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ASSESSMENT OF REPRODUCTIVE TOXICITY OF CONCOCTION OF *ANDROGRAPHIS PANICULATA* IN MALE WISTAR ALBINO RATS

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ABSTRACT: Objective: To assess the reproductive toxicity of concoction of *Andrographis paniculata* in male Wistar albino rats. **Methods:** 18 male Wistar albino rats were divided into 3 groups with 6 animals in each group. Group 1 served as control and received distilled water. Group 2 and 3 received 0.5 ml of a concoction of *Andrographis paniculata* and Nilavembu kudineer (a concoction of 9 herbs) respectively for 30 days. Serum testosterone levels were measured at the baseline and at the end of the study. Animals were sacrificed at the end of the study, and histopathological examination of testes and sperm analysis were carried out. **Results:** There were no significant changes in the body weight and general behavior of the animals within the groups and between the groups. The differences noted in mean sperm count, as well as the qualitative characteristics such as progressive & non-progressive sperms and percentage of immotile sperms among the 3 groups, were not significant. Histopathology of testes did not show any difference between the control and treated groups. Similarly, serum testosterone levels also did not show significant changes. **Conclusion:** This study shows that concoction of *Andrographis paniculata* and Nilavembu kudineer in male Wistar albino rats, administered for 30 days, did not produce any significant adverse reproductive effects in terms of sperm count, sperm quality and testicular histology.

INTRODUCTION: Nilavembu kudineer or kariyat (kalamegha) is a Siddha concoction of nine herbs, namely, 1. Nilavembu (*Andrographis paniculata*), 2. Vilamichaiver (*Plectranthus vetiver*), 3. Vetiver (*Vetiveri azizaniodes*), 4. Chukka (*Zingiber offcinale*). 5. Milagu (*Piper nigrum*), 6. Koraikizhangu (*Cyperus rotundus*), 7. Santanam (*Santalum album*), 8. Peyputtal (*Trichosanthes cucumerina*) and 9. Parpadagam (*Mollugo cerviana*).

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Though it has 9 ingredients, the main ingredient is *Andrographis paniculata*. It is one of the most commonly used traditional concoctions in viral infections and febrile illnesses. It is used in malaria, dysmenorrhoea, worm infestations, eczema, jaundice, wound infections, dengue and chikungunya [1].

Experimental studies indicate that the extract of *Andrographis paniculata* and the concoction of 9 herbs have antimicrobial, anti-inflammatory, antiallergic, antioxidant and immunostimulant activities [2, 3]. The outbreak of dengue in Tamil Nadu in 2017 was a serious issue pushing the Government of Tamil Nadu to promote the use of Nilavembu kudineer in dengue fever. The fact that there was no drug available in the allopathic system for dengue fever made people look for alternate

remedies, and Nilavembu kudineer became popular. It was being distributed freely in all Government hospitals, schools, eateries, and Siddha clinics. Lakhs of people consumed Nilavembu kudineer with the belief that it might be useful in treating or preventing dengue fever.

At the same time, few newspapers [4] reported that Nilavembu kudineer might cause male infertility by quoting a few journal articles. These reports made the general public, especially males, to suspect whether it was safe to consume the concoction and whether their reproductive potential would remain unaffected by the concoction. The Government insisted that it was used for hundreds of years and no such observations were reported from traditional practice, and it was indeed safe to consume the concoction. However, the literature search provides conflicting reports, a few articles [5, 6, 7] reports that the concoction affects the male reproductive system and a few [8, 9] report that the concoction does not affect the male reproductive system.

Dayang Nurul Fathihah *et al*., (2015) investigated the fertility suppression effects of methanolic extract of *Andrographis paniculata* in male Sprague Dawley rats. They used 2 doses of the extract – low dose (800 mg/kg) and high dose (1600 mg/kg). Sexual behavior, fertility test, sperm quality analysis, serum testosterone, and histology of testes were assessed in the animals. Rats treated with both low and high dose *Andrographis paniculata* extract showed a significant reduction in the number of mountings compared to control group. The seminal fluid analysis showed significantly reduced sperm count, and motility in the *Andrographis paniculata* treated rats. Histopathology of tests showed degenerated spermatogenic elements, with the lumen of seminiferous tubules partially filled with spermatozoa in both the test groups. The authors concluded that the methanolic extract of leaves of *Andrographis paniculata* had male reproductive toxicity and could be used as a plant-based contraceptive agent for male rats to control rodent pest problem [5].

