

ORIGINAL RESEARCH ARTICLE

**Efficacy of *Nicotianatabacum* Extract on Infertility in male *Albino* Rats**

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**ABSTRACT**

The present study was designed to evaluate the fertility effect of *Euphorbia hirta* Linn. and the association between smokeless tobacco and infertility in rats. *Euphorbia hirta* L. is one of the well-known folklore medicinal plants. Many people use smokeless tobacco (AEST) in different forms worldwide. AEST is addictive and their use is harmful to health. It contains different varieties of toxic substances. Adequate data is available on hazardous effect of tobacco. However, data on the harmful effect of smokeless tobacco on reproduction is scanty. This work is an effort to study the harmful effects of the consumption of aqueous extract of tobacco and its effects on reproduction in male rats. The degree of motility of sperm, sperm count and volume was considerably decreased and abnormality of the head and tail of the sperm was increased in the experimental rats by the administration of aqueous extract of tobacco when compared with rats of normal control group. Consequently, the sperm count, motility and volume was increased ( $P < 0.05$ ) and the abnormality in head and tail of sperm was decreased in the rat treated with the aqueous extract of *Euphorbia hirta* when compared with the rats of experimental group (toxic control).

**Key words:** Smokeless tobacco, fertility, *Euphorbia hirta*, sperm quality, motility

**1. INTRODUCTION**

*Nicotianatabacum* is used in different forms such as smoking, chewing, local applications, drinking and gargling, which leads to harmful health effects such as increased prevalence of mortality from cardiovascular disease, cerebrovascular aortic aneurysm and peripheral arterial disease, respiratory diseases and cancer (Bolinder *et al.*, 1994; Henley *et al.*, 2005; Hergenset *et al.*, 2007; Hergenset *et al.*, 2008;). Smokeless tobacco (ST) is placed in the mouth, cheek, or lip, and is sucked (dipped) or chewed. Usage of ST is a growing global problem, particularly in Asian countries, especially among adolescent boys and young men. These are about 100 million users of STE products in India and Pakistan (Gupta and Ray, 2003). In addition to these dangerous affects, reproductive outcomes, dental and oral disease also causing impairments in normal functions (Christen., 1992; Tomar, 1999).

Tobacco contains specific chemicals such as nitrosamines, formaldehyde, acetaldehyde,

crotanaldehyde, hydrazine, arsenic, nickel, cadmium, benzopyrene, and potassium which are cancer causing. It also contains nicotine which is absorbed by all the tissues of body including the mucous membranes of the mouth, nose and intestines, respiratory epithelium and skin (National Cancer Institute Fact Sheet 1997).

Tobacco products also cause chromosomal aberrations (Mahimkar and Bhisey, 1995; Zhu *et al.*, 1999). Tobacco causes health and reproductive impairments in human males. Cigarette smoking is associated with reductions in quality of semen including sperm concentration, motility and morphology (Said *et al.*, 2005). Epidemiological data reports show that cigarette smoking is strongly associated with reductions in the quality of semen.

Oral cavity is the most common site of cancer in the body of tobacco chewers as observed by Indian registries (ICMR, 1987-1989). Consumption of tobacco is responsible for 50% of

all the cancers in men and a one fourth of all cancers in women (WHO, 1997). Consumption of smokeless tobacco leads to oral cancer, leukoplakia, cancer of esophagus, pharynx, stomach, and pancreas (Hatsukami and Severson, 1999).

WHO international Agency for Research on Cancer concluded that smokeless tobacco users have an 80% higher risk of developing oral cancer and a 60% higher risk of developing pancreatic and esophageal cancer (Boffetta, 2008). Reproductive diseases are also included because of the important cross-generational effects of smoking. Consumption of tobacco is associated with infertility in male. Many studies have shown that chewing of tobacco detrimentally affects the quality of semen, concentration of sperm, motility, morphology, viability and damages of DNA in men undergoing infertility evaluation (Stillman et al., 1986; Vine et al., 1996; Saleh et al., 2002; Kunze et al., 2003). Addition to this, cigarette smoking has been correlated with reduced sperm function (Close et al., 1990; Sofikitset et al., 1995). Cigarette smoking slows spermatogenesis and causes reduced steroidogenesis in men (Aydos et al., 2001; Mlynarcikova et al., 2005). The aim of this study was to evaluate the connection between the use of smokeless tobacco and quality of sperm in male rats.

Many parts of the world especially the developing and under developed countries are even now using herbal medicines for preventing and curing various ailments. (Kirtikaret al., 1956; UNESCO, 1996; Newman et al., 2003; Gilani et al., 2005; Srivastava et al., 2011; Thirumalai et al., 2012; Jayavelu et al., 2013). Various plants have been used for the treatment of many diseases such as skin infection, ulcers, diabetes and diseases related to male reproduction (Das et al., 2004; Senthilkumaret al., 2013).

## 2. MATERIALS AND METHODS

### Plant material

The leaves of *Euphorbia hirta* Linn. (Euphorbiaceae) were collected from places in and around Vellore district, Tamil Nadu and authenticated at the Department of Botany, C. Abdul Hakeem College, Melvisharam, Vellore District, Tamil Nadu. The plant materials were cleaned with distilled water and shade dried at room temperature. A voucher specimen has been deposited in the Department of Zoology, Thiruvalluvar University, Serkadu, Vellore. 632115.

