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INVESTIGATION OF ANTIFERTILITY ACTIVITY OF ETHANOL EXTARCT OF *ACHYRANTHES ASPERA* ON MALE ALBINO RAT

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ABSTRACT
The aim of the present study was to investigate the antifertility activity of *Achyranthes aspera* family (Achyranthaceae) ethanolic extract on male albino ratresulted in reducedtestesweight, sperm count,sperm viability, sperm abnormality and sperm motility.It indicates, *Achyranthes aspera*(Achyranthaceae) have significant antifertility activity and it suppresses the process of spermatogenesis which can lead to infertility in male albino rat. As a whole it can be postulated from study that the extract appears to be promising male contraceptive agent in order to develop the potential of herbal medicine.

Key Words: *Achyranthus aspera*,rat, testes, sperm count,sperm viability, sperm abnormalityand sperm motility.

INTRODUCTION

Fertility control is an issue of global and national public health concern. Contraceptive vaccines, and inhibitors of spermatogenesis and sperm motility, provide a potential for non-hormonal male contraceptive. Use of anti-fertility agent is one of the methods in controlling human population. In recent years, there has been a concern about the use of plant products in affecting fertility of humans. India has vast resources of natural products people have been using many of the medicinal plants for inducing abortion and permanent sterility **(Dixit,1992).** A large number of herbal drugs are used to control fertilization with considerable success.

The folklore information and the ancient literature about the plants and herbs can help the anti-fertility program. In the recent past, a number of plants have been identified as antifertility and evaluation of extracts and active principles from different parts of plants like seeds, root, leaves, flowers, stem or stem barks have been confirmed by various researchers**(Henshaw,1953; Chopra *etal*., 1958).**

Achyranthes aspera family (Amaranthaceae), is found on road sides, field boundaries and waste places as a weed throughout India, in South Andaman Islands.It is pungent, antiphlegmatic, antiperiodic, diuretic, useful in oedema, dropsy and piles, boils and eruptions of skin etc. Principal constituents of *Achyranthes aspera* are alkaloids, betaine and achyranthine, from the whole plant (**Priya*et al*.,2012**).The composite extract of *Achyranthes aspera* reported to possess spermicidal activity in male rats. It has been reported that a protein present in the ethnolic extract of *Achyranthes aspera* is responsible

for the sperm toxicity and spermicidal activity in male rats.The medicinal value of plants lies in their bioactive phytochemical constituents that produce definite physiological actions in the human body (**Atessahin*et al*., 2006**). The chemical constituents include flavonoids, alkaloids, essential oils, saponins, terpenoids, tannins, phenolic compound etc.

MATERIAL AND METHODS

Selection of herbal plant

Achyranthes aspera was selected for the present study. It is commonly known as "Aghada" in marathi and in Sanskrit called as "Apamarga" is widely available in field area of Vidarbha region and it is a common weed. The plant was identified by authenticated Department of Botany, Govt. Vidarbha Institute of Science and Humanities, Amravati.

Alcoholic extraction

Mature and young Inflorescence of *Achyranthes aspera* was collected and shadow dried. The shade-dried whole material was subject to pulverization to get coarse powder. The coarsely powder whole material (1kg) of *Achyranthes aspera* was used for extracted with ethanol in Soxhlet apparatus. The extract was evaporated to dryness under vacuum and dried in vacuum desiccators (15.5% w/w). The ethanol extract of *Achyranthes aspera* material was obtained and stored in refrigerator at 40C for future use.

Experimental Animals

Normal healthy male albino rats (250-300 g) were used for the present investigation. Animals werehoused separately in cages underenvironmental standard conditions for acclimatized 1 week underat temperature (25 ± 20C) and light and Dark (12 : 12 h). Rats were fed with standard pellet and water week at the end after doses will start of the experiment period upto 15 days.

Chemical :

Clofert 50content Clomiphene citrate IP 50 mg, ManeeshPharmaceutical Ltd. purchased from., India.

Experimental set up

The rats were divided into 5 groups and each group contains of 4 rats and were given the following treatment schedule

Group – I (Normal/control group) Rats were given distilled water for 15 days.

Group – II (Standard control) Rats were treated with standard drug, clomiphene citrate 50mg/ Kg for 15 days.

Group – III Rats were treated with selected plant extract for 15 days once a day (200 mg/Kg)

Group – IV Rats were treated with selected plant extract for 15 days once a day (400mg/kg)

Group – V Rats were treated with selected plant extract for 15 days once a day (600mg/kg)

Testis and Epididymal sperm count, viability and abnormality:

Sperm counts were performed under the microscope using the improved Neubauer chamber (**Fein Optik, Blankenburg, Germany**).

