

## Gonadosomatic Index and Spermatozoa Morphological Characteristics of Male Wistar Rats Treated with Graded Concentration of Aloe Vera Gel

M.O. Oyeyemi and A.P. Fayomi

Department of Veterinary Surgery and Reproduction, Faculty of Veterinary Medicine,  
University of Ibadan, Ibadan, Nigeria

**Abstract:** Male Wistar rats were used to study the effects of graded concentration of Aloe vera gel on the gonadosomatic index and the spermatozoa morphological characteristics. Ninety six rats (140 to 255 g) were randomly grouped into four: A (Control), B (200 mg/kg), C (300 mg/kg) and D (400 mg/kg); and were treated for one, two and three weeks. Samples were collected after each of these periods. The results revealed significant increase ( $p < 0.05$ ) in the gonadosomatic index of the testis and the epididymis as well as significant increase ( $p < 0.05$ ) in the percentage of spermatozoa abnormalities in the test groups when compared with the control. These increase ( $p < 0.05$ ) were concentration-dependent for each week and increases ( $p < 0.05$ ) with duration of administration from the first week to the third week. It was therefore concluded that Aloe vera gel should be used with caution in breeding bulk, stud, ram and bull; and should be less than 200 mg/kg when being administered for up to 7 consecutive days.

**Key words:** Aloe vera gel, gonadosomatic index, graded concentration, male wistar rats, morphological characteristics, spermatozoa

### INTRODUCTION

People interest is on the increase on medications that are/or contain components of natural origin, as such, certain plants have gained popularity in the world (Holanda *et al.*, 2009). Aloe vera is one of these popular plants (Rajasekaran *et al.*, 2005). Aloe vera has been used in the traditional medicine of many cultures (Chithra *et al.*, 1998). The genus Aloe (belonging to the family Liliaceae) is a shrubby tropical or sub tropical plant which has succulent and elongated leaves (Pugh *et al.*, 2001; Rajasekaran *et al.*, 2005).

Among the over 360 Aloe species known, Aloe Barbadensis Miller (Aloe vera) is the most widely used (Pugh *et al.*, 2001; Vogler and Ernst, 1999). Aloe vera is a cactus-like plant that grows readily in hot, dry climates and currently, because of demand is cultivated in large quantities (Vogler and Ernst, 1999). The plant comprises of yellowish exudate (containing anthraquinone derivatives) which is called the juice and a clear mucilaginous fluid from the parenchyma known as the gel (containing polysaccharides mainly) (Pugh *et al.*, 2001; Holanda *et al.*, 2009; Vogler and Ernst, 1999). The fresh gel, juice or formulated products are being used for medical and cosmetic purposes and for general health (Chithra *et al.*, 1998) but most commercially available products are based on the gel (Vogler and Ernst, 1999).

Aloe vera (Av) gel contains not less than 75 potentially active constituents including alkaloids, glycoproteins, resins, enzymes, vitamins, amino acids,

minerals, salicylic acids, sterols, genolins, saponis, and lignin (Atherton, 1998; Holanda *et al.*, 2009; Ni *et al.*, 2004). Consequently, Aloe vera gel has been used for the treatment of burns and other wounds (Pugh *et al.*, 2001; Grindlay and Reynolds 1986; Joshi, 1998) arthritis, acne, gout (Chithra *et al.*, 1998; Grindlay and Reynolds, 1986); dermatitis, cutaneous leishmaniasis (Holanda *et al.*, 2009; Capasso *et al.*, 1998); eczema, coldsores, ulcers, asthma, candida, chronic fatigue syndrome, digestive and bowel disorders (Newall *et al.*, 1996; Holanda *et al.*, 2009; Vogler and Ernst, 1999).

The observed pharmacological activities of Av gel include anti-inflammatory, antifungal, antimicrobial, antiviral, antidiabetic and anticancer activities (Vogler and Ernst, 1999; Holanda *et al.*, 2009; Capasso *et al.*, 1998; Reynolds and Dweck, 1999) with physiologic hypoglycemic, hypolipidemic (Holanda *et al.*, 2009; Patel and Mengi, 2008) and Immuno stimulatory (Pugh *et al.*, 2001) activities.

Recently, Aloe vera gel has been associated with some side effects which include burning after topical application (Fulton, 1990), contact dermatitis (Williams *et al.*, 1996) and mild hitching (Syed *et al.*, 1996); hepatotoxicity in man (Rabe *et al.*, 2005) and rat (Holanda *et al.*, 2009). In the mice, high dose was associated with decreased red cell count as well as sperm damage (Shah *et al.*, 1989).

