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TOXICOLOGICAL APPROACH OF *PARTHENIUM HYSTEROPHORUS* (LINN.) ON SPERM DYNAMICS AND MALE FERTILITY OF *RATTUS NORVEGICUS*

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ABSTRACT

Parthenium hysterophorus Linn., an exotic plant has now dominated most of the herbaceous flora of many countries. It is a weed of national significance belongs to family Asteraceae. The compound *Parthenin extracted from parthenium hysterophorus* was orally administrated to *Rattus norvegicus* at 60.0 mg/kg b.wt. for acute and 2.o mg/kg b.wt. for sub-chronic (30 days) treatment to study its impact on testicular fertility parameters to different groups of animals. Results from the present study have elucidated that sperm parameters (Fertility index, sperm count, sperm motility and sperm abnormality) showed a marked decrease in their functioning, and toxic effects on male reproductive functions. The observed testicular changes are suggestive of testes dysfunction and additional mode of action of *Parthenium* leaf extract.

Key Words- Parthenin, Testes, Sperm Count, Motility, Abnormality, *Parthenium hysterophorus*. *Rattus norvegicus.*

INTRODUCTION-

Parthenium hysterophorus Linn. is an annual or short-lived perennial herb of neotropical origin which now has a pantropical distribution[1], belongs to family Asteraceae. It is known by a number of common names including congress grass, false ragweed, starweed and bastard feverfew, depending on the country infested. It has been introduced in India in 1951 in Pune (Maharashtra), recognised by Dr. H.P. Paranjpe, and now has spread over an area of about 2,05,000 hectares[2]. Several aspects of the ecology of *Parthenium* weed contribute to its success, including the production of large numbers of seeds and its aggressiveness, which invades roadsides, railway tracks, pastures, pond sites and canal ditches and agricultural land associated with every crop[3].

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More than 45 sesquiterpene lactones were identified from leaves and flower among them the major is sesquiterpene lactone parthenolide, which is up to 0.9% of total constituents[4]. 23 compounds, representing 90.1% or more of the volatile oils, have been identified from *Parthenium*[5]. The main isolated bioactive compounds from *Parthenium hysterophorus* are parthenolide parthenin and coronopilin of psuedoguainolide class. Distribution of parthenin in different parts of *Parthenium hysterophorus* shows that leaf contains maximum amount i.e. 3.40 percent of wet weight as compared to other parts of the plant[6].

Although *Parthenium* has toxic and adverse effect on human, livestock and crops, it has also been regarded as beneficial plant. It has potential use in pharmaceutical and clinical industry. It can be used for fever, anaemia, hepatic amoebiosis, dysentery and purification of blood[7]. *Parthenium* can be used for biogas production and green manure. Extracts have been used or trialed as a flea-repellent and as herbicide. Therefore, study was undertaken to assess the toxicity of *Parthenin*on male fertility and sperm dynamics of *Rattus norvegicus*.

Plant Material-

Parthenium hysterophorus plant were collected from M.S.J. College campus, vicinity of Keoladeo National Park and residential colonies of Bharatpur, Rajasthan (India), in the month of August and September, when there was luxuriant growth of plant after rainy season. The leaves were dried at room temperature for 15 days. Dried leaves were ground in mixer grinder to make a fine powder. The powder was mixed with distilled water and extraction was carried out with the help of a soxhelet apparatus for 48 hours. A brownish sticky resinous material was obtained. The isolation and characterization of parthenin was performed by HPLC Method, Department of Chemistry, Indian Institute of Technology (IIT) New Delhi India. The sub lethal doses were prepared by adding distilled water.

Experimental Animal-

Normal healthy *Rattus norvegicus* were obtained from CDRI (Central Drug Research Institute), Lucknow (U.P, India), and were housed in air cooled room. Animals were maintained in metal cages under controlled temperature 25° ± 5°c, relative humidity 65 ± 5% and photoperiod 12

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hours/day. The rats were fed on Gold Mohar rat feed (23.5% protein, 5% fat and 4.5% fiber) purchased from Hindustan Lever Ltd., Calcutta. The water was provided ad libitum.

Experimental Protocol-

The rats of almost same weight (170 ± 10 gm) were selected randomly irrespective of age while male and female in ration (1:3) were used for the fertility test. The *rattus norvegicus* were grouped into five sets having four individuals in each set. One set for acute study and four sets for sub-chronic studies. The control set of four rats was run simultaneously for acute and sub-chronic treatment. The study was approved by the ethical committee of the Department of Zoology, M.S.J. Govt. P.G.College, Bharatpur (Raj.). The "guidance for the Care and use of animals for scientific research" was strictly followed (INSA, 2000).

