

# Effect of Apamarga Kshara of Whole Plant of *Achyranthes Aspera* Linn on Fertility in Male Albino Rats (*Rattus Norvegicus*)

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## ARTICLE INFO

### Article History:

Accepted: 03 March 2024

Published: 15 March 2024

### Publication Issue :

Volume 11, Issue 11

March-April-2024

### Page Number :

01-06

## ABSTRACT

*Achyranthes aspera* Linn. (Amaranthaceae) is an abundant medicinal and Ayurvedic herb found throughout India. *A. aspera* has occupied a pivotal position in Indian culture and folk medicine. From the ancient time the tribal and rural people of our country commonly use this herb to treat various disorders. Based on the literature reviews, it shows a potential antifertility effect on laboratory animals. The main objective of the present study was to evaluate the effect of the whole plant of *A. aspera* Linn. on fertility in male albino rats. A total of 24 proven fertile male albino rats were equally divided into four groups. The three Experimental groups (E1, E2 and E3) were orally administered with the kshara of the whole plant of *A. aspera* about 1g/kg b.wt /day for 7, 14, and 21 days respectively. Normal saline was orally given to the control group rats for the same period. On the 8th, 15th and 22nd days of the autopsy, all the animals were sacrificed and reproductive organs were removed and their weights were recorded. Sperm analysis was done to observe the effect of Apamarga kshara of the whole plant on fertility. Weight of testes and epididymis and sperm counts were significantly decreased as dose exceed in respective days in all the treated animals in comparison with control group animals. Hematological parameters were studied to see the toxic side effects on blood in treated animals. These haematological parameters showed very slight significant changes in treated animals. Hence, it is concluded that the Apamarga kshara of the whole plant of *A. aspera* shows an antifertility effect on male albino rats with less effects on blood parameters.

**Keywords:** Apamarga Kshara , *Achyranthes aspera* , Antifertility, Haematological parameter.

## I. INTRODUCTION

The current world population is around 8.1 billion and that of India in particular is around 1.42 billion. One of the critical problems of the developing countries like India is its geometrical increase in the human population. This population explosion will have negative impact on our economic policies and would simultaneously misbalance our socio-economic infrastructure. Thus the control of human fertility in the sense of its limitation is the most important and urgent requirement.

In this search several potential approaches for induction of infertility have been investigated over a long period, including chemical, hormonal, and immunological approaches. However, no suitable method has emerged that is effective and free from side-effect (Raka & Gupta *et al.*, 2003). Hence, there is a need for development of new fertility regulating drug from medicinal plants because from times immemorial humans have relied on plant products as sources of drugs and therapeutic agents. In recent times due to low toxicity and long standing experience of exposure, these drugs are used in ethnic medicine system like Ayurveda (Deshpande *et al.*, 1980).

*Achyranthes aspera* L. (Family Amaranthaceae) is a common plant of the study area abundantly found in wastelands. It is known as "Prickly chaff flower" in English and "Chirchita", "Onga", "Latjeera" or "Apamarga" in local language and dialects (Khan A.V 2002). Kshara is the water-soluble ash of drug which is in the form of solutions, powders which is alkaline in nature prepared from Apamarga (*A. aspera*) in powder form (Sushruta *et al.*, 2013). The plant is highly esteemed by traditional healers and used in treatment of asthma, bleeding, in facilitating delivery, boils, bronchitis, cold, cough, colic, debility, dropsy, dog bite, dysentery, ear complications, headache, leucoderma, pneumonia, renal complications, scorpion bite, snake bite and skin diseases etc. (Jain, 1991). Traditional healers claim that addition of *A.*

*aspera* would enhance the efficacy of any drug of plant origin.

A literature study reveals that no systematic advent has been made in the past to study the antifertility activity of whole plants of *A. aspera* in male albino rat (*Rattus norvegicus*). Therefore, this study was designed to investigate the antifertility activity of Apamarga Kshara of whole plants of *A. Aspera* with haematological parameter.



Fig 1: *Achyranthes aspera* (Whole plant)

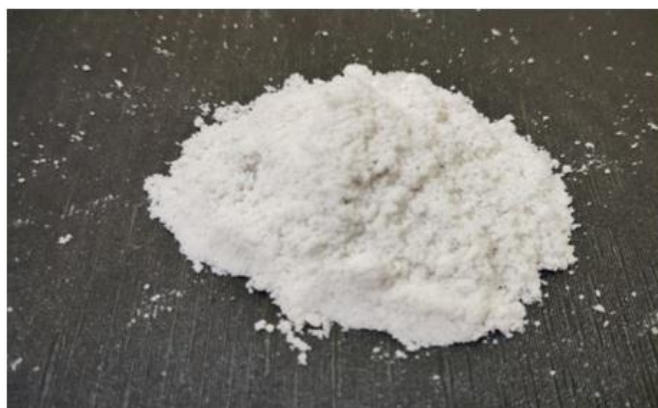


Fig 2: Apamarga Kshara of *A. aspera* (Whole plant)