There is, however, dearth of information on the effects of Av gel on the reproductive potential of the male

wistar rat especially with focus on the testis, epididymis and morphological characteristics of the spermatozoa. Therefore, this study was designed to investigate the gonadosomatic index and spermatozoa morphological characteristics of the male wistar rats treated with graded concentration of Aloe vera gel.

## MATERIALS AND METHODS

**Experimental animal:** Ninety six (96) sexually mature male wistar rats were used to study the gonadosomatic index and spermatozoa morphological characteristics of male wistar rats treated with graded concentration of Aloe vera gel. Each weighed between 140 to 255 g and aged 16 to 20 weeks.

**Experimental animal management:** These rats were housed in the Experimental Animal Unit of the Faculty of Veterinary Medicine, University of Ibadan, Oyo State, Nigeria. University of Ibadan is about 6 kilometer to the North of Ibadan city at latitude 3°54' E and latitude 7°26' N at mean altitude of 277 m above sea level. The annual rainfall is about 1200 mm most of which fall between April and October, and a dry season from November to March during which this work was done.

They were kept in cages made of a circular plastic of about 60 cm in diameter and depth of about 20 cm with wooden and wire meshes top. Wood shaven was used as bedding and was replaced every week. The rats were fed *ad libitum* with commercially prepared rat feed containing 21% protein, 3.5 fat, 6% fibre, and 0.8% phosphorus by Ladokun feeds Limited, Ibadan; and water from hygienic source was given *ad libitum*. These feeds and water were supplied using the earthen troughs.

**Aloe vera gel extracts preparation:** The Aloe vera stem was harvested on each day of administration. This was thoroughly washed and rinsed under flowing tap water. It was then finally rinsed with distilled water.

The sharp edge of the Aloe vera stem was cut and the fleshy mass carefully opened up. The flowing gel was carefully collected into a beaker (which had its weight predetermined) and weighed. 2.0, 3.0 and 4.0 g of Aloe vera gel were weighed using a digital microsensitive weighing scale. Each of these was then diluted with 100ml of distilled water (measured by the measuring cylinder) to form 200, 300 and 400 mg/kg concentration. These were gently stirred with spatula to achieve homogenous solution.

**Gel administration:** These rats were randomly grouped into 4 groups (A to D) of 24 rats each. The group A was used as control given distilled water while the treatment Groups B, C and D were given 200, 300 and 400 mg/kg of the gel respectively. These rats were dosed orally in the morning (07:00-09:00 h) using oral cannula and tuberculin syringe.

**Sample collection:** Samples were collected after one week, 2 weeks and 3 weeks of gel administration.

Rats from each group were randomly selected; weighed and euthanized by decapitation. The testes were immediately exteriorized through a mid-caudoventral abdominal incision with a sterile scapel blade. Sperm cells were then collected from the cauda epididymis while the two testes and the epididymis were immediately weighed using a sensitive electronic weighing machine.

Smears were prepared from the collected epididymal sample and stained with Wells and Awa stain for morphological studies as previously described (Zemjanis, 1970).

**Data analysis:** The mean percentages and standard error of means were calculated for Gonadosomatic index (calculated as Gonad weight/body weight x100%) and morphological characteristics of the spermatozoa. One way ANOVA (Analysis of Variance) and Duncan<sup>a</sup> multiple comparison test of the Statistical Package for Social Sciences (SPSS) were used to establish any significant difference at 95% confidence interval. P values less than 0.05 were considered significant.

## RESULTS

**Gonadosomatic index:** The results are presented as mean  $\pm$  standard error of the mean as shown in Table 1. A concentration - dependent increase ( $p < 0.05$ ) gonadosomatic index was observed (Fig. 1).

**The Epididymis:** Significant difference ( $p < 0.05$ ) exist between the control group and the groups C and D after one week of gel administration. There is, however, a significant difference ( $p < 0.05$ ) in the GSI of the control group and each treatment group and between the treatment groups after two weeks of gel administration. After three weeks of gel administration, the significant difference ( $p < 0.05$ ) observed was between the control group and the test groups.

**The testes:** Significant difference ( $p < 0.05$ ) between the control group and the test groups; and between the groups B and C and the group D after one week of gel administration exist. Similar results of significant difference ( $p < 0.05$ ) between the control and the treatment groups as well as significant difference ( $p < 0.05$ ) in between the treatment groups were observed after the second and the third week of gel administration.