The doses were selected after determining LD_{50} by the log Probit method[8]. The LD_{50} was calculated as 67.40 mg/kg b.wt. through oral route of exposure. *Parthenium hysterophorus* leaf extract was orally administered to *Rattus norvegicus* at 60.0 mg/kg b.wt. for acute and 2.0 mg/kg b.wt. for each sub-chronic treatment by stomach tube feeding. The vehicle distilled water was given to control set of rats.

The rats were reweighted and excised out after the termination of the experimental period i.e. 1, 3, 7, 15 and 30 days. Testes (male reproductive organ) of both sides were weighed and processed for testicular studies. Change in body weight and organ weight was determined. Fertility Test was performed using healthy and fertile males cohabited with proestrous females in the ratio of 1:3. Both sides of testes were excised out. One testis used for sperm count and motility test and other was used for sperm abnormality determination. Detailed sperm dynamical and fertility studies were processed and data were subjected to statistical evaluation using student's t-test. Statistical significances were used at $P < 0.05$ and $P < 0.01$ compared to control.

Body-Organ Weight Ratio-

Body weight of each animal was recorded weekly. As the protocol conducted animals were sacrificed and organ testes were weighed. Calculated the organ weight ratio (organ wt. / Body wt. X 100).

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Fertility Test-

The mating exposure test of all the animals were performs. Sexually receptive males cohabited with proestrous females in 1:3 ratio (4 males and 12 females). The vaginal plug and presence of sperms in the vaginal smear was checked for positive mating. The mated females were separated to note the implantation sites on day 16th of pregnancy.

$$\text{Male Fertility Index} = \frac{\text{No. of males impregnating females}}{\text{No. of mated males}} \times 100$$

Sperm Analysis-

The left testes was used for sperm count and motility whereas right testes was used for sperm shape morphology.

Sperm Count-

Sperm count was assayed by the method of Prasad (1972)[9]. Total number of sperms were counted using haematocytometer after diluted (1:20) testes fluid with physiological saline. Sperm suspension was pipette very gently, placed on Neubauer Chamber and the total number of sperm head counted.[10] sperm count were expressed a million/ml.

Sperm Motility-

Sperm motility was assayed by Prasad (1972). Motility was determined by counting both motile and non-motile spermatozoa per unit area expressed as percent motility.

$$\text{Motility \%} = \frac{\text{Motile sperms}}{\text{Motile sperm + non-motile sperms}} \times 100$$

For sperm motility drops of sperm fluid were for sperm on to the microscopic slide and drops of normal saline were added to mobilize the sperm cells.

Sperm Abnormality-

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Sperm morphology test was done from a smear of the testicular filterate prepared on a clean glass slide by addition of a drop of 1% eosin. Abnormalities of either head or tail were noted. Total morphological abnormalities were observed as described by Linde *et al*.[11]

Result and Discussion-

The study revealed that administration of Partheninto male rats resulted in reproductive toxicity. A significant reduction in the body weight with decrease in testes weight. A significant decrease in testes-body weight ratio has been observed after acute and sub-chronic treatment of Partheninin the present study. It is evident that Partheninhas caused adverse effects on the testes and body weight ratio. (Table-1)

Sherin and Hawards and Takihara et al, have also studied similar study reported decrease in testicular weight that may be due to loss of spermatogenic elements and spermatids in the tubule[12,13]. Hess *et al*, have reported a decrease in testicular weight and sloughing of germinal cells treated with benomyl[14]. Choudhary and Joshi reported significant reduction in the testes weight after exposure of rats to endosulfan[15]. The reduction in testes-body weight ratio by the administration of extract may reflect a decreased bioavailability and production of androgens[16].

In the present investigation oral administration of Partheninreduces the number of pregnant females by treated males after acute treatment and also followed negative fertility to 30 days protocol, so that reduces the number of implantations. Decrease in pregnancy in untreated female rats which were mated with treated males may be due to failure of fertilization as indicated by reduction in sperm production. Negative fertility test may be attributed to lack of forward progressive and reduction in density of spermatozoa and altered biochemical mileu of testes. Montanari *et al*, reported similar type of study as antispermatogenic effect of

(1)Effect of *Parthenin* on Body wt., Testes wt., and Testes/Body wt. ratio.