Kalyan Batra Santra *et al*., (2013), in their experiment on Indian wild rats (*Rattus rattus*) evaluated the reproductive toxicity of aqueous leaf extract of *Andrographis paniculata*. 250 mg/kg of aqueous extract was given to 2 groups for 30 and 45 days, respectively. There was a significant quantitative and qualitative reduction of spermatozoa in both groups. Histochemical analysis showed that the levels of Δ5-3β – hydroxysteroid dehydrogenase (HSDH), 17β - hydroxysteroid dehydrogenase (HSDH), alkaline phosphatase and acid phosphatase were significantly decreased in the testes of extract treated groups. The authors concluded that the aqueous extract of *Andrographis paniculata* disrupted spermatogenesis in rats [6].

Dasuki MS *et al*., (2015) studied the effects of 50% ethanolic extract of *Andrographis paniculata* on the sexual behavior in male Sprague Dawley rats. Sexual behavior was found to be decreased in the test group when compared to the control group in terms of mount latency, ejaculation latency, and intromission latency. There was a significant increase in the serum testosterone levels in *Andrographis paniculata* treated groups [7].

Burgos RA *et al*., (1997) studied the testicular toxicity of *Andrographis paniculata* in male Sprague Dawley rats. The rats were treated with 20, 200, 1000 mg/kg of dried extracts of *Andrographis paniculata* for 60 days. They observed that *Andrographis paniculata* did not produce any testicular toxicity. Their conclusion was based on testicular weight and its histological examination, analysis of leydig cells and serum testosterone levels [8].

Mkrtchyan A *et al*., (2005) conducted a phase I clinical study in adult males to investigate the effect of *Andrographis paniculata* special extract on spermatogenesis and sperm quality. They did not observe any antifertility effect in the subjects treated with *Andrographis paniculata*. They, moreover, reported that the subjects had more of active spermatozoids, less inactive spermatozoids and better fertility indices [9].

Shahid Akbar (2011), in his review article on the pharmacological activities and clinical effects of *Andrographis paniculata* stated that it was not possible to conclude whether it has male reproductive toxicity or not with the existing evidences as they were conflicting in nature. He was of the opinion that further fertility studies were

needed to understand more about the reproductive toxicity of *Andrographis paniculata* [10]. Because of the conflicting reports of *Andrographis paniculata* on male fertility, this study was systematically planned to investigate the male reproductive toxicity of concoction of *Andrographis paniculata* and concoction of the 9 herbs in male Wistar albino rats.

The study aimed to investigate the male reproductive toxicity of Nilavembu kudineer (a concoction of 9 herbal ingredients) and the concoction of the main ingredient, *Andrographis paniculata*, in male Wistar albino rats.

The primary objectives were to evaluate the sperm count, sperm quality, serum testosterone and testicular histology in male Wistar albino rats followed by consumption of Nilavembu kudineer (a concoction of 9 herbal ingredients) and the concoction of the main ingredient, *Andrographis paniculata*. The secondary objectives were to assess the renal and liver function tests followed by exposure to the herbal concoctions.

MATERIALS AND METHODS: The study was conducted after getting approval from the Institutional Animal Ethics Committee, vide letter no. 21/A.Lr: 05 dated 01.03.2018.

The experiment was conducted using 18 male Wistar albino rats weighing 100-200 grams. The rats were clinically healthy when they were selected for the study. They were kept in 12 hours dark and light cycle at the room temperature of 22 ^{o}C. They were fed with normal pellets with continuous drinking water supply. The animals were divided into three groups with each group consisting of 6 animals. First group was the control group, while the second and third groups were experimental groups.

First Group / Control Group (6 Animals): 0.5ml of distilled water, everyday for 30 days.

Second Group (Test-1, 6 Animals): 0.5ml of concoction of *Andrographis paniculata*, every day, for 30 days.