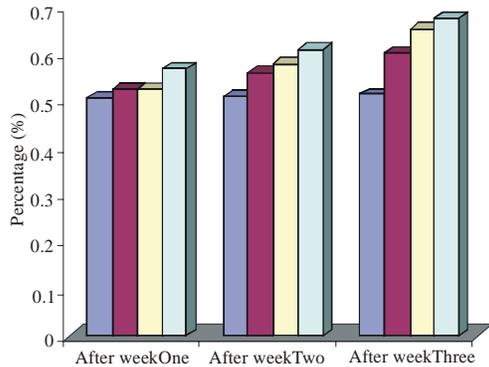
**Morphological characteristics:** The results are presented as mean + standard error of the mean as shown in Table 2. A concentration - dependent increase ( $p < 0.05$ ) in percentage abnormality of the spermatozoa was observed (Fig. 2).

Table 1: Gonadosomatic index

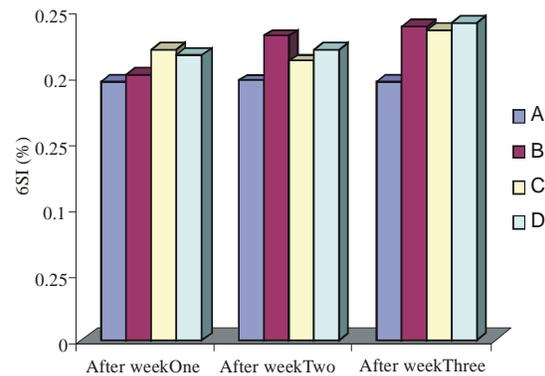
After one week	Groups	Right testis (%)	Left testis (%)	Epididymis (%)
After one week	A	0.505±0.007 <sup>c</sup>	0.505±0.0011 <sup>c</sup>	0.195±0.0007 <sup>b</sup>
	B	0.526±0.009 <sup>b</sup>	0.523±0.0096 <sup>b</sup>	0.2±0.0013 <sup>b</sup>
	C	0.525±0.0014 <sup>b</sup>	0.525±0.0014 <sup>b</sup>	0.22±0.0021 <sup>a</sup>
	D	0.57±0.0021 <sup>a</sup>	0.555±0.0023 <sup>a</sup>	0.216±0.0026 <sup>a</sup>
After two weeks	A	0.51±0.0045 <sup>d</sup>	0.51±0.0043 <sup>d</sup>	0.196±0.0007 <sup>d</sup>
	B	0.56±0.0012 <sup>c</sup>	0.545±0.0014 <sup>c</sup>	0.229±0.0031 <sup>a</sup>
	C	0.58±0.001 <sup>b</sup>	0.573±0.0015 <sup>b</sup>	0.21±0.0023 <sup>c</sup>
	D	0.614±0.0011 <sup>a</sup>	0.635±0.0011 <sup>a</sup>	0.22±0.0021 <sup>b</sup>
After three weeks	A	0.513±0.0038 <sup>d</sup>	0.513±0.0033 <sup>d</sup>	0.195±0.0007 <sup>b</sup>
	B	0.601±0.0038 <sup>c</sup>	0.611±0.0025 <sup>c</sup>	0.236±0.0038 <sup>a</sup>
	C	0.654±0.0026 <sup>b</sup>	0.65±0.0028 <sup>b</sup>	0.235±0.001 <sup>a</sup>
	D	0.676±0.002 <sup>a</sup>	0.675±0.0023 <sup>a</sup>	0.24±0.002 <sup>a</sup>

