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Studies on the histological changes in testes and analysis of serum hormonal level of *Aloe vera* treatd male albino rat, *Rattus norvegicus*

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Abstract –Many herbal plants have medicinal value and are used throughout the world as safe source of medicine. *Aloe vera* is a succulent herb used for various ailments and contains many healing properties. Its beneficial effect on diabetes, burns, wounds and gastrointestinal diseases has been proved. But there is scarce information of its effects on male reproductive system. In the present study the effect of crude extract of *Aloe vera* on testes and serum gonadotropins of male albino rats have been investigated. In this study 12 male albino rats weighing between 180-240 gm and aged 3-4 months were randomly divided into 2 groups of 6 animals each. The group I served as control, provided normal saline and group II is experimental and are treated with 25 mg/kgbw of crude extract of *Aloe vera* gel daily for 30 days orally. Histopathological studies showed that there were atrophic tubules, germ cell debris, vacu- olization of sertoli cells and interstitial cells, disrupted basement membrane, empty lumen of seminiferous tubules and intercellular spaces in seminiferous tubules in testes of *Aloe vera* treated groups as compared to control group. Analysis of serum level of luteinizing hormone, follicle stimulating hormone and testosterone was found to be significantly decreased in treated group. Thus it is concluded that *Aloe vera* cause adverse effect on the testes of rat by affecting the secretion of reproductive hormones.

Key words: *Aloe vera*, testes, histoarchitechture, luteinizing hormone, follicle stimulating hormone, testosterone and albino rats.

Introduction:

Aloe is a cactus-like perennial herbaceous plant which grows easily in arid warm regions of Africa, North America, Europe and Asia. *Aloe vera* is reproduced via seed, leaves cuttings and other parts of origin plant. This plant contains many vitamins including antioxidant vitamins like A and C, vitamins of B group like thiamin, niacin, riboflavin, cobalamine and folic acid. Sodium, potassium, calcium, magnesium, manganese, copper, chromium and iron are found in *Aloe vera*. *Aloe vera* is a pharmaceutical plant which can be useful for curing various diseases and improving body's physiology. It can be used as a natural antioxidant with high potential of reducing fats oxidation and oxidative stresses (Vinson *et al.*,2005).

The main chemical constituents of the *Aloe vera* 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 also showed that hydro-alcoholic extract of this plant has an anti-androgenic property that can reduce androgen-dependent parameters including secretion of gonadotropins and probably cause oligospermia (Shariati *et al.*, 2009). There are very few literature available related to antifertility potential of *Aloe vera* on male reproductive system and also considering different compositions of *Aloe vera* plant including Aloe Amodin, and Phytoestrogens such as Beta-cytosterol, it is possible that these compounds could affect sex hormones (Poorfarid *et al.*, 2013). Thus, the present study was conducted to examine the effect of *Aloe vera* gel extract on serum gonadotropin level and histological changes in testes of male albinorats.

Materials and Methods:

Experimental location: The present experiment was performed in the Research Laboratory, PGTD of Zoology, MJF Campus, RTM Nagpur university, Nagpur.

Experimental Animal: For this study 12 male albino rats weighing between 180-240 gm 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.

Preparation of *Aloe vera* gel extract: Fresh *Aloe vera* gel extracted daily as follows: The fresh *Aloe vera* leaf cut down with the help of sharp sterilized knife, washed with clean water and cut transversely into slices and then the gel extracted by squeezing the thick epidermis and collected in a small petriplate.

Immediately 25mg *Aloe vera* gel weighed and mix with 2gm jowar flour to make small pellets by using little distill water and given orally.

Experimental design:

The 12 male albino rats were randomly divided into 2 groups of 6 animals each. The first group is control and provided normal saline while the second group is experimental and is treated with 25mg/kg bw of *Aloe vera* gel daily for 30 days orally. 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±4^{0}C and relative humidity. They werefedwithstandarddietpelletsandwateradlibitumthroughouttheexperimentalperiod.

Histopathological study:

After the completion of 30 days treatment, on 31st day the rats were sacrificed under chloroform 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.

