www.ajbrui.org

Afr. J. Biomed. Res. Vol. 25 (January, 2022); 95 - 99

Research Article

Semen Quality and Gonadometric Assessment of Male Wistar Rats treated with Aqueous Leaf Extract of *Chasmathera dependens* (Hochst)

***Ajani, O.S. and Omoyeni J.E.**

Department of Theriogenology, Faculty of Veterinary Medicine, University of Ibadan, Nigeria

ABSTRACT

Chasmanthera dependens (Hochst), a well-known medicinal plant widely used as pain-killer in Africa, however, its effects on fertility have not been fully studied. The effect of aqueous leaf extract of *Chasmathera dependens* (Hochst) (ALECD) on sperm quality and gonadometric indices of the male Wistar rats was studied. Twenty-five adult male Wistar rats were randomly assigned into 5 groups A-E. Groups A-D were orally treated with 25mg/kg, 50mg/kg, 100mg/kg and 200mg/kg of the extract respectively. Group E served as the control and was given only distilled water. The treatment lasted for 21 days after which the rats were humanely sacrificed and samples (blood, semen, testes and epididymis) were collected for laboratory analysis. The treatment did not show any deleterious changes in the haematological parameters studied. There was an increase in sperm motility, livability and sperm count of rats treated with 100 mg/kg. Conversely, there was a decrease in the semen parameters of groups treated with 25 mg/kg, 50 mg/kg and 200 mg/kg of the ALECD. The treatment also caused a significant decrease in the number of morphologically abnormal sperm cells across the groups. There were no significant changes in the testicular and epididymal biometry of the treated rats across the groups. The aqueous leaf extract of *Chasmathera dependens* possesses fertility enhancing effect at100 mg/kg by improving the semen quality and maintaining gonadometric integrity of male rats. Therefore, the plant can be used to improve the fertility of the male breeding stock at 100 mg/kg body weight.

Keywords: *Fertility, semen, rats, testes, Epididymis*

*Author for correspondence: Email: drgoforth09@yahoo.com; *Tel: +234-8038087625*

Received: October 2021; Revised version accepted: December, 2021

INTRODUCTION

The use of folk medicine in treating human ailments and animal diseases has gained a wide acceptance globally in the recent past. In 1993, the Director of WHO Traditional Medicine reported that the use of plant based traditional medicine provided the primary healthcare need for 80 % of the human population (Akerele, 1993; Elujoba *et al*, 2005). Today, the use of folk medicine and herbal remedies is gaining a rapid momentum of interest both in developed and the developing world (Haq, 2004).

Chasmanthera dependens (Hochst) is a well-known medicinal plant that is widely available in a number of African countries like the Sierra Leone, Somalia, Congo, Eritrea, Tanzania, Nigeria and others (Iwu *et al*., 1999, Stanley, 2012). It is classified under the family of Menispermaceae, and commonly known as *Chasmanthera*, while it is locally called by the Igbo and Yoruba tribes of Nigeria as "Ogbo" and "Atoo" respectively. The medicinal property of *Chasmathera dependens* (Hochst has been widely suggested; the treatment of malaria, and fracture management (Oguniesi, *et al*., 2008); anticonvulsant, anti-epileptics, treatment of red-eye infection, dementia and as antidote of snakebite toxins (Odugbemi, 2008).

The pain ameliorating and anti-inflammatory activities of the plant was established in laboratory animals (Morebise, *et al*., 2001) and (Adekunle and Okoli, 2002) proposed that the ethanolic crude extract of the leave of the plant can be used to treat fungal infections Although extensive research has been carried out on the medicinal properties of *Chasmathera dependens* (Hochst), nonetheless, very little is known about its effect on reproduction in both animals and man. A few available studies on its reproductive effects in animals only made use of the root extracts of the plant (Quadri and Yakubu, 2017). Also, it is believed that a mixture of the leaf extract of the plant and alcohol can be used as an aphrodisiac, but this claim has not been substantiated scientifically.

Whereas, both the leaf and root of *Chasmathera dependens* (Hochst) are largely consumed, yet so little is known about its effects on fertility in animals and man. Therefore, the study was designed to investigate the effect of aqueous leaf extract of *Chasmathera dependens* (Hochst) on sperm quality and gonadometric indices of the male Wistar rats.