Table 2: Morphological characteristics (percentage abnormalities) of spermatozoa

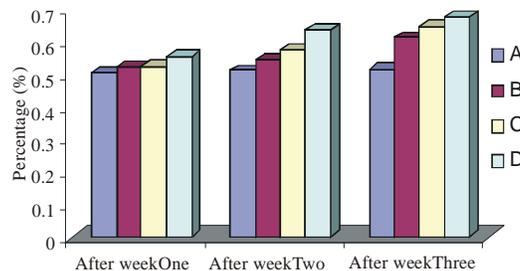
Groups	Rudimentary Tail (%)	Bent Tail (%)	Looped Tail (%)	Head without Tail (%)	Tail without Head (%)	Coiled Tail (%)	Curved Mid piece (%)	Bent mid piece (%)	Curved Tail (%)	Total (%)	
After one week	A	0.373±0.0097 <sup>d</sup>	1.128±0.235 <sup>c</sup>	0.421±0.0028 <sup>d</sup>	0.56±0.1517 <sup>c</sup>	0.582±0.0167 <sup>c</sup>	1.256±0.0222 <sup>c</sup>	1.856±0.0483 <sup>c</sup>	1.6±0.0071 <sup>d</sup>	1.43±0.041 <sup>b</sup>	9.232
	B	0.648±0.0159 <sup>c</sup>	2.268±0.0394 <sup>b</sup>	0.614±0.0077 <sup>c</sup>	0.716±0.0258 <sup>b</sup>	0.736±0.0316 <sup>b</sup>	1.612±0.0116 <sup>b</sup>	2.516±0.0327 <sup>b</sup>	2.23±0.0288 <sup>c</sup>	1.426±0.0051 <sup>b</sup>	12.766
	C	0.874±0.0051 <sup>b</sup>	2.3±0.0247 <sup>b,c</sup>	0.6586±0.082 <sup>b</sup>	0.844±0.0144 <sup>a</sup>	0.0848±0.0086 <sup>a</sup>	1.676±0.00715 <sup>b</sup>	2.826±0.0068 <sup>a</sup>	2.376±0.0078 <sup>b</sup>	1.452±0.034 <sup>b</sup>	13.854
	D	1.004±0.035 <sup>a</sup>	2.374±0.0136 <sup>c</sup>	0.828±0.0235 <sup>a</sup>	0.874±0.0093 <sup>a</sup>	0.852±0.0073 <sup>a</sup>	1.79±0.0481 <sup>a</sup>	2.878±0.0058 <sup>a</sup>	2.512±0.0198 <sup>a</sup>	1.642±0.0139 <sup>a</sup>	14.804
After two weeks	A	0.392±0.0054 <sup>d</sup>	1.094±0.025 <sup>d</sup>	0.426±0.0037 <sup>d</sup>	0.596±0.0147 <sup>d</sup>	0.57±0.017 <sup>c</sup>	1.306±0.0196 <sup>d</sup>	1.854±0.0279 <sup>d</sup>	1.808±0.0092 <sup>c</sup>	1.384±0.0133 <sup>d</sup>	9.23
	B	0.714±0.0157 <sup>c</sup>	2.362±0.0334 <sup>b</sup>	0.78±0.0207 <sup>c</sup>	0.838±0.0086 <sup>c</sup>	0.852±0.0116 <sup>b</sup>	1.708±0.0107 <sup>c</sup>	2.864±0.0093 <sup>c</sup>	2.39±0.0324 <sup>b</sup>	1.512±0.0447 <sup>c</sup>	14.02
	C	0.68±0.0086 <sup>b</sup>	2.483±0.0218 <sup>b</sup>	0.862±0.18 <sup>b</sup>	0.91±0.0098 <sup>b</sup>	0.91±0.0095 <sup>b</sup>	1.964±0.0132 <sup>b</sup>	2.964±0.0224 <sup>b</sup>	2.564±0.0225 <sup>a</sup>	1.716±0.0103 <sup>b</sup>	15.32
	D	1.166±0.0238 <sup>a</sup>	2.752±0.053 <sup>a</sup>	1.238±0.0388 <sup>a</sup>	1.222±0.0441 <sup>a</sup>	1.256±0.0328 <sup>a</sup>	2.12±0.0506 <sup>a</sup>	3.184±0.0206 <sup>a</sup>	2.67±0.0472 <sup>a</sup>	1.87±0.0205 <sup>a</sup>	17.478
After three weeks	A	0.398±0.005 <sup>d</sup>	1.136±0.025 <sup>d</sup>	0.427±0.0031 <sup>d</sup>	0.606±0.0172 <sup>d</sup>	0.58±0.0145 <sup>d</sup>	1.318±0.028 <sup>c</sup>	1.836±0.0353 <sup>d</sup>	1.624±0.166 <sup>d</sup>	1.378±0.0231 <sup>d</sup>	9.303
	B	0.862±0.0171 <sup>c</sup>	2.57±0.04 <sup>c</sup>	0.878±0.0136 <sup>c</sup>	0.944±0.0121 <sup>c</sup>	0.944±0.0093 <sup>c</sup>	2.154±0.0269 <sup>c</sup>	2.932±0.0186 <sup>c</sup>	2.552±0.0139 <sup>c</sup>	1.77±0.0222 <sup>c</sup>	15.606
	C	1.068±0.032 <sup>b</sup>	2.92±0.025 <sup>b</sup>	1.218±0.0662 <sup>b</sup>	1.287±0.0236 <sup>b</sup>	1.318±0.0254 <sup>b</sup>	2.248±0.0416 <sup>b</sup>	3.2±0.0216 <sup>b</sup>	2.83±0.0114 <sup>b</sup>	2.016±0.037 <sup>b</sup>	18.102
	D	1.326±0.0367 <sup>a</sup>	3.2±0.169 <sup>a</sup>	1.506±0.0385 <sup>a</sup>	1.562±0.076 <sup>a</sup>	1.47±0.0239 <sup>a</sup>	2.832±0.0402 <sup>a</sup>	3.368±0.0451 <sup>a</sup>	3.172±0.0346 <sup>a</sup>	2.256±0.0825 <sup>b</sup>	20.692