## II. METHODS AND MATERIAL

### A. Plant collection and Identification:

*Achyranthes aspera* Linn was freshly collected during Nov- Dec, 2022 from in and around of campus of R.T.M Nagpur University, Nagpur (India) as it is abundantly available. The plant was authenticated identified by a taxonomist of Department of Botany, Govt. Institute of Science, Nagpur and voucher

specimen (Herbarium number BOT-442A) was preserved.

### B. Preparation of Apamarga Kshara of *A.aspera* (Whole plant):

Fresh plants consisting of all parts was collected and cleaned. The plants will be dried completely under shade and packed with due care. After complete drying and weighing, plants was taken for burning in an iron vessel and burned completely in open air till a white ash were obtained. The weight of this ash was recorded and transfer to a glass container to which distilled water four times the weight of the ash was added. The mixture was stir well by glass rod and keep overnight. The solution filtered by ordinary filter paper by passing the supernatant solution on the filter paper. The filtrate was clear and collected in a clean pot. This filtrate was subjected to slow evaporation till all the water get evaporate. At the end of this process it is necessary to keep the mixture stirring with glass rod to avoid the formation of lumps. This mixture is dried to get the white crystalline kshara. Since the kshara are hygroscopic in nature, it stored in a tightly closed glass bottle. (The Sushruta Samhita Suthrasthanam, 1907)

### C. Treatment of animals:

Wistar strain male albino rats of proven fertility (200-220g) were used for the study and maintained in standard environmental condition (temperature  $22 \pm 20^\circ\text{C}$  and 12hr light/12hr dark cycle & 45-60% humidity) fed a commercial diet and water ad libitum. All the experiments were performed according to the CPCSEA norms after obtaining the approval of the Institutional animal's ethics committee (IAEC). They were acclimatized for laboratory conditions for one week.

### D. Experimental design:

The animals were equally divided into four groups each containing six animals. The treatment groups (E1, E2 and E3) were subjected to oral administration of

Apamarga kshara about 1g/kg b.wt /day for 7, 14 and 21 days respectively. The control group animals received vehicle only (Normal saline).

### E. Body weight and fertility parameters:

Body weights of animals were recorded before the experiment. After 24 hours of the last dose, the rats were weighed, anesthetized and blood was collected by puncturing the retro-orbital venous plexus. The rats were sacrificed by cervical dislocation, sperms were collected from the cauda epididymis immediately after sacrifice for sperm count (Adhinkary *et al.*, 1990).

Group	Table 1 : Weights of Reproductive organ			
	Average body weight			
	Treatment Weight of Organ(mg/100 gm b.w)			
	Before	After	Testis	Cauda epididymis
Control	216 $\pm$ 7.87	255 $\pm$ 5.94	560 $\pm$ 1.76	168 $\pm$ 2.23
(E1)1g/kg/day for 7 Days	220 $\pm$ 7.05	250 $\pm$ 9.95	531 $\pm$ 4.13	147 $\pm$ 3.11
(E2)1g/kg/day for 14 Days	218 $\pm$ 7.29	257 $\pm$ 7.22	523 $\pm$ 3.34	145 $\pm$ 3.90
(E3)1g/kg/day for 21 Days	214 $\pm$ 6.55	255 $\pm$ 6.24	520 $\pm$ 5.57	142 $\pm$ 2.30

Values expressed as mean  $\pm$  S.D for 6 animals in each group

The target organs like testes, epididymis, were dissected, trimmed off from fat and weighed. The relative weights of the organ per 100 g of body weight were calculated.

### F. Haematological parameters:

The safety of Apamarga Kshara of *A.aspera* were evaluated at the tested dose levels of consecutive days by comparing the hematological parameters of treated group animals with the control group. Using the Haematology Analyzer machine, the procedure for blood cell (full blood count) determination was performed as follows: EDTA samples were placed in a hematology blood mixer for three minutes and the blood cells were automatically counted through a probe fitted in the Analyzer. After one minute, the results of the blood cell count were displayed on the color LCD screen of the machine. The haematological indices values of the negative control group were used as the reference range for the estimated parameters in this study.

### Statistical analysis:

Results were expressed as mean  $\pm$  standard deviation (S.D.). Where applicable, the data were subjected to one way analysis of variance (ANOVA). P values at 0.05 were regarded as significant and the comparison between the control and experimental groups was done using the Dunnett's test.