Treatment	Days	Body wt (gm)	Testes wt (gm)	Testes-Body wt Ratio %
		Mean ± S.E	Mean ± S.E	
Control	1	184.80 ± 0.015	1.27 ± 0.035	0.68 %*
Acute	1	176.78 ± 0.020	1.19 ± 0.040	0.67 %*

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SUBCHRONIC	3	175.55 ± 0.245	0.98 ± 0.325	0.55 %*
	7	174.30 ± 1.005	0.89 ± 0.038	0.51 %*
	15	170.51 ± 0.915	0.86 ± 0.020	0.50 %*
	30	168.72 ± 1.640	0.77 ± 0.030	0.45 %*

Each values of SEM of 4 animals $p<0.05$ *

(2)Effect of *Parthenin* on male fertility index.

Treatment	Days	No. of Treated Males	No. of Treated Females	Pregnant Females	Fertility Index %
Control	1	4	12	12	100 %*
Acute	1	4	12	10	83.33 %*
SUBCHRONIC	3	4	8	6	75 %*
	7	4	7	4	57.14 %*
	15	4	5	2	40 %*
	30	4	5	1	20 %*

Each values of SEM of 4 animals $p<0.05$ *

(3)Effect of *Parthenin* on testes, sperm count, motility and abnormalities.

Treatment	Days	Sperm Count (mil/ml)	Sperm Motility (%)	Sperm Abnormalities	
		Mean ± S.E	Mean ± S.E	Head (%)	Tail (%)
Control	1	80.35 ± 0.852	76.68 ± 1.711	2.35 ± 0.29	5.78 ± 0.23
Acute	1	76.00 ± 1.724*	72.93 ± 1.174*	2.05 ± 1.13*	3.10 ± 0.07*
SUBCHRONIC	3	59.16 ± 1.496**	59.43 ± 0.789**	19.23 ± 2.36*	26.58 ± 3.21*
	7	61.02 ± 0.535**	36.31 ± 0.554**	38.17 ± 2.10*	40.32 ± 0.17*
	15	40.79 ± 0.206**	19.95 ± 0.584**	52.91 ± 2.11*	58.37 ± 0.31*
	30	27.34 ± 0.330**	18.45 ± 0.350**	61.34 ± 1.98*	63.26 ± 2.12*

Each value of SEM of 4 animals $P < 0.05$* and $P < 0.01$ **

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Achillea millefolium L. in mice[17]. Pant et al, observed a dose and age-dependent decrease in the sperm motility and count and a significant increase in the abnormal sperms on oral administration of Carbaryl in male albino rats[18].

Sperm count and motility in testes decreases at significantly level after acute treatment of *Parthenin*. A high significance depletion in counting and motility of sperms after sub-chronic exposure up to 30 days noted in the present study. Sperm motility and density (counting) is introduced as an important factor in the success of natural and experimental fertilization. In fertile individuals, sperm motility levels especially progressive sperms are directly related to the ability of fertilization[19]. Reduced sperm count may be due to insufficient secretion of testosteron, degeneration of seminiferous tubules and leydig cells, severally damaged spermatocytes and spermatids[20].

Carica Papaya was reported antifertility effect by reduction of testicular mass, sperm count and sperm motility when benzene extract of the seeds was administered to male albino rats[21]. Another factor which caused decrease in sperm motility may be androgen deprivation effect of the extract. Das *et al*, found similar type of results treated with nickel-sulphate rats[22]. Sperm count and motility is considered to be one of the important factors that affect fertility. Similar results observed by Rajamohan. T *et al*, in their study of role of coconut water on Nicotine-Induced reproductive dysfunction in male rats[23]. (Table-3).

Sperm morphology is an essential parameter that reflects the degree of normality and maturity of the sperm population and correlated with fertility[24]. In our study we evaluated the toxicity of *Parthenin* on rat testes. We found a significant increase in sperm shape anomalies. Sperm shape abnormalities (head and tail) were more frequent in treated rats than those of normal control. The head abnormalities were amorphous, banana-shaped, hookless and occasionally double-headed. The tail abnormalities were mainly coiled and double tailed. The majority of abnormalities include the changes of tail shape. Damage of genetic material in spermatogonia and spermatocytes has been related to the increased sperm abnormalities. These alterations bring about possibilities ofgenetic disorders if passed down to offsprings[25]. Our results are in agrrement with Abd. EL-Rahim *et al.*, who reported that the diabetic condition of male rats significantly increased sperm shape abnormalities[26] (Table-3).

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It may be concluded from the present study that sub-chronic oral exposure of male rats to *Partheninh*as caused testicular toxicity having negative impact on sperm parameters and fertility. It can be resulted that leaf extract has the potential to be developed into a male contraceptive agent. The negative consequences of parthenin isolated from *Parthenium hysterophorus* on the sperm may be taken as an advantage for further study.

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Annexure-1

(1)Effect of *Parthenin* on Body wt., Testes wt., and Testes/Body wt. ratio.

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