Hormone analysis:

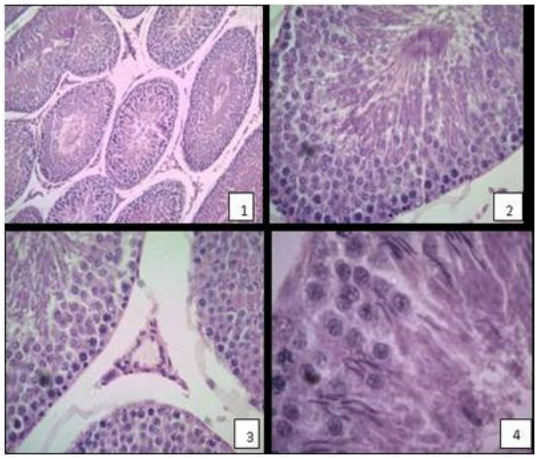
For hormonal assay 2ml blood was withdrawn by cardiac puncture with the help of disposable syringe and collected in a non EDTA tubes. Serum level of follicle stimulating hormone (FSH), luteinizing hormone (LH) and testosterone (T) were assessed by CMIA Architect Abbott method method.

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. Differences between groups were considered significant at ($P<0.05$).

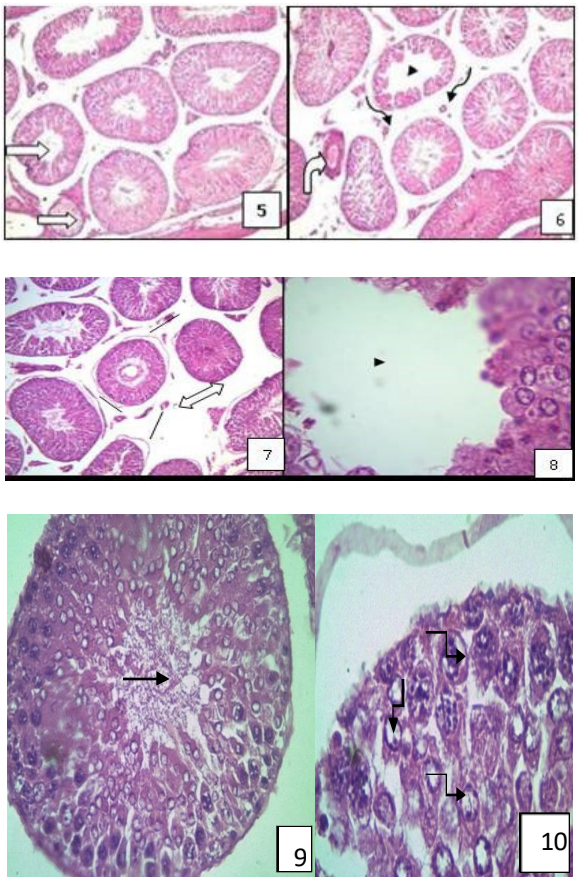
Result:

Histopathological analysis: Histopathological examination of the testes of control rats showed normal structure (Figures 1, 2, 3 and 4)**.** The rats treated with 25mg/kg bw of *Aloe vera* showed marked tissue damage in the form of atrophic seminiferous tubules, increase in interstitial spaces due to shrinkage of seminiferous tubules and atrophic Leydig cells. The blood capillaries shows thickening of perivascular wall, extrudation of fluid in the interstitial space, lumen without sperms and vacuolation of spermatogonia. The sertoli cells are highly affected. Their breakage into pieces, bilateral compression, detachment from the basement membrane, sloughing and shedding of cellular material from the tip into lumen forming cell debris was observed as compared to control (Figures 5, 6, 7, 8, 9 and 10).



Transverse section of photomicrographs (Figures 1,2,3 and4) showing normal histoarchitecture of testes of control group (Stained with HE X10, X40 and X100).