MATERIALS AND METHODS

Experimental animals: Twenty-five (25) adult male Albino rats (Wistar strain), each weighing 150-180 ± 10g were purchased from a local breeder in Ibadan, Oyo State, Nigeria. The animals were stabilized for three weeks at the Animal house section, Veterinary Biochemistry Department, Faculty of Veterinary Medicine, University of Ibadan, under normal photoperiod of (12-hours light and 12- hours dark), controlled conditions of temperature (25±2.0°C) and relative humidity (50±15%).

Plant material and extraction: Fresh leaves of *Chasmathera dependens* (Hochst) was collected from a location at the Faculty of Veterinary Medicine, University of Ibadan. Plant authentication was done at the herbarium unit of the Department of Botany, University of Ibadan (UIH-23115).
The leaves were air-dried at room temperature for 7 days, after which they were milled into powder and kept for extraction. The aqueous extraction was done as described by Gupta, *et al*., (2005).

Study design: The rats were randomly assigned into 5 groups containing five (5) animals each (n=5). There were four treatment groups; A to D which were dosed, using oral cannula, with 25mg/kg, 50mg/kg, 100mg/kg and 200mg/kg of the aqueous leave extract of the plant respectively. Group E was the control and was given only distilled water (Quadri and Yakubu, 2017). The animals were treated for twenty-one (21) days, after which they were sacrificed through cervical dislocation for sample collection.

Blood Collection: Blood sample was collected from the retro orbital plexus of the rats using an heparinized capillary tube. The haematological analyses were done using conventional methods, as described by Mehdi *et al*. (2019).

Collection of the testes and epididymis: The animal was first humanely sacrificed, after which a caudoventral mid-abdominal incision was made using a sterilized scissors in order to access the internal organs. The testes were located once pushed upward from the scrotum. Then, the testes were detached from the epididymis and harvested using the method described by (Oyeyemi and Fayomi, 2011).

Semen collection and analysis: After the animals were sacrificed, semen sample was collected from the caudal epididymis and analysed as modified by Ajani and Oyeyemi (2014) to determine the sperm motility, livability and the sperm count.

Gonadometric Assessment: The testicular and epididymal biometry was done as described by Oyeyemi and Ajani, 2015. This includes weight of left testis, weight of right testis, length of left testis, length of right testis, diameter of left testis, diameter of right testis and weight of epididymis.

Data analysis: Mean and standard deviation were used to express quantitative data. Comparison of means was done using one-way analysis of variance (ANOVA) and followed by post hoc Turkey's test. Normality of the data and homogeneity of variance between the treatments means were expressed at 5% confidence level using the Duncan Multiple Range Test. Microsoft Office Excel 2016 (Microsoft® Corporation, 2016) and IBM SPSS Statistics Version 20 were used to carry out data management and analyses.

RESULTS

Haematology: The results of haematological profiles revealed that white blood cell, platelets, lymphocytes, Plasma protein and MCHC were statistically significant ($P < 0.05$) (Table 1). For WBC; Group C treated with 100mg/kg has the lowest mean value across all the groups and Group A, B and D treated with 25mg/kg, 50mg/kg and 200mg/kg of aqueous extract of *Chasmathera dependens* (Hochst) have significant increased mean difference compare with the control group. For Platelets; Group C treated with 100mg/kg has highest mean value, followed by Group D treated with 200mg/kg compare with the control group while Group A and B treated with 25mg/kg and 50mg/kg have lower significant mean value compare with the control group. For Lymphocytes; all the treatment groups (A - D) have lower significant mean value compare to the control group (E). For Plasma Protein; Group C has the highest significant mean value in all the groups, while Group A, B and D have lower mean values compare with the control group E. For MCHC; all the treatment group (A - D) have lower mean values compare with the control group E while Group C treated with 100mg/kg has the highest mean value among all the treatment groups (Table 1).