Sperm motility and progressive sperm motility

Sperm motility progression was observed by standard methods (**Slott *et al*.,1991)**

Statistical analysis

Data were described by proportion, mean, SD, range etc. The data were statistically analyzed by using one-way analysis of variance (ANOVA). The Statistical analysis was done by using student t test for estimation of significant results in experimental and control group of rats. of <0.05, 0.01 and 0.001 were considered as significant, more significant and highly significant respectively to discard the null hypothesis at 5% precision and 95% confidence interval.

Results and Discussion

Testis weight

The weight of the testis was significantly lower in the experimental groups, treated with 200 mg/kg, 400 mg/kg, and 600 mg/kg than the control except the standard group. However, the weights of testis of the standard group treated with the pharmaceutical drug, clomiphene citrate significantly increased in the present study as compared to a control group of rats (Table 1). Significant results were obtained in all groups with a negative impact in the treated group and a positive impact was found in the standard group of rats.

Table 1: Impact of Inflorescence extract of *Achyranthes aspera* and Standard drug on weight
of Testis of rat (M ± SD, n=4)

Treatment	Testis (g/bw)
Control	2.757±15.93
200mg/kg	1.827±8.04
400mg/kg	1.726.6±4.91
600mg/kg	1.523.2±3.14
Standard clomiphene citrate	2.958.1±15.67

Results given as Mean ± S.D., Means in the column with * are significant at (P=0.05).

Sperm count

The sperm count in the treatment groups was significantly lower than that of the control group treated at 200 mg/kg, 400 mg/kg, and 600 mg/kg of body weight for 15 days of periods. Additionally, the standard groups of clomiphene citrate showed a favorable positive result as compared to the control group (**Table and Fig. 4.10**).

Sperm viability

From **(Table 2)** it was observed that the sperm viability percentage was found to be significantly lower at 200 mg/kg, 400 mg/kg, and 600 mg/kg of body weight after 15 days of treatment in rats and the result was significantly varied than the control.

In addition, compared to the control group, the clomiphene citrate standard groups exhibited greater viability throughout the treatment period.

Sperm abnormalities

The results of the treatment are documented in **(Table 2).**Sperm abnormalities percentage was a significant increase in ethanolic leaf extract of *Achyranthes aspera* treated groups with 200mg/kg,400mg/kg, and 600mg/kg body weight when compared to control groups. Particularly, in all treated groups following abnormalities were observed in the sperm morphology characters sticky sperm, double head, detached head, coiled tail, Macrocephaly head, acephali head, and bent tail regions, etc. In contrast, no abnormalities in sperm are seen in the clomiphene citrate standard group.

It was also found that the effect was significantly altering the sperm abnormality of rats as compared to untreated rats. The change was observed in all treated groups of rats including the standard group of rats.

Table 2: Effect of Inflorescence extract of Achyranthes aspera and Standard drug on percentage of sperm count ,sperm viability and abnormal sperm (M ± SD, n=4)

Treatment	Sperm count (Million/ml)	Sperm Viability	Sperm abnormalities
Control	34.50±0.66	84.16±0.78	11.23±0.33
200mg/kg	28.10±1.16	72.25±1.08*	42.43±0.73*
400mg/kg	20.02±1.12*	65.40±1.86*	44.30±0.79*
600mg/kg	15.04 ±1.03*	53.15 ±0.59*	65.15±1.08*
Standard clomiphene citrate	35.71±1.11	90.15±0.18*	05.11±1.03*

Data exposed as Results given as Mean ± S.D., Means in the column with * are significant at (P<0.05).

Sperm motility

In the present data from (table 3) showed that the percentage of sperm motility significantly decreased as per amount of concentration of extract increased as 200mg/kg and 400mg/kg, 600mg/kg body weight when compared to the control group. In another group, a selected drug Concentration was given, at the end of the experiment non-motile functions was recovered after 15, 30, 45 60, 75 and 90 minutes of time intervals and it was found that functional motility was decreased while increasing the periods interval in this group of rats but when compared to control groups the motile functions was decreased.

Table-3: Effect of Inflorescence extract of *Achyranthes aspera* and Standard drug on percentage of sperm motility (M± SD, n=4).

Treatment	Duration (minutes) (%)					
	15	30	45	60	75	90
Control	91.2±1.51	89.31±0.95	73.73±0.3	61.31±2.6	48.8±2.4	26.54±1.89
200mg extract	60.54±0.25*	10.49±0.48	NIL	NIL	NIL	NIL
400mg extract	45.25±0.99*	NIL	NIL	NIL	NIL	NIL
600mg extract	45.25±0.99*	NIL	NIL	NIL	NIL	NIL

Standard clomiphene citrate	93.59±0.33*	92.52±1.95	75.7±3.28	67.5±2.12	50.4±0.48	35.71±0.42

Data exposed as Results given as Mean ± S.D., Means in the column with * are significant at ($P<0.05$).

