academicJournals

Vol. 9(15), pp. 510-514, 17 April, 2015
DOI: 10.5897/JMPR2014.5800
Article Number: 98B080852767
ISSN 1996-0875

http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

Haematological parameters, semen characteristics and sperm morphology of male albino rat (wistar strain) treated with *Aloe vera* gel

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Received 24 March, 2015; Accepted 14 April, 2015

Haemograms, semen characteristics and sperm morphology of the male albino rat (wistar strain) treated with *Aloe vera* gel was studied. *A. vera* gel has been widely used for both medicinal and non-medicinal purposes. Fifteen male clinically healthy albino rats weighing between 0.15 to 0.24 kg and 16 to 20 weeks of age were randomly assigned to three groups A, B and C. Groups A and B were the experimental groups while Group C was the control group. The experimental groups A and B were treated with *A. vera* gel at 300 and 200 mg/kg body weight, respectively for 10 days. The findings showed that the sperm motility from group A (64.00 ± 6.80) and B (66.00 ± 8.74) were lower (P < 0.05) than group C (89 ± 2.46). The percentage liveability of the sperm cells of group A (88.00 ± 6.74) and B (85.00 ± 4.48) were also lower (P < 0.05) than the control, 96.8 ± 0.74. The sperm concentration in the experimental group A (53.2 ± 2.27) and B (59.4 ± 2.43) were also lower (P < 0.05) than the control group 84.6 ± 2.14. The blood parameters across the groups were within the normal range of values in rats, and there was no significant difference (P>0.05). It was concluded that the use of *A. vera* gel for 10 days consecutively has an adverse effect on sperm cells characteristics and morphology, and can precipitate infertility in the male wistar strain albino rats when administered at concentration higher than 300 mg/kg body weight. However, it has no negative effect on the blood parameters.

Key words: *Aloe vera* gel, albino rats, semen characteristics, sperm morphology, haematology.

INTRODUCTION

Aloe vera is the name given to a variety of perennials of the families *Liliaceae*. There are over 325 species in this genus most of which are native to South Africa, Madagascar and Arabia. Examples of medicinal species are *Aloe barbadensis*, *Aloe vulgaris, Aloe arborescens, Aloe ferox* (Kathi and Chron, 1999). *A. vera* grows well in flower pots; it is light green in colour with white spots. It has long triangular fleshy leaves that have spike along the edges (Kathi and Chron, 1999).

A. vera gel is 99% water with pH 4.5 and the major carbohydrate fraction in the gel is a water soluble long chain mannose polymer which accelerates wound healing, modulate immune functions and demonstrate antiviral effects (Zhang and Tizard, 1996). The gel also contains bradykininase: an antinflammatory, magnesium lactate which helps to prevent itching, glucomannan a

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good moisturizer, salicylic acid and other anti-prostaglandin compounds which relieve inflammation (Yagi et al., 1982).

The leaf lining contains anthraquinone glycosides that are potent stimulant and laxatives (Schilcher, 1997). These water soluble glycosides are split by intestinal bacteria into aglycones which affect the laxative action. The laxative effect from *A. vera* is stronger than any other herb, including senna, cascara or rhubarb root, it also has more severe side effects such as cramping, diarrhoea and nausea (Schilcher, 1997). The juice is soothing to digestive tract irritations, such as colitis and peptic ulcers (Schilcher, 1997). The Chinese describe *Aloe's* skin and the inner lining of its leaves as a cold, bitter remedy which is downward draining, and used to clear constipation due to accumulation of heat, the gel is considered cool and moist (Bensky et al., 1993).

In traditional medicine of India, *A. vera* is used internally as laxative, anthelminthic, haemorrhoid remedy and uterine stimulant. It is used topically, often in combination with licrocice root, to treat eczema or psoriasis (Ghazanfar, 1994). In Arabian medicine, the fresh gel is rubbed on the forehead as a remedy or rubbed on the body to cool it in case of fever, as well as being used for wound healing, conjunctivitis and as a disinfectant and laxative (Ghazanfar, 1994). *A. vera* gel is an active ingredient in hundreds of skin lotions, sun blocks and cosmetics (Grindlay and Reynolds, 1986). According to Danhof (1993), *A. vera* gel in cosmetics has a similar anti-ageing effect to vitamin A derivatives. Recently, *A. vera* extracts have been used to treat canker sores, stomach ulcers and even acquired Immune deficiency syndrome (AIDS). Some natural health enthusiasts promote gel as a cleaning juice (McGuffin et al., 1997).