Sperm Quality Assessment: The results in Table 2 showed that treatment Group A, B and D given 25mg/kg, 50mg/kg and 200mg/kg of aqueous extract of *Chasmathera dependens* (Hochst) leave respectively have considerable decreased in motility and livability compare with the control group E, although the dcreased in Group D is significant. Group C treated with 100mg/kg of aqueous extract of *Chasmathera dependens* (Hochst) has significant increase in mean percentage motility and livability compared with the control group.

For sperm count, Group A, B and C showed comparable non-significant increase mean percentage to the control group, with the highest increase in Group C treated with 100mg/kg but Group D treated with 200mg/kg has a decreased mean percentage compare with the control group.
The overall stats (F-stats) showed that none of the semen characteristics are statistically significant among the five group (Table 2)

Sperm Morphological characteristics: Table 3 showed that there was no significant mean percentage difference in head defect within the five groups, there was a significant mean percentage difference in tail defects, the total abnormal cell counts and percentage abnormality were also significantly difference. The control group has the highest morphological defects in all the parameters compare with Group A – D.

Table 1:
Haematological Profiles of Wistar Strain Abino Rats in Different Treatment Groups

Parameters	Groups							
	A (25mg/kg)	**B (50mg/kg)**	**C (100mg/kg)**	**D (200mg/kg)**	**E (control)**	**F-stats**	**P-value**	**Remark**
PCV (%)	52.80 ± 2.65	48.40±2.66	52.80 ± 6.03	54.20 ± 2.65	57.40 ± 1.57	0.878	.495	NS
H.b (g/dl)	16.54 ± 0.53	15.38 ± 0.72	16.20 ± 1.89	17.10 ± 0.77	18.62 ± 0.47	1.407	.268	NS
RBC ($\times10^6$µl)	8.12 ± 0.33	7.80 ± 0.32	7.95 ± 0.96	8.68 ± 0.36	8.94 ± 0.17	0.925	.469	NS
WBC ($\times10^3$µl)	11780.00 ± 288.71	12760.00 ± 408.17	8130.00 ± 671.12	11560.00 ± 858.69	9010.09 ± 283.90	12.840	.000	*
Platelets (µl)	105400.00 ± 6446.70	111400.00 ± 5784.46	164800.00 ± 16411.58	143000.00 ± 11886.97	137400.00 ±6961.32	5.516	.004	*
Lymphocytes (%)	76.40 ± 1.86	73.20 ± 0.92	80.20 ± 3.12	79.80 ± 1.80	82.60 ± 0.93	3.712	.020	*
Neutrophil (%)	20.06 ±2.70	24.20 ± 1.02	21.40 ± 5.11	17.80 ± 1.46	13.40 ± 0.87	2.418	.082	NS
Monocyte (%)	2.00 ±0.32	1.60 ± 0.40	2.20 ± 0.37	1.40 ± 0.24	2.20 ± 0.49	0.943	.460	NS
Eosinophil (%)	1.60 ± 0.51	1.00 ± 0.55	2.20 ± 3.37	1.00 ± 0.55	1.80 ± 0.37	1.193	.344	NS
Plasma Protein (g/dl)	63.93 ± 0.85	61.89 ± 1.05	66.77 ± 0.80	62.39 ± 0.95	64.21 ±1.12	4.232	.012	*
MCV (fl)	31.45 ± 0.67	31.84 ± 0.39	30.63 ± 0.19	31.58 ± 0.26	32.44 ± 0.23	2.836	.052	NS
MCHC (%)	20.40 ± 0.19	19.69 ± 0.19	20.45 ± 0.22	19.69 ±0.14	20.84 ± 0.82	4.046	.015	*
MCH (pg)	9.40 ±0.28	8.66 ± 0.39	8.60 ± 0.14	8.68 ± 0.37	8.50 ± 0.10	1.659	.199	NS

*NS = no significant mean difference at 5% level. ; * At least two treatment groups are significantly different at .05 level.*

Table 2:
Semen Characteristics of Wistar Rats in different treatment groups.