Gurumani*et al*., (2004) in his result ethanolic extract of *Achyranthes aspera* (ACE) treatment caused reduction in reproductive organ weights in rats. The effects brought by *Achyranthes aspera* extract are non-toxic and transient. **Abhaykumar *et al*., (2017)** evaluated that contraceptive potential of *Achyranthes aspera* leaves MeOH extract in male albino mice and has shown promising results of anti-spermatogenic, antiandrogenic and hypolipidaemic activities. In the present study, *Achyranthes aspera* leaves extract resulted in decrease the weight of testis 1.523±3.14 significantly reduced. Spermatogenic elements like, spermatogonia, spermatocytes and spermatids in the testis were reduced significantly as well as Leydig cells count in testis when compared to control.

The present results are supported by **Shahid and Akbar (2020)** who find that feeding male rats with a 50% ethanolic extract of *Achyranthes aspera* resulted in reduced sperm counts and serum testosterone levels, and a protein isolated from the roots exhibited irreversible spermicidal effect.

Similarly, in present study, treated with ethanolic extract of *Achyranthes aspera* to male rats resulted in reduced sperm counts, the treatment groups were significantly lower than that of the control group treated at 200 mg/kg, 400 mg/kg, and 600 mg/kg of body weight for 15 days of periods, weight of epididymis, However, the weights of the standard drug treated group showed increased and it was showed positive result as compared to control group. The results suggest that ethanolic extract of *Achyranthes aspera* caused reproductive toxicity in male rats and the action may be by suppressing the synthesis of androgen. Similar findings were obtained in our study which suggest that *Achyranthes aspera* may have a negative impact on sperm count. In our study when we used the standard clomiphene citrate the sperm count get increased by the process of gametogenesis. Aqueous and ethanol extracts exhibit significant anti-inflammatory effect, which is comparable to diclofenac at higher dose of the aqueous extract. **Sandhyakumary*et al.*, (2002).**

Our findings suggest that *Achyranthes aspera* extract impairs male reproductive performance and may contribute to infertility in male rats. The reduction in sperm viability was accompanied by a significant decrease in serum testosterone levels. significantly lower at 200 mg/kg, 400 mg/kg, and 600 mg/kg of body weight after 15 days of treatment to rat and the result was significantly varied than the control.

Abu *et al*., (2014) reported similar result as sperm viability in *Achyranthes aspera* was found to be significantly reduced in male rats after exposure to hydroethanolic extract of the plant. Seminal vesicle and prostate showed there was a lower population of spermatozoa in the epididymis. Similarly**, Paul *et al*., (2006)** also observed the effect of a 50% ethanolic extract of the leaf of *Stephania hernandifolia* and the root of *Achyranthes*

aspera on sperm motility and function in a ratio of 1:3 by weight at different concentrations. Concentration of 0.08 g/mL of the extract affected the motility, and at a concentration of 0.16 g/mL, the sperm motility was reduced to 20% immediately (within 20s). At a concentration of 0.32 g/mL, this composite extract showed the most promising results by complete sperm immobilization within 2 min after the application of the extract. The effects were spermicidal but not spermiostatic as sperm immobilization effect was found to be irreversible. Sperm viability was decreased significantly and was found to be nonviable after 30 min when treated with the composite extract at a concentration of 0.32 g/mL. The hypo-osmotic swelling of these sperm was reduced significantly at this highest concentration, indicating that the crude extract may probably cause injury to the sperm plasma membrane.

Conclusion:

The testis weight of treated groups was reduced at 200 mg/kg, 400 mg/kg, and 600 mg/kg body weight concentration of extract compared to control. Significantly altered testis weight was observed in all treated groups of rats than the control group. Opposite to treated group, the standard group showed an increase in body weight in comparison to the control group. The Standard group does not show any adverse effect of Clomiphene citrate on their body weight and this drug showed a positive impact on rats due to its fertile activity. Reducing the weight in testis by activating proteolytic enzyme by the stimulation of alkaloids like phytochemicals, which was adversely affecting on protein synthesis mechanism of rat.

The findings of this study indicate that the *Achyranthes aspera*, exerted significant effects on various reproductive morpho physiological parameters, including sperm count, motility, viability and progression of sperm motility. The *Achyranthes aspera*, demonstrated a significant decrease in sperm count, indicating that the formulation has the potential to reduce male fertility. Decreasing of sperm count due the effect of plant extract and its phytochemicals on the process of spermatogenesis and overall effect on synthesis and formation of mature sperm process and was also showing adverse effect on biochemically and physiologically parameters of rat. But in case of standard drug, shows positive effect in formation of sperm and it may positively act at hormonal level for secreting male hormone from endocrine gland for boosting and balanced functioning of male reproductive system. Additionally, the recession in sperm motility and morphology were observed, further supporting the potential usefulness of the herbal formulation in diminishing male reproductive function.

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