Third Group (Test-2, 6 Animals): 0.5ml of Nilavembu kudineer (concoction of 9 herbs), every day, for 30 days.

The treatments were administered via animal feeding tube. Complete blood count, urea, creatinine, liver function test, and serum testosterone were measured before the start of the study and 30 days after administration of study interventions. 0.5 ml of blood was drawn by retro-orbital venous puncture after the animals were anesthetized using low dose halothane and used for the analysis of blood parameters.

At the end of the study, the animals were sacrificed by administering a high dose of halothane anesthesia. The testis, along with epididymis, was dissected and kept in 4 ml of Phosphate Buffer Solution (PBS). The seminal fluid from the epididymis and testis was aspirated, and the concentration of spermatozoa was assessed by adding 10 µl of seminal fluid in makler's chamber and counting under a compound microscope (10X). The sperm quality was assessed under a compound microscope (40X) by studying seminal fluid (10 µl) in a glass slide under coverslip. The testes and epididymis were further subjected to histopathological examination.

Preparation of Extracts: The dried powder of leaves of *Andrographis paniculata* and the dried powder of 9 herbs (powder of Nilavembu kudineer) are commercially available and were purchased from an Ayurvedic store. Plant authentication was not applicable as the dried powder of the leaves, sold for preparation of concoction, were purchased from established Ayurvedic store. 10 grams of the respective powders were taken in 240 ml of water and boiled for 60 min to concentrate to 60 ml. The concentrated solution was filtered, and the filtrate solution was used to dose the animals of respective test groups.

RESULTS: The study was conducted in 18 animals with 6 animals, each allocated to control, test-1, and test-2 groups. One animal in the control group died on day 1, at the time of blood withdrawal via retro-orbital venous puncture and only 5 animals in the control group completed the study. One animal in the test-2 group died at the time of blood withdrawal on day 30 and hence sperm analysis, liver function tests and renal function tests were not carried out for this animal, but complete blood count was done with the available blood sample on day 30. The body weight

and general behavior were assessed during baseline and at the end of the study, and there were no significant changes in the body weight and behavior within the groups and between the groups.

Sperm Analysis: After the end of the study, the animals were sacrificed, and seminal fluid was collected from testes and epididymis. The mean sperm count in the control animals at the end of the study was 73.4 million/mL. It was 85.83 million/ml in test-1 group and 121.6 million/ml in test-2 group. The difference noted among the 3 groups was analyzed using one-way ANOVA, and it was not statistically significant.

The percentage of progressive sperms was 18.63% in the control group, 23% in test-1 group and 29.75% in test-2 group. The non-progressive sperms constituted 17.38% in control group, 11.58% in test-1 group and 16.25% in test-2 group. The percentage of immotile sperms was 65.38% in control group, 63.17% in test-1 group and 52.88% in test-2 group. These variations observed among the three groups were not statistically significant (One-way ANOVA).

The data about the quantitative and qualitative analysis of spermatozoa is provided in **Table 1** and **2,** respectively.

Histopathological Examination of Testes: The histopathological examination of the tests showed no significant changes. The seminiferous tubules and spermatozoa appeared normal in all the three groups. The representative figures of the histopathological examination of tests are shown in **Fig. 1**.

Serum Testosterone: Serum testosterone measured during baseline and at the end of the study showed no significant difference within the groups (paired t-test) and among the three groups (one-way ANOVA). The mean testosterone was 386.34 ng/dl (±129.57) before the initiation of the study and 419.99 ng/dl (±214.91) at the end of the study in the control group. It was 358.50 ng/dl (±128.28) and 250.21 ng/dl (±134.34) before and after the study in a test-1 group, respectively.

In a test-2 group, the testosterone level was 323.97 ng/dl (±223.76) and 316.55 ng/dl (±177.34) before and after the study, respectively. The serum testosterone levels are provided in **Table 3**.

Other Blood Parameters: The following blood parameters were measured in this study during the baseline and at the end of the study.