(a) Gonadosomatic index of right testis



(b) Gonadosomatic index of epididymis

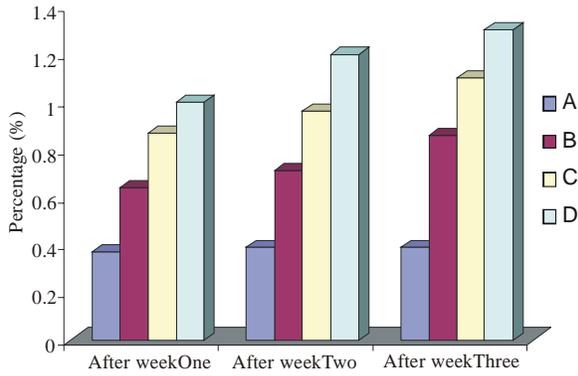


(c) Gonadosomatic index of left testis

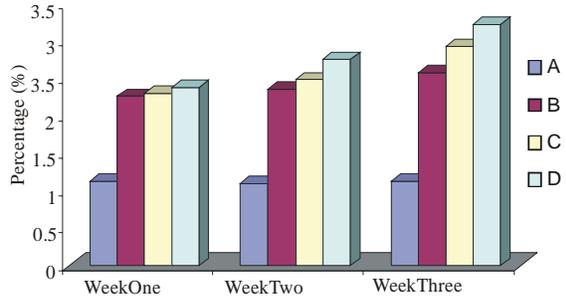
Fig. 1: Gonadosomatic index

**Rudimentary tail abnormality:** There are significant differences ( $p<0.05$ ) between the control and the treatment groups as well as in between the treatment groups after one week, two weeks and three weeks of gel administration.

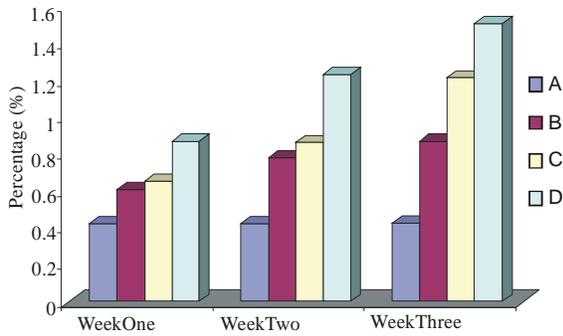
**Bent tail abnormality:** Significant difference ( $p<0.05$ ) exist between the control group and the treatment groups, and only between the group B and D of the treatment groups after one week of gel administration. The significant differences ( $p<0.05$ ) observed after two and



