Alalwani, (2014). Results of group III in the present investigation for short and longer duration revealed significant histological alterations such as unevenly distributed seminiferous tubules, atrophic tubules, degenerated interstitial connective tissue, depletion of spermatogonia and germinal epithelium, degeneration of Leydig cells, accumulation of interstitial fluid between the seminiferous tubules was prominent and lumen with cell debris. These results are consistent with (Asuquo *et al*., 2007 and 2009) showing adverse effect of C on histopathology of testes. Various authors mentioned similar effects of different synthetic drugs on testes (Izunya *et al*., 2010) artesunate, (Murdakai *et al*., 2011) cotecxin, (Mutalip *et al*., 2013) testosterone, nandrolone, and stanozolol.

Semen analysis is the cornerstone of the evaluation of male fertility. Routine semen analysis can many times allow a definitive diagnosis of infertility. Therefore, the typical measurements done in a semen analysis include sperm concentration, sperm motility, progressive sperm motility, sperm morphology and foreign cell contamination (Baqir and Sodani, 2014). The findings of the present study revealed negligible change in sperm morphology of group I for 45 day's duration whereas group II and III showed significant increase in the sperm morphological abnormalities as compared to group Il. These results are in agreement with (Oyeyemi and Fayomi 2011; Ahbab *et al*., 2014; Oyeyemi and Ajani, 2015) AV, (Desai *et al*., 2018) C, (Gupta *et al*., 2013; Alaa-Eldin *et al*., 2017) combined effect of sulphasalazine and ampicillin, chlorpyrifos and cypermethrin respectively. The mid- piece abnormalities are associated with the deficiency of zinc. Since zinc and folate are involved in the synthesis of DNA and RNA its deficiency has been shown to cause impaired male fertility in the form of reduced sperm motility, reduced percentage motility of sperm, morphological abnormalities and reduced spermatogenesis (Wong *et al*., 2000). Similar observations of increased sperm morphological abnormalities caused due to different herbal and synthetic drugs has been reported (Mishra and singh, 2005; Nwanjo *et al*.,2006; Patil *et al*., 2010; and Oyedeji *et al*., 2013;) on effect of *Azadirachta indica*, dihydroartemisinin, *Terminalia bellirica* and aspirin. Thus AV and C individually has potential to exert deleterious effect on sperm morphology of male albino rats and may impair the fertility.

The functioning of the reproductive process is under the control of hormonal pathways. Luteinizing hormone (LH) acts exclusively on Leydig cells in the testis and is the primary regulator of Testosterone (T) secretion (Creasy and Foster 2002). 45 days duration of treatment with AV and

C results in significant decline in FSH, LH and T levels as compared to control. Present study results are in accordance with (Asuquo *et al*., 2012) *Spondias mombin*; (Onyeka *et al*., 2013) *Chrysophyllum albidum*; **(**Alaa-Eldin *et al*., 2017) of chlorpyrifos and cypermethrin: (Mali *et al*., 2002) *Martynia annua* and (Onyeka *et al*., 2013) *Chrysophyllum albidum*. AV (Ahbab *et al*., 2014); Jussiaea repens (Ghosal and Pradhan 2016); (Yakubu *et al*., 2007); Chromolaena odorata (Yakubu, 2012).

Conclusion

The findings of the present study concluded that herbal product AV and synthetic drug C individually have potential to cause toxic effect on testes and sperm morphology by unbalancing the hormonal levels in a dose and duration dependent manner.

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