## III. RESULTS AND DISCUSSION

The result obtained from the present study are discussed below:

### A. Body weight and fertility parameters:

During the period of experiment, the rats were healthy, growing at normal growth rate. Their body weight gain was similar to that of control group (Table 1).

The result of the current study showed that, animal treated with Apamarga Kshara of whole plant of *A. aspera* to male rat at dose 1g/kg b.wt/day for 7, 14, 21 days brought about a highly significant reduction in testis and epididymis, especially in 21 days experiment group showed remarkable reduction in the size of testis and epididymis weight. In the similar study of methanol extract of *Nyctanthes arbortristis* stem bark in male albino rat caused decreased in the

weight of testes and accessory reproductive organs at dose 100mg/kg b.wt for 60 days whereas body weight of the animal did not show any significant change when compared to control group (Gupta *et al.*, 2006). Hence, the results of the present study indicated the correlation of reduction in weights of testes and epididymis with the antifertility effect on rats.

A significant decrease ( $P < 0.05$ ) in sperm concentration about 67.77%, 73.33% and 88.88% was also found in Experimental groups E1, E2 and E3, respectively in comparison with control group animals (Table 2). In earlier study, it was found that feeding 50% ethanolic extract of *A. aspera* to male rats resulted in reduced sperm counts (Sandhyakumary and Bobby, 2002). Based on the present study, it could be concluded that the Apamarga kshara of whole plant of *A.aspera* shows antifertility property with consecutive reduction in sperm concentration.

**Table 2 : Sperm Concentration Analysis**

Group	Sperm Concentration (millions/ml)	Percentage Change
Control	90 $\pm$ 4.45	NIL
(E1) 1g/kg/day for 7 Days	29 $\pm$ 3.09	-67.77
(E2) 1g/kg/day for 14 Days	24 $\pm$ 2.19	-73.33
(E3) 1g/kg/day for 21 Days	10 $\pm$ 2.24	-88.88

Values expressed as mean  $\pm$  S.D for 6 animals in each group

### B. Haematological parameters:

All the haematological variables were within the normal range (Table 3) showed very less significant changes in treated animals in comparison with the control group animals. In a similar study administration of *aloe-vera* gel at 300 & 200 mg/kg body weight for 10 days reduced the sperm concentration, motility and liveability and the haematological parameters were within the normal

range of values in rat indicating no toxic side effects on normal metabolism at the tested dose levels (Ajani and oyeyemi., 2015). Hence, the present study shows that Apamarga kshara of whole plant of *A.aspera* are safe without any toxic side effects at the dose levels for 7, 14 and 21 days.

**Table: 3 Haematological Parameter**

	Control	Treatment Dose 1g/kg b. wt/day		
		(E1)	(E2)	(E3)
		7 days	14 days	21 days
Hb(g/dl)	14.1±0.39	14.8±0.12	12.1±0.82	12.8±1.63
PCV (%)	41.21±1.05	46.58±1.24	45.03±1.48	40.50±3.40
MCV (fl)	54.19±1.97	57.20±1.56	56.60±1.07	58.40±1.99
MCH(pg)	17.50±2.60	18.73±2.24	17.69±2.64	16.99±1.57
MCHC(gr/dl)	30.63±0.51	32.18±0.42	30.90±1.63	30.02±2.54
RBC(x10 <sup>6</sup> /mm <sup>3</sup> )	7.36±0.03	7.38±0.02	7.46±0.02	8.12±0.94
WBC(x10 <sup>3</sup> /mm <sup>3</sup> )	5.45±0.96	5.12±2.25	4.06±1.77	4.80±1.80
Platelets(x10 <sup>3</sup> /mm <sup>3</sup> )	780.52±71.94	819.5±131.5	845.42±83.50	896.19±106.6
Lymphocytes	72.6±0.5	74.1±1.90	81.1±1.6	87.4±0.83
Neutrophils	13.5±0.8	12.5±2.6	15.6±3.7	12.58±0.4
Monocytes	14.3±0.18	13.1±1.4	8.4±0.41	6.4±1.12

PCV=Packed cell volume; MCV=Mean corpuscular volume;

MCH= Mean corpuscular hemoglobin;

MCHC= Mean corpuscular hemoglobin concentration.

Values expressed as mean ± S.D for 6 animals in each group

#### IV.CONCLUSION

The oral administration of Apamarga kshara of *A.aspera* of whole plant results in changes at a dose level in Body weight, Fertility of male albino rat. The ingestion of 1g/kg b.wt/day for 7, 14, 21 days causes antifertility activity and slight change in haematological parameter as compared to control group. However, it has no more than negative effect on Haematological parameter as compared to control group. Hence, the kshara of whole plant needs to be studied further to identify and isolate the active compounds to develop new effective, potent and safe antifertility agent.

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