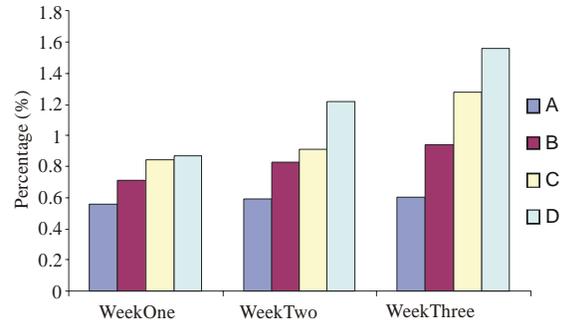
(a) Rudimentary tail abnormality



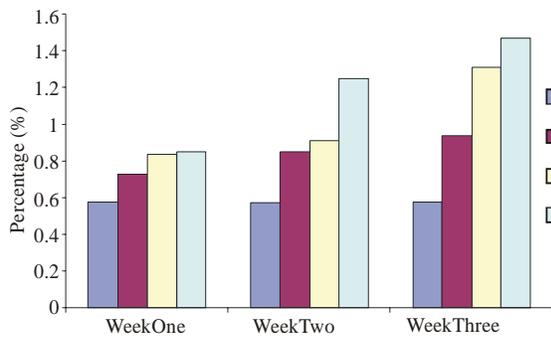
(b) Bent tail abnormality



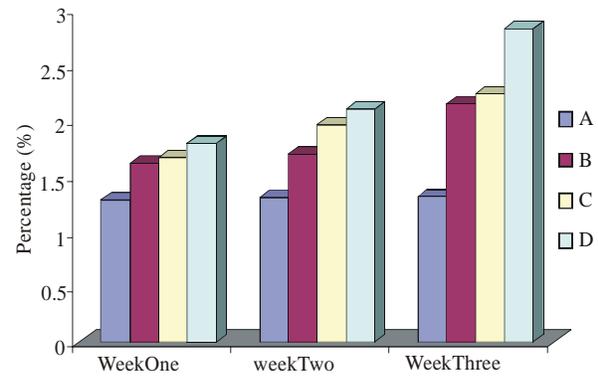
(c) Looped tail abnormality



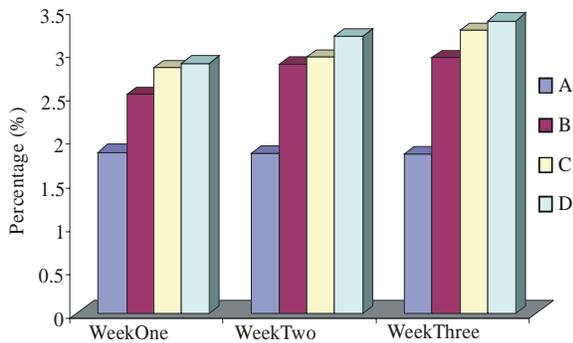
(d) Normal head without tail abnormality



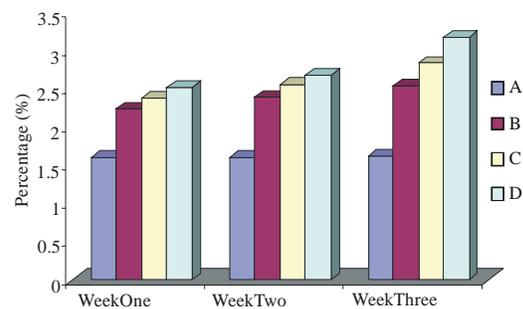
(e) Normal tail without head abnormality



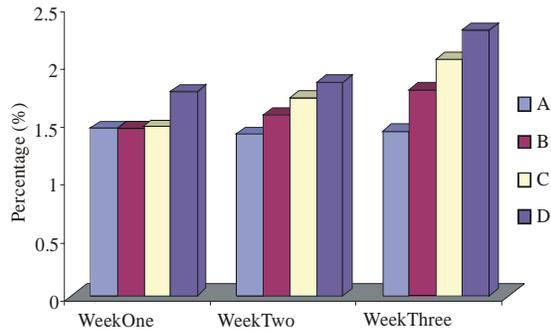
(f) Coiled tail abnormality



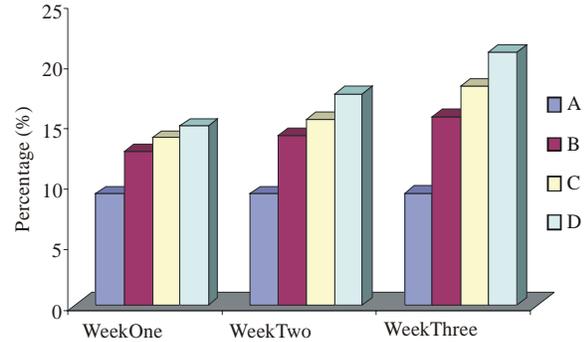
(g) Curved mid piece abnormality



(h) Bent mid piece abnormality



(i) Curved tail abnormality



(j) Total percentage abnormality

A: Control Group  
 B: 200 mg/kg Group  
 C: 300 mg/kg Group  
 D: 400 mg/kg Group

Fig. 2: Morphological characteristics (percentage abnormalities) of spermatozoa

three weeks of gel administration is between the control and the treatment groups and in-between the treatment groups.