Parameter	Groups					F-stats	P-value	Remark
	A (25mg/kg)	**B (50mg/kg)**	**C (100mg/kg)**	**D (200mg/kg)**	**E (Control)**			
Sperm Motility (%)	76.00 ± 7.97^a	67.20 ± 5.03^a	83.20 ± 9.59^b	71.20 ± 18.47^c	83.20 ± 5.87^a	0.458	.765	NS
Sperm Livability (%)	72.00 ± 3.74^a	66.80 ± 2.71^a	82.20 ± 3.57^b	67.20 ± 17.30^c	77.00 ± 5.39^a	0.599	.668	NS
Sperm Counts (%)	105.00 ± 4.56^a	103.40 ± 3.67^a	109.00 ± 5.72^a	95.40 ± 2.48^a	98.40 ± 3.265^a	1.734	.182	NS

Data are from five replicates ± SEM. Test values for each parameter with superscripts b,c different from control, a are significantly different (P < .05); NS = no significant mean difference at 5% level.

Table 3:
Sperm Morphological Abnormalities of Wistar Strain Albino Rats in Different Treatment Groups

Parameters	**Groups**							
	A (25mg/kg)	**B (50mg/kg)**	**C (100mg/kg)**	**D (200mg/kg)**	**E (Control)**	**F-stats**	**P-value**	**Remark**
Double head	0.20 ± 0.20	0.20 ± 0.20	0.20 ± 0.20	0.20 ± 0.20	0.40 ± 0.25	0.556	0.697	NS
Double tail	0.40 ± 0.25^a	0.20 ± 0.20^a	0.20 ± 0.20^b	1.60 ± 0.51^c	0.60 ± 0.40^a	3.036	.042	*
Bent tail	8.40 ± 2.11^a	0.20 ± 0.56^a	14.50 ± 0.74^b	13.40 ± 0.51^c	17.80 ± 1.50^a	9.826	.000	*
Coiled tail	3.60 ± 1.08^a	2.40 ± 0.51^a	7.40 ± 0.17^b	8.80 ± 1.28^c	13.40 ± 1.08^a	17.25	.000	*
Looped tail	7.20 ± 1.50^a	9.60 ± 1.08^a	8.20 ± 1.02^b	10.00 ± 1.14^c	13.60 ± 1.33^a	3.968	.016	*
Abnormal head	1.40 ± 0.25	0.20 ± 0.20	1.10 ± 0.08	0.03 ± 0.20	1.60 ± 0.51	1.500	.240	NS
Rudimentary tail	0.40 ± 0.25	0.06 ± 0.25	1.80 ± 0.80	0.40 ± 0.25	0.60 ± 0.40	1.776	.173	NS
Curved Mid-piece	4.80 ± 0.74^a	4.00 ± 0.89^b	7.60 ± 0.68^a	5.60 ± 0.81^b	10.20 ± 1.02^a	8.869	.000	*
Tailless head	0.60 ± 0.25	0.40 ± 0.25	1.40 ± 0.93	1.60 ± 0.68	2.20 ± 0.74	1.384	.275	NS
Headless tail	3.60 ± 1.34^b	3.00 ± 0.84^a	3.60 ± 0.74^b	0.40 ± 0.68^a	8.00 ± 0.32^a	5.514	.004	*
Total abnorma cell	30.40 ± 6.28^b	28.80± 1.93^c	46.0 ± 3.11^d	48.80 ± 1.66^e	68.40 ± 5.67^a	14.793	.000	*
Total cell counted	456.80 ± 18.79	444.27±23.04	477.00±16.82	444.60 ±1147	453.60±15.10	0.585	.677	NS
Percentage abnormality (%)	6.63 ± 1.39^a	6.62 ± 0.70^a	9.81 ± 0.84^b	10.98 ± 0.28^c	15.20 ± 1.56^a	11.256	.000	*

*NS = no significant mean difference at 5% level; * At least two treatment group are significantly different at .05 level.*

Table 4:
Testicular Biometrics of Wistar Strain Abino Rats in Different Treatment Groups