Figure 1: Shows seminiferous tubules in transverse section separated from one another by interstitial connective tissue containing Leydig cells. Figure2: Indicates seminiferous tubule with all the stages of spermatogenesis and whorl like arrangement of sperms in the lumen. Figure 3: Shows the space between the seminiferous tubule filled with interstitial connective tissues, nerve fibers, blood capillaries and lymphatic vessels. Connective tissue composed of rounded or polygonal shaped cells with central nucleus called Leydig cells. Figure 4: Magnified view of seminiferous tubule showing basal cells, spermatogonia, primary spermatocytes, Spermatids, Spermatozoa and sertolicells



Transverse section of photomicrographs (Figures 5,6,7,8,9 and10) showing structure of testes of treated group (Stained with HE X10, X40 and X100)

Figure 5: shows atrophic seminiferous tubules ( ), Figure 6: shows thickening of perivascular wall of bloodcapillaries( ) and vacuolation in interstitial cells( ) , Figure7:shows detached basement membrane ( ) and increase in interstitial spaces between seminiferous tubule ( ), Figure 8: shows central lumen of seminiferous tubule without sperms ( ), Figure 9: shows accumulated germinal cell debris in lumen ( ), Figure 10: shows vacuolation of spermatogonia , spermatocyte and sertoli cells ( )

Hormonal analysis:

The serum level of LH, FSH and T was found to be decreased in *Aloe vera* treated group for 30days as compared to control.

Table 1: Serum FSH, LH and Testosterone levels in control and *Aloe vera* treated male albino rats:

Groups	duration	Dose	FSH	LH	Testosterone
Control	30days	Normal saline	0.121±0.003	0.098±0.004	45.183±0.11
Aloe vera	30days	25mg/kgbw	0.105±0.003*	0.086±0.004*	42.560±0.41*

n=6 for each group, $p<0.05$ significantly different from control group.

Discussion:

The results obtained from this study showed that *Aloe vera* could cause reproductive impairment in male albino rats. In the present study, control group showed normal histoarchitechture of testes. It is observed that seminiferous tubules appear to be perfectly rounded to oval in shape, separated from one another by interstitial connective tissue containing Leydig cells. All the stages of spermatogenesis and whorl like arrangement of sperms in the central lumen of seminiferous tubule are observed. The space between the seminiferous tubule filled with interstitial connective tissues, nerve fibers, blood capillaries and lymphatic vessels. Connective tissue composed of rounded or polygonal shaped cells with central nucleus called Leydig cells. Further it showed the basal cells- spermatogonia, primary spermatocytes, Spermatids, Spermatozoa and

sertoli cells. Histopathological analysis of the treated group showed atrophic seminiferous tubules, increase in interstitial spaces due to shrinkage of seminiferous tubules, atrophic Leydig cells as compared to control.

Other histological changes observed due to *Aloe vera* treatment are thickening of perivascular wall of blood capillaries, extrudation of fluid in the interstitial space, lumen without sperms, Vacuolation of spermatogonia. The sertoli cells are highly affected. Their breakage into pieces, bilateral compression, and detachment from the basement membrane, sloughing and shedding of cellular material from the tip into lumen forming germinal cell debris was observed as compared to control. These results are similar with the results of (Müfide *et al.,*2014)

In the present study it is observed that 25 mg / kg bw of *Aloe vera* treatment for 30 days caused significant degenerative changes such as central lumen of seminiferous tubules without spermatozoa in testes of albino rats and degeneration of interstitial cells. These findings correlates with the study done by (Murdakai *et al.*, 2011).

Hormonal analysis of the present study showed decreased level of serum FSH, LH and Testosterone as compared to control group are considered to be most useful indicators to detect adverse effects of *Aloe vera* on the process of spermatogenesis.These results are in agreement with the finding of (Müfide, *et al.,* 2014) showed LH level decreased in all treated groups whereas testosterone decreased only in high dose treated group.

Previously (Karimi *et al.,* 2012) showed that *Aloe vera* extract significantly reduced the level of testosterone at doses of 100 and 200 mg/Kg body weight and also lowers the serum level of FSH in experimental groups, although the reduction was not significant Further (Ghosal *et al.*, 2016) observed the fluctuation in the level of sialic acid in reproductive tissues indicates the altered levels of testosterone or FSH and LH, needed for the functioning of gonads in their study.

Conclusion: Results of the present study bring us to the conclusion that oral administration of *Aloe vera* has antifertility potential causing significant decreased in serum level of gonadotropins which ultimately affects the histological integration of testes in male albino rats.

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