**Looped tail abnormality:** This also gave significant difference ( $p < 0.05$ ) between the control and the treatment groups as well as in-between the treatment groups after one week, two weeks and three weeks of gel administration.

**Normal head without tail abnormality:** The observed abnormality here includes a significant difference ( $p < 0.05$ ) between the control and the test groups as well as between group B and groups C and D after 1 week of gel administration. The significant difference ( $p < 0.05$ ) observed after two and three weeks of gel administration is between the control and the treatment groups and in-between the treatment groups.

**Normal tail without head abnormality:** There are significant differences ( $p < 0.05$ ) between the controls and the Treatment groups after one, two and three weeks of gel administration. The in-between treatment groups differences ( $p < 0.05$ ) are between the group B and groups C and D after one week of gel administration; groups B and C and group D after two weeks of administration and between groups B, C and D after 3 weeks of gel administration.

**Coiled tail abnormality:** There are significant differences ( $p < 0.05$ ) between the controls and the treatment groups after one, two and three weeks of gel administration. Significant difference between the groups B and C and the group D (after one week), between groups B, C and D (after two weeks) and groups B and C and D (after 3 weeks) were also observed.

**Curved mid-piece abnormality:** A significant difference ( $p < 0.05$ ) exist between the control group and the treatment groups and between group B and the groups C and D after one week of gel administration. The significant differences ( $p < 0.05$ ) observed after two and three weeks of gel administration were between the controls and the treatment groups and in-between the treatment groups.

**Bent mid piece abnormality:** There were significant differences ( $p < 0.05$ ) between the controls and the treatment groups after weeks one, two and three with significant differences in-between the treatment groups after week one and three. Significant difference ( $p < 0.05$ ) only exist between the group B and the groups C and D after two weeks of gel administration.

**Curved tail abnormality:** Significant difference ( $p < 0.05$ ) only exist between the control group and the group D after one week of gel administration. While significant differences ( $p < 0.05$ ) between the control groups and the treatment groups as well as in-between the treatment groups were observed after two and three weeks of gel administration.

## DISCUSSION

The observed concentration-dependent increased GSI after one, two and three weeks of gel administration was probably due to concentration-dependent excessive flow of fluid into the epididymis and the testis as administration of Aloe Vera gel has been observed to increase the bio-distribution of sodium ions in the testis of wistar rat (Holanda *et al.*, 2009) and Cellular migration of sodium ions is linked with fluid movement into the cells (Byers and Graham, 1989). This fluid accumulation

increases with increased concentration of aloe vera gel intake and prolonged duration of administration.

The change in osmolarity due to excessive inflow of sodium ions as well as the change in tonicity due to the excessive migration of fluid accompanying the sodium ions migration is only mildly tolerated by the spermatozoa, and when these deviate considerably; spermatozoa abnormalities increase as observed in this study (Foote, 1970). These results corroborate previous work (Oyeyemi and Fayomi, 2009) where we observed concentration dependent reduction in spermatozoa motility, livability and count after one, two and three weeks of gel administration in the male wistar rats.

This work, therefore, shows that the administration of Aloe vera gel up to and above 200 mg/kg for at least one week will increase the weight of the gonads and impair normal structure of the spermatozoa which will definitely affect the fertilization capacity of the spermatozoa in the wistar rat. It is, therefore, recommended that further work using other animals should be carried out to ascertain the reproductive safety of Aloe vera gel at these concentrations. Also, active component of the gel responsible for this need be investigated.

### CONCLUSION

Oral administration of Aloe vera gel up to 200, 300 and 400 mg/kg for 7, 14 and 21 consecutive days affects fertility of male wistar rat. Hence, it should be used with caution in breeding buck, stud, ram, bull and man but not up to these concentrations for these numbers of days.

### ACKNOWLEDGMENT

We acknowledge that this work was done by us i.e. Oyeyemi M.O. and Fayomi A.P. in the location and time stated in the manuscript. We made contributions to this work alone.