Parameters	Groups							
	A (25mg/kg)	**B (50mg/kg)**	**C (100mg/kg)**	**D (200mg/kg)**	**E (control)**	**F-stats**	**P-value**	**Remark**
Weight of left testis (g)	1.23 ± 0.08	1.21 ± 0.03	1.28 ± 0.05	1.08 ± 0.10	1.29±0.03	1.752	.178	NS
Weight of right testis(g)	1.26 ± 0.05	1.08 ± 0.15	1.32 ± 0.58	1.06 ± 0.95	1.28±0.07	1.622	.208	NS
Length, left testis (mm)	18.02 ± 0.56	17.41± 0.45	18.37 ± 0.30	17.06 ± 0.72	18.05±0.47	1.05	.407	NS
Length rgt testis (mm)	18.39 ± 0.53	16.81 ±1.14	18.22 ± 0.43	16.16 ± 0.67	17.62±0.39	2.159	.111	NS
Dia. of left testis (mm)	9.98 ± 0.32	9.61 ± 0.32	9.96 ± 0.31	9.54 ± 0.48	9.78 ± 0.19	0.448	.772	NS
Dia. of right testis (mm)	10.08 ± 0.35	8.55 ± 051	10.15 ± 0.19	9.23 ± 0.43	9.23 ± 0.38	2.585	.068	NS
Epididymal weight (g)	00.54 ± 0.04	0.53 ± 0.11	0.57 ± 0.09	0.54± 0.06	0.48 ± 0.03	0.216	.927	NS

*NS = no significant mean difference at 5% level. * At least two treatment group are significantly different at .05 level.*

Group D has the highest mean percentage abnormality in the treatment group treated with 200mg/kg while Group B has the lowest mean percentage treated with 50mg/kg (Table 3).

Gonadometric indices

Biometrical assessment of the testes and epididymis: Results in Table 4 showed that Group C treated with 100mg/kg has the highest mean value among all the treatment groups (A - D) and has increased mean value compare to the control group in all the parameters except in the mean value of weight of left testis. Group D treated with 200mg/kg was observed to have the lowest mean value across the experimental groups. The results revealed that none of the testicular biometrics have a statistically significant mean different ($P < .05$).

DISCUSSION

In experimental animals, haematological parameters are indices for predicting or investigating the toxic (and otherwise, beneficial) effect of extracts from plants or other natural products (Ladokun, *et al*., 2015; Sunmonu and Oloyede, 2010)

In this study, the effect of the aqueous leaf extract on the haematological parameters appeared to be dose-dependent. There was an increase in WBC at 25 mg/kg, 50 mg/kg and 200 mg/kg doses, compared to the control group E. This increase may be due to an improvement in the rate of entrance of the blood cells from the bone marrow into circulation, or otherwise, from a decrease in the rate of removal of cells from circulation.

There was a significant reduction ($P<0.05$) in the lymphocytes count across the treated groups (A-D), compared to the control group E. This finding may be suggestive of the fact that the aqueous leaf extract of the plant does not adversely affect the effector cells of the immune system. This study also revealed a dose-dependent significant ($p<0.5$) increase in the platelet mean values at (100 mg/kg BW) and (200 mg/kg BW), which suggests that the aqueous extract of the leaves may improve the ability to stop or control bleeding in the animals, when given at these doses.

Also, in this study, there was a noticeable decrease in sperm motility and live/dead ratio at the dosage of 25 mg/kg, 50 mg/kg and 200 mg/kg, while the sperm count was reduced at a higher dosage value of 200 mg/kg, but all the semen characteristics studied are increased at (100mg/kg BW) dosage of the aqueous leave extract.

This result was justified by the findings made by Quadri and Yakubu, (2017) that the administration of the aqueous extract of the root of *Chasmathera dependens* (Hochst), at (100mg/kg BW) significantly increased all the semen parameters. The administration of the aqueous leaf extract of *Chasmathera dependens* (Hochst) caused a significant decrease iin the number of morphologically abnormal sperm cells. The total abnormal cells count and the percentage abnormality was significantly ($p < 0.05$) reduced in all the treatment groups, compared to the control.

This finding was in agreement with the observation that the aqueous extract of *Chasmathera dependens* (Hochst) root has no negative effect on the morphology of the normal sperm cells, and decreased the morphological defects of the head, neck and tail in the sperm cells (Quadri and Yakubu, 2017).