Some nature-pathologists promote *A. vera* juice as a way to prevent and treat renal stone (Murray and Pizzorno, 1991). According to Cithra et al. (1998), topical application on the wound surface or by oral administration of the *A. vera* gel in diabetic rats may enhance the process of wound healing by influencing phases such as inflammation, fibroplasia, collagen synthesis, maturation and wound contraction.

There is dearth of information on the reproductive implication of *A. vera* gel administration especially on haematology, semen characteristics and morphology in the male wistar strain albino rats. This study, therefore, was designed to investigate the haemogram, semen picture, and sperm morphology of the male wistar strain albino rats treated with *A. vera* extract.

MATERIALS AND METHODS

Experimental animal

Fifteen male Albino rats (Wistar strain) were used for this study, each weighing between 0.15 to 0.24 kg and aged 16 to 20 weeks, they were housed in the experimental animal unit of the Faculty of Veterinary Medicine, University of Ibadan Oyo State, Nigeria. The rats were kept in circular plastic cages of about 60 cm in circumference with depth of about 20 cm, and these were covered with wooden and wire meshes. Beddings were provided using wood-shaven and replaced weekly. They were fed *ad libitum* with a purchased rat feed, a ration containing 21% crude protein, 3.5% fat, 3% crude fibre, and 0.8% phosphorus, from Ladokun Feeds Limited, Ibadan. Water was supplied *ad libitum.* The feed and water were given using earthen troughs. All the rats used in this study were handled in accordance with the Good Animal Practice requirements of the Animal Ethics Procedures and Guidelines and was approved by the Animal Ethics Committee of the University of Ibadan, Nigeria.

Aloe vera gel preparation

A. vera were harvested in the University of Ibadan, Botanical garden and identified at the herbarium unit of the Botany Department, University of Ibadan. These were thoroughly washed and followed by rising under flowing tap water and later with distilled water. The fleshy mass of the *A. vera* was carefully opened by cutting the sharp edges. The gel was funnelled into a sterile beaker. 2 and 3 g of *A. vera* gel were weighed using a digital micro-sensitive scale. Each of these was then diluted with 100 ml of distilled water (measured by the measuring cylinder) to constitute 200 and 300 mg/kg concentrations respectively. These were gently stirred with spatula to achieve homogenous solution.

Restraints of rats

The rats were picked up gently by the tail with the right hand placing the rat on a surface, the left hand was placed over the head and using the thumb and index finger to grasp the skin below the two ears firmly. The rat was then turned with the stomach towards the handler and the skin along the back is firmly grasped with the remaining three fingers

Administration of gel

The rats were in three groups A, B and C of five rats each. Group C was used as the control. Group A and B were dosed with *A. vera* gel for 10 consecutive days. Each rat was marked for proper identification. The dosage used by McGuffin et al. (1997) was used as a guide. The dose range was between 50 to 300 mg in a single dose. In this study, the rats in Group A were dosed 300 mg of *Aloe vera* gel in a single dose per day while group B rats were dosed 200 mg of *A. vera* gel in a single dose *per os* by oral gavage.

Sample collection

Blood was collected from each rat post-treatment from the orbital sinus called the infraorbital lateral canthus method. This was done by using heparinised capillary tubes, a glass chamber, anaesthetic ether, cotton wool, 2.0 ml heparinised sample bottles and a recovery cage. Cotton wool was soaked in anaesthetic ether and placed in the glass chamber. Gauze was placed over the cottonwool and the rat was put in the glass chamber and allowed to be anaesthetized one at a time for about 2 min. The rat was then removed, placed on lateral recumbency and capillary tube inserted infraorbitally at the lateral canthus, the infraorbital groove is located and pressure is applied to puncture the infraorbital artery and blood flows through the capillary tube. This was collected into a heparinised sample bottle and was properly labelled.

Table 1. Semen characteristics of male albino rats (Wistar strain) in *A. vera* gel treated rats.

Identification	Motility (%)	Percentage liveability (%)	Sperm count (X10^6 cells/ ml)
Group A (300 mg)	64.0±6.80^a	88.0±6.74^a	53.2±2.27^a
Group B (200 mg)	66.0±8.74^a	85.0±4.48^a	59.4±2.43^a
Group C (control)	89.0± 2.46^c	96.8±0.74^c	84.6±2.14^c

$^{a, b, c:}$ Mean difference is significant at (P<0.05).

Semen collection

The rats from each group were weighed and euthanized by decapitation. The testes were immediately exteriorized through a mid-caudoventral abdominal incision with sterile scalpel blade. Sperm cells were then collected from the caudal epididymis while the two testes, and the epididymis were immediately weighed using a sensitive electronic weighing machine.