- **Complete Blood Count:** Haemoglobin, Packed cell volume, Red blood cells, White blood cells, Differential white cell count, Platelet count.
- **Liver Function Tests:** Bilirubin (direct, indirect and total), SGOT, SGPT, Alkaline phosphatase, Total protein, Albumin, Globulin
- **Renal Function Tests:** Urea, Creatinine

The statistical analysis of these parameters within the groups was done by paired t-test. The changes observed after the completion of the study were not statistically significant compared to the baseline data. Similarly, the differences between the baseline data and end of the study data among the three groups were analyzed using one-way ANOVA, and it did not show any statistical significance in these parameters between groups.

TABLE 1: SPERM COUNT ANALYSIS (MILLIONS / mL)

Animal	Control			Test-1, *Andrographis paniculata*			Test -2, Nilavembu kudineer		
	Total sperm count	Epididymis	Testis	Total sperm count	Epididymis	Testis	Total sperm count	Epididymis	Testis
1	41	27	14	43	25	18	185	110	75
2	65	39	26	106	61	45	100	55	45
3	54	38	16	153	101	52	61	43	18
4	164	120	44	110	86	24	174	114	60
5	43	31	12	47	30	17	88	58	30
6	-	-	-	56	36	20	-	-	-
Mean	73.4	51.00	22.40	85.83	56.50	29.33	121.6	76.00	45.60
SD	51.55	38.89	13.22	44.12	31.58	15.20	54.85	33.37	22.79

TABLE 2: SPERM QUALITY ANALYSIS (%)

Animal	Control group								
	Total sperm			Epididymis			Testis		
	Progressive	Non Progressive	Immo-tile	Progressive	Non Progressive	Immotile	Progressive	Non Progressive	Immotile
1	13	18.5	69.5	22	21	58	4	16	81
2	15.5	19	65.5	24	19	57	7	19	74
3	7.5	21	71.5	7	26	67	8	16	76
4	38.5	11	55	69	7	31	8	15	79
5	19	14	67	28	7	65	10	21	69
6	-	-	-	-	-	-	-	-	-
Mean	18.63	17.38	65.38	30.50	18.25	53.25	6.75	16.50	77.50
SD	13.66	4.39	7.35	26.89	10.74	27.34	3.44	7.53	34.76
Animal	**Test -1, *Andrographis paniculata***								
	Total sperm			Epididymis			Testis		
	Progressive	Non Progressive	Immo-tile	Progressive	Non Progressive	Immotile	Progressive	Non Progressive	Immotile
1	7	15	78	11	22	67	3	8	89
2	28.5	17.5	54.5	44	10	46	13	25	63
3	47	14.5	39.5	55	5	42	39	24	37
4	27	7.5	65.5	44	7	49	10	8	82
5	6	4.5	89.5	7	6	87	5	3	92
6	22.5	10.5	52	31	14	55	14	7	49
Mean	23.00	11.58	63.17	32.00	10.67	57.67	14.00	12.50	68.67
SD	15.28	4.96	18.32	19.41	6.44	16.80	12.99	9.48	22.62
Animal	**Test -2, Nilavembu kudineer**								
	Total sperm			Epididymis			Testis		
	Progressive	Non Progressive	Immo-tile	Progressive	Non Progressive	Immotile	Progressive	Non Progressive	Immotile
1	27	20.5	52.5	37	21	42	17	20	63
2	37.5	20.5	42.5	38	19	44	37	22	41
3	17	18	60	23	25	42	11	11	78
4	37.5	6	56.5	45	5	50	30	7	63
5	32.5	10	57.5	48	8	44	17	12	71
6	-	-	-	-	-	-	-	-	-
Mean	29.75	16.25	52.88	35.75	17.50	44.50	23.75	15.00	61.25
SD	9.84	6.93	7.56	17.87	10.86	20.17	14.78	9.14	30.41

TABLE 3: SERUM TESTOSTERONE LEVELS (ng/dl)

Animal	Control		Test -1, *Andrographis paniculata*		Test -2, Nilavembu kudineer	
	Baseline	End of study	Baseline	End of study	Baseline	End of study
1	331.3	435.34	347.7	200.25	126.45	407.34
2	603.6	527.9	218.23	448.46	198.28	140.64
3	315.3	697.42	542.62	274.6	436.36	399.4
4	278.6	131.22	398.78	200.34	664.56	517.38
5	402.9	308.1	432.04	326.34	194.22	118
6	-	-	211.68	51.3	-	-
Mean	386.34	419.99	358.50	250.21	323.97	316.55
SD	129.57	214.91	128.28	134.34	223.76	177.34