In conclusion, this study has shown that, the aqueous leaf extract of *Chasmathera dependens* (Hochst) demonstrated enhanced spermatogenic effect at 100 mg/kg by improving the semen quality and maintaining the testicular sizes, it has not exhibited any toxic effects on the male fertility and the reproductive system, especially at a dose not more than 100 mg/kg in the male rats. Therefore, the study provides an empirical basis for the common claim that the plant has a fertility enhancing potential in the male animals. Howbeit, a low dose not more 100mg/kg is recommended.

REFERENCES

Adekunle, A. A., Okoli, S. O., (2002): Antifungal activity the crude extracts of Alafia barteri oliver (Apocynaceae) and *Chasmanthera dependens* Hochst. (Menispermaceae). Hamdard Medicus (Pakistan), 45(3), 52–56.

Ajani, O.S., Oyeyemi, M.O. and Moyinoluwa, O.J., (2015): Correlation between age, weight, scrotal circumference and the testicular and epididymal parameters of Red Sokoto bucks. Journal of Veterinary Medicine and Animal Health, 7(5), 159-163

Akerele O., 1993. Nature's Medicinal Bounty: don't throw it away. World Health Forum, 14:390-395.

Elujoba, A. A., Odeleye, O. M. and Ogunyemi, C. M. (2005): Traditional medicine development for medical and dental primary health care delivery system in Africa. African Journal of Traditional, Complementary and Alternative Medicines, 2(1), 46-61.

Gupta, R.K., Kesari, A.N., Watal, G., Murthy, P.S., Chandra, R., Maithal, K. and Tandon, V., (2005): Hypoglycaemic and antidiabetic effect of aqueous extract of leaves of Annona squamosa (L.) in experimental animal. Current Science; 1244-1254.

Haq, I., (2004): Safety of medicinal plants. Pak J Med Res, 43(4), 203-10.

Iwu, M. M., Duncan, A. R., Okunji, C. O. (1999): New antimicrobials of plant origin. In: Janick, J., (Ed.), Perspectives on New Crops and New Uses. ASHS Press, Alexandria, VA, 457-462.

Ladokun, O., Ojezele, M., Arojojoye, O. (2015): Comparative study on the effects of aqueous extracts of Viscum album (mistletoe) from three host plants on hematological parameters in albino rats. African Health Sciences, 15(2), 606–612.

Mehdi, M. A. H., Alarabi, F. Y., Omar, G. M. N. and Pradhan, V. (2019): Effect of extracts on haematological parameters in albino rats Tamarindus indica infected with parasite Entamoeba histolytica. Asian Journal of Pharmacy and Pharmacology, 5(5), 889-894.

Oyeyemi, M.O. and Ajani, O.S., (2015): Haematological parameters, semen characteristics and sperm morphology of male albino rat (wistar strain) treated with Aloe vera gel. Journal of Medicinal Plants Research, 9(15), 510-514.

Odugbemi, T. (2008): A textbook of medicinal plants from Nigeria. Lagos: University of Lagos Press, 558

Ogunlesi, M., Okiei, W, Ademoye, M., (2008): Medicinal plants used in treating eye infections in Nigeria.

Ogunlesi, M., Okiei, W, Ademoye, M., Ogunlesi, E. A. O. M. (2010): Analysis of essential oil from the stem of Chasmanthera dependens. Journal of Natural Products, 47–53.

Oyeyemi, M.O. and Fayomi, A.P., (2011): Gonadosomatic index and spermatozoa morphological characteristics of male wistar rats treated with graded concentration of Aloe vera gel. Int J Anim Vet Adv, 3(2), 47-53.

Quadri AL, Yakubu MT. (2017): Fertility-enhancing activity and toxicity profile of aqueous extract of Chasmanthera dependens roots in male rats. Andrologia ;00: e12775. https://doi.org/10.1111/and.12775

Stanley, I. (2012): Isolation and characterization of antimicrobial compounds from the plants, erythrina abyssinica DC. and Chasmanthera dependens hochst (Doctoral Dissertation, School of Pure and Applied Sciences, Kenyatta University).

Sunmonu, T. O., Oloyede O. B., (2010): Performance and haematological indices in rats exposed to monocrotophos contamination. Human and Experimental Toxicology, 29(10), 845–850.