Semen analysis

Smears were prepared from the collected epididymal sample and stained with Wells and Awa stain for morphological studies, Eosin-Nigrosin stains for sperm percentage livability. Also, percentage motility was carried out using 2 to 3 drops of 2.9% warm buffered sodium citrate kept at body temperature as previously described by Zemjanis (1977).

Percentage livability

This was done by staining one drop of semen and one drop of warm Eosin-Nigrosin stain on a warm slide. A thin smear was then made of mixture of semen and stain. The smear was air dried and observed under the microscope. The ratio of the *in vitro* dead sperm cells was observed and it is based upon the principle of Eosin penetrating and staining the dead autolysing sperm cells whereas viable sperm repel the stain (Zemjanis, 1977).

Percentage motility

It was evaluated with a drop of semen with drop of 2.9% buffered sodium citrate on a warm glass slide covered with a glass slip and viewed at a magnification of ×40. Only sperm cells moving in a unidirectional motion were included in the motility rating, while sperm cells moving in circles, in backward direction or pendulating movement were excluded (Zemjanis, 1977).

Blood analysis

Blood sample was collected for haematological analysis, packed cell volume (PCV), haemoglobin (Hb) concentration, red blood cell (RBC) count, total white blood cell (WBC) count and differential leucocyte count (DLC) according to the methods described by Schalm et al. (1975) and Reece (1997).

Data analysis

The mean and standard error of means were calculated for the semen characteristics and heamograms, while the morphological characteristics were presented in percentages. One way ANOVA (Analysis Of Variance) and Duncan 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

Table 1 shows that the percentage liveability of sperm cells of Group A (88.0±6.74%) and B (85.0±4.48%) were significantly lower (P<0.05) than the group C value (96.8±0.74%). The percentage liveability at group A (88.0±6.74%) compare to group B (85.0±4.48%) was not significant (P>0.05). The mean percentage motility followed the same trend. The sperm count for group A (53.2±2.27 × 10^6 sperm cells/ml) and group B (59.4±2.43 × 10^6 sperm cells/ml) are significantly lower (P<0.05) than group C value (84.6±2.14 × 10^6 sperm cells/ml). Table 2 presents the sperm morphological abnormalities of Male albino rats (Wistar Strain) in group A to C.

Tailless head (Normal head without tail)

There is a significant difference (P<0.05) between the values of tailless head in group A, 29 (1.43%) group B, 24 (1.18%) and group C, 23 (1.12%). However, there was no significant difference (P>0.05) between group B and group C.

Headless tail (Normal tail without head)

There is a significant difference (P<0.05) between the values of headless tail in group A, 30 (1.48%) group B, 21 (1.04%) and group C, 23(1.12%). However, there was no significant difference between group B and group C..

Rudimentary tail

There is a significant difference (P<0.05) between the values of rudimentary tail in group A, 33 (1.63%), group B, 28 (1.38%) and group C, 19 (0.92%). However, the difference between the values of group A and group B was not significant (P>0.05).

Bent tail

There is a significant difference (P<0.05) between the

Table 2. Sperm morphological characteristics of male albino rats (Wistar Strain) in *A. vera* gel treated rats.

Parameters	Tailesshead	Headless tail	Rudimentary tail	Bent tail	Curved tail	Curved mid piece	Bent mid piece	Coiled tail	Looped tail	Total abnormal Cells	Total normal Cells	Total Count
Group A	29.00*	30.00*	33.00**	41.00*	43.00*	39.00*	43.00*	7.00*	4.00*	269.00	1756.00	2025.00
(%)	(1.43%)	(1.48%)	(1.63%)	(2.03%)	(2.12%)	(1.93%)	(2.12%)	(0.35%)	(0.19%)	(13.28%)	(86.72%)	(100%)
Group B	24.00**	21.00**	28.00**	29.00**	36.00**	33.00	32.00**	0.00**	1.00**	204.00	1824.00	2028.00
(%)	(1.18%)	(1.04%)	(1.38%)	(1.43%)	(1.78%)	(1.63%)	(1.58%)	(0%)	(0.05%)	(10.06%)	(89.94%)	(100%)
Group C	23.00**	23.00**	19.00*	29.00**	31.00**	29.00**	32.00**	0.00**	0.00**	186.00	1869.00	2055.00
(%)	(1.12%)	(1.12%)	(0.92%)	(1.41%)	(1.51%)	(1.41%)	(1.56%)	(0%)	(0%)	(9.05%)	(90.95%)	(100%)

* Mean difference is significant at (P<0.05),**Mean with the same superscript are not significantly different at P<0.05 level. Morphological exadffcffgg vp[;[;;[;mination.

values of bent tail in group A, 41 (2.03%), group B, 29 (1.48%) and group C, 29 (1.41%), but the difference between the values of group B and group C was not significant (P>0.05).