### REFERENCES

- Atherton, P., 1998. Aloe vera revisited. *Br. J. Phytotherapy*, 4: 176-183.
- Byers, S. and R. Graham, 1989. Distribution of sodium-potassium ATPase in the rat testis and epididymis. *Am. J. Anaomy*, 188: 31-43.
- Capasso, F., E. Borrelli, R. Capasso, G. Di Carlo, A.A. Izzo, L. Pinto, N. Mascolo, S. Castaldo and R. Longo, 1998. *Aloe* and its therapeutic use. *Phytother. Res.*, 12: 124-127.
- Chithra, P., G.B. Sajithlal and G. Chandrakasan, 1998. Influence of *Aloe vera* on the healing of dermal wounds in diabetic rats. *J. Ethnopharm.*, 59: 195-201.
- Foote, R.H., 1970. Fertility of bull semen at high extensive rates in tris-buffered extenders. *J. Dairy Sci.*, 53: 1475-1477.
- Fulton, J.E., 1990. The stimulation of postdermabrasion wound healing with established aloe vera polyethylene oxide dressing. *J. Dermatol. Surg. Oncol.*, 16: 460-467.
- Grindlay, D. and T. Reynolds, 1986. The *Aloe vera* phenomenon: A review of the properties and modern uses of the leaf parenchyma gel. *J. Ethnopharmacol.*, 16: 117-151.
- Holanda, C.M.C.X., M.B. Costa, N.C.Z. Silva, M.F. Silva Junior, V.S. Arruda Barbosa, R.P. Silva and A.C. Medeiros, 2009. Effect of an extract of *Aloe vera* on the biodistribution of sodium pertechnetate ( $\text{Na}^{99m}\text{TcO}_4$ ) in rats. *Acta Cir. Bras.*, 24: 5.
- Joshi, S.P., 1998. Chemical constituents and biological activity of *Aloe barbadensis* - A review. *J. Med. Aromat. Plant Sci.*, 20: 768-773.
- Newall, C.A., L.A. Anderson and J.D. Phillipson, 1996. *Herbal Medicines. A Guide for Health-Care Professionals*. The Pharmaceutical Press, London.
- Ni, Y., D. Turner, K.M. Yates and I. Tizard, 2004. Isolation and characterization of structural components of *Aloe vera* L. leaf pulp. *Int. Immunopharm.*, 4: 1745-1755.
- Oyeyemi, M.O. and A.P. Fayomi, 2009. Reproductive potential of Wistar rats treated with aloe vera gel extract. Proceeding of the 13<sup>th</sup> East, Central and Southern African Commonwealth Veterinary Association, Regional Meeting and International Conference, Kampala, Uganda, 8-13, November.
- Patel, P.P. and S.A. Mengi, 2008. Efficacy studies of *Aloe vera* gel in atherosclerosis. *Atherosclerosis Supp.*, 9: 210-211.
- Pugh, N., S.A. Ross, M.A. El-Sohly and S. David, 2001. Characterization of aloeride, a new high-molecular-weight polysaccharide from *Aloe vera* with potent immunostimulatory activity. *J. Agric. Food Chem.*, 49: 1030-1034.
- Rabe, C., A. Musch, P. Schirmacher, W. Kruis and R. Hoffmann, 2005. Acute hepatitis induced by an *Aloe vera* preparation: A case report. *J. Gastroenterol.*, 11: 303-304.
- Rajasekaran, S., K. Sivagnanam and S. Subramanian, 2005. Antioxidant effect of *Aloe vera* gel extract in streptozotocin-induced diabetes in rats. *Pharmacol. Reports*, 57: 90-96.
- Reynolds, T. and A.C. Dweck, 1999. *Aloe vera* leaf gel: A review update. *J. Ethnopharm.*, 68: 3-37.
- Shah, A.H., S. Qureshi, M. Tariq and A.M. Ageel, 1989. Toxicity studies on six plants used in the traditional Arab system of medicine. *Phytother. Rea.*, 3: 25-29.
- Syed, T.A., K.M. Cheema, A. Ashfaq and A.H. Holt, 1996. Aloe vera extract 0.5% in a hydrophilic cream versus *Aloe vera* gel for the management of genital herpes in males. A placebo-controlled, double-blind, comparative study. (Letter) *J. Eur. Acad. Dermatol. Venerol.*, 7: 294-295.

- Vogler, B.K. and E. Enerst, 1999. *Aloe vera*: A systematic review of its clinical effectiveness. *Br. J. Gen. Pract.*, 49: 823-828.
- Williams, M.S., M. Burk and C.L. Loprinzi, 1996. Phase III double-blind evaluation of an *Aloe vera* gel as a prophylactic agent for radiation induced skin toxicity. *Int. Radiat. Oncol. Biol. Phy.*, 36: 345-349.
- Zemjanis, R., 1970. Collection and Evaluation of Semen. In: *Diagnostic and Therapeutic Techniques in animal Reproduction*. 2nd Edn., The William and Wilkins Company, Baltimore, pp: 139-153.