Andrographis Paniculata **Nilavembu Kudineer** **Control**

FIG. 1: HISTOPATHOLOGICAL EXAMINATION OF TESTES

Note: The testes of all the three groups show normal seminiferous tubules and spermatozoa

DISCUSSION: This study was conducted to investigate the male reproductive toxicity of concoctions of commercially available powder form of *Andrographis paniculata* and Nilavembu kudineer in Wistar albino rats. This experiment was planned due to the controversy over the Govt. of Tamil Nadu publicly recommending Nilavembu kudineer as the treatment for dengue fever while few magazines are reporting that it could be associated with male reproductive toxicity.

Nilavembu kudineer is the concoction prepared by the process mentioned in the methodology. The basic raw material consists of 9 plants and the concoction is a mixture of ingredients from these 9 plants, though *Andrographis paniculata*is the main ingredient. In this experiment, the concoction of the 9 plants (Nilavembu kudineer) along with the concoction of only *Andrographis paniculata* leaves experimented to evaluate the male reproductive toxicities of *Andrographis paniculata* and Nilavembu kudineer. The animals received 0.5 ml, the maximum volume that could be comfortably administered to the rats via feeding tube, every day for 30 days.

The results show that there were no significant changes in the male reproductive system of the animals treated with *Andrographis paniculata* concoction and Nilavembu kudineer compared to the control group. The sperm count and the quality of the sperm in terms of percentage of progressive, non-progressive, and immotile sperms were not significantly different from control animals, in the treated animals. In fact, the number of sperms in the test-2 group that received Nilavembu kudineer was 121.6 million/ml and it was 85.83 million/ml in *Andrographis paniculata* group (test-1), which were higher than the number observed in control group (73.4 million/ml). But these increases were not statistically significant (ANOVA, $p > 0.05$). Further, the baseline sperm analysis was not carried out due to methodological issues since the rats had to be sacrificed to do sperm analysis. Due to this limitation, it cannot be ensured whether there was any actual increase in the sperm count when the baseline sperm data was not available.

The quality of spermatozoa did not show any significant change in the *Andrographis paniculata* group and Nilavembu kudineer group compared to the control group. The percentage of motile sperms that includes both progressive and non-progressive sperms was 36.01% in control group, 34.58% in *Andrographis paniculata group* and 46% in Nilavembu kudineer group.

The sperm count more than 20 million/ml and percentage of motile sperms more than 40% are considered to be normal [11, 12]. In this study, the sperm count was more than the limit and hence it can be said that *Andrographis paniculata* and Nilavembu kudineer did not affect the sperm count. But the percentage of motile sperms was less than 40% in control and *Andrographis paniculata* groups, while in Nilavembu kudineer treated group, it was more than 40%. Though it is easy to conclude that Nilavembu kudineer increased the sperm quality, the quality was less than normal in the control group. The control group is supposed to have normal sperm quality, and when the control data is not normal, the data of treated animals cannot be compared with control, and any interpretation should be guarded.

Hence, this increase in the percentage of motile sperms observed with Nilavembu kudineer is only considered as incidental in this study and not significant clinically as well as statistically. The histopathological examination of tests showed only normal histological findings with normal seminiferous tubules and spermatozoa. Serum testosterone levels at baseline and after the study were not significantly different within groups and between groups. The hematological parameters, renal function tests, and liver function tests were also within the normal limits at baseline and after the study.

CONCLUSION: This study shows that administration of 0.5 ml of a concoction of *Andrographis paniculata* and Nilavembu kudineer in male Wistar albino rats for 30 days did not produce any significant adverse reproductive effects in sperm counts, sperm quality and testicular histology. However, further studies are needed in large sample size and different doses to ascertain the outcomes of this study.

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CONFLICT OF INTEREST: The authors declare that there is no conflict of interest.

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