Curved tail

The values obtained for group A, group B, and group C were 39 (1.93%), 33 (1.63%) and 29 (1.41%) respectively. When compared, there was a significant difference (P<0.05) between the values obtained for group A, group B and group C, but there was no significant difference (P>0.05) between the values of group B and group C.

Curved midpiece

There was a significant difference (P<0.05) between group A value 39 (1.39%) and group C value 29 (1.41%).

Loped tail

There was a significant difference (P<0.05) between group A: 4 (0.19%) and group C: 0 (0%).

Bent midpiece

There was a significant difference (P<0.05) between the values of group A, 43 (2.12%) when compared with Group B: 32 (1.58%) and Group C: 32 (1.56%). However, there was no significant difference (P>0.05) between the values of group B and group C.

Total abnormal cell

There was a significant difference (P<0.05) between group A: 269 (13.28%), group B: 204 (10.06%) and group C: 186 (9.05%). However, there was no significant difference (P>0.05) between the values of group B and group C. It was observed that there was a significant difference (P<0.05) between the total number of cell counted for each group. This indicated a higher number of abnormal cells in rats treated with higher dose of *A. vera* gel (Table 3).

Result of haemogram

The PCV of group A (300 mg/kg) was 43.6±1.200%, higher than that of group B (200 mg/kg) 41.8±0.5% and group C (control) 40.8±0.80%. However, these PCV values as well as other blood parameters are within the normal range.

DISCUSSION

The semen picture observed in this study showed that the percentage progressive motility of group A rats treated with 300 mg/kg body weight and group B rats treated with 200 mg/kg body weight of *A. vera* gel were significantly (P<0.05) lower than the control group. This indicates that increased treatment with *A. vera* adversely affect sperm motility. This finding supports the report of Oyeyemi et al. (2011) in which treatment with *A. vera* gel significantly (P<0.05) reduced the sperm motility of West African Dwarf bucks.

The mean percentage liveability of sperm cells decreased significantly (P<0.05) from control group C to group B treated with 200 mg/kg body weight of *A. vera* gel, and further decreased significantly (P<0.05) in group A rats treated with 300 mg/kg body weight of *A. vera* gel. This implies that the continuous administration of *A. vera* extract would result in decreased fertility in the male wistar strain albino rats. The reports of

Table 3. Haematological result of of male albino rats (Wistar strain) in *A. vera* gel treated rats.

Identification	PCV (%)	Hb (%)	RBC ($\times10^{12/7}$)	WBC ($\times10^{9}$/1)	LYM (%)	NEUT (%)
A	43.6±1.20[a]	14.08±0.33[a]	9.9±0.96[a]	20.9±2.94[a]	48.6±4.27[a]	51±4.38[a]
B	41.8±0.58[a]	13.7±0.20[a]	8.9±0.32[a]	12.9±2.94[a]	48.6±4.27[a]	52±2.50[a]
C	40.8±0.80[a]	13.4±0.23[a]	9.9±0.96[a]	20.9±2.94[a]	48.6±4.29[a]	53.4±3.0[a]

[a] Mean with the same superscript are not significantly different at (P<0.05) level.

Oyeyemi et al. (2011) alluded to this fact. The percentage liveability value across the groups was higher than 47.50% reported by Oyeyemi et al. (2006) when 50 mg of Clomiphene citrate was administered to male Albino rats but Farombi et al. (2007) had a higher percentage in their reports when male Albino rats were treated with Curumin and kolaviron.

The sperm cell concentration value ($84.6\pm2.14\times10^{6}$ sperm cells/ml) of control group C was higher than the value of group A and group B value. The difference in the experimental values are significant (P<0.05). This indicates that *A. vera* gel administration reduces sperm count in a fertile male Albino rats (Wistar strain). The total abnormal cells value 186 (9.05%) of control group C was lower than the value of group A rats and the value of group B rats, and there was a significant difference (P<0.05) within the experimental values. This indicated that there was an increase in total percentage spermatozoa abnormalities for group A, B and C 186 (9.05%) when compared to the values reported by Farombi et al. (2007) when curcumin and kolaviron were administered in rats. The blood analysis result obtained for the haemogram are within the normal range of values in rats as reported by Harkness and Wagner (1989), and there was no significant difference (P>0.05) in the blood parameters.

Conclusion

It was concluded that the use of *A. vera* gel for 10 days consecutively will induce a deteriorative effect on sperm cells characteristics and morphology, and can precipitate infertility in the male wistar strain albino rats when administered at concentration higher than 300 mg/kg body weight. However, it has no negative effect on the blood parameters.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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