### Preparation of Smokeless Tobacco Extract

Aqueous extract of smokeless tobacco (AEST) was prepared as described in the literature (Lam et al., 2003) with slight modification. Commercially available khaini was finely powdered, and 20 g was dissolved in 50 ml of PBS (pH 7.4) and incubated at 37°C for 30 min with thorough shaking. The dissolved contents were filtered twice through filter paper and quickly frozen at -80°C before lyophilization. The dried yield of AEST was found to be around 1 mg/10 mg of khaini containing about 24% salt concentration (NaCl, KCl, and phosphates). The required amount of lyophilized extract was reconstituted in 300 µl distilled water and was orally administered through gavage to the rats at the desired doses for different time periods (Avtiet et al., 2010)

### Preparation of Plants extract

The shade dried plant materials were powdered separately in an electrical blender. The powdered material of the leaves was extracted in a Soxhlet apparatus using distilled water (500ml for 100gms) as solvent for 3hrs. The extract was filtered and concentrated under reduced pressure on rotary evaporator to obtain (10%) the extract. The powder obtained was then subjected to phytochemical analysis to determine the chemical constituents present in the extract and the remaining was stored at 5° C for further use.

### Animals

Adult male *albino* rats weighing around 175-200g were purchased from Tamil Nadu Veterinary and Animal Sciences University, Chennai, India. The animals were kept in polypropylene cages (three in each cage) at an ambient temperature of 25±2°C and 55-65% relative humidity. 12±1 hr light and dark schedule was maintained in the animal house till the animals were acclimatized to the laboratory conditions. They were fed with commercially available rat chow (Hindustan Lever Ltd., Bangaluru, India). Water was provided *ad libitum*. Experimental protocols and procedures with respect to the animals employed in this study were approved by the Animal Ethics Committee of Thiruvalluvar University, Serkadu, Vellore, Tamil Nadu, India.

### Experimental design

Tobacco was obtained from the local market at Vellore. The animals were divided into three main groups consisting of six animals each for different experiments.

Group I: Normal rats administered with 0.9% saline

Group II: Rats treated with aqueous extract of tobacco [(AEST) (30 mg, 60 mg and 120 mg /kg body wt.,)] for 60 days orally by intra gastric tube.

Group III: The aqueous extract of tobacco (AEST) [30 mg, 60 mg and 120 mg/kg body wt.,] intoxicated rats treated with aqueous leaf extracts (60, 120, and 240 mg/kg body wt.) of *Euphorbia hirta* Linn. for 45 days (from 61<sup>st</sup> to 105<sup>th</sup> day) orally by intra gastric tube.

#### Induction of Testes damage

Testes damage was induced by the administration of aqueous extract of tobacco (AEST) orally for 60 days (30mg, 60 mg and 120mg/kg body weight) in experimental rats. After the end of the experimental course of therapy rats were fasted over night and samples were collected by cervical dislocation under light ether anesthesia. Samples were used for the determination of various parameters.

#### Separation of epididymal sperm

The Brooks modified method was used for the separation of Epididymal spermatozoa (Brooks, 1976). Caudal portion of epididymis was cut into small pieces. Spermatozoa from epididymal pieces were removed by vortexing gently in Krebs Ringer phosphate buffer (pH7.4) for 10 min. Suspension was used for sperm count.

#### Sperm volume

The volume and color were determined by reading out the volume in a calibrated measuring cylinder, while the color was determined by visual assessment.

#### Sperm motility

For the estimation of sperm motility spermatozoa were expressed out by cutting the distal end of caudaepididymal tubule (Srikanthet al., 1999).It was diluted with physiological saline placed on a glass slide and the motility of sperm was studied according to Abouaetal., 2009. Ten random fields were manually scored for the number of motile and non-motile sperms. Motility was expressed as a percentage of motile sperm compared to total sperm counted.

#### Statistical analysis

Data were expressed as mean  $\pm$  standard deviation (SD) and analyzed using one-way analysis of variance (ANOVA), followed by Scheffe post hoc test. The data were analyzed with SPSS version 16 software (SPSS Inc., Chicago, USA). Statistical significance of difference was accepted at the p-values of less than 0.05.

### 3.RESULTS

#### Sperm count and volume

The extract of tobacco leads to the impaired function of testes. The different doses of AST (30 mg (Table 1), 60 mg (Table 2) and 120 mg/kg body wt., (Table 3) decreased the sperm count and volume in epididymis. The result was more significant at higher concentrations 120 mg/kg body wt. than 60 mg and 30 mg ( $P < 0.05$ ). The potential toxic effect of AST was decreased by aqueous extract of *Euphorbia hirta* Linn. Therefore, the decreased levels of the sperm count and volume were increased near to the normal control group ( $P < 0.05$ ).

#### Sperm motility

The grade of motility of spermatozoa of rats in the normal control group was significantly ( $P < 0.05$ ) higher, than what was observed in rats in each of the experimental groups i.e. 30 mg, 60 mg and 120 mg/kg body wt., i.e. group II and III. The percentage of motility was  $92.33 \pm 3.46\%$  in normal control group. It was significantly decreased in rats administered AEST 30 mg/kg body weight (-12.02%) [Table I], in 60 mg (-24.46%) [Table II] and in 120 mg (-43.46%) [Table III] in rats of experimental group (Group II).

#### Head abnormality

The rats in the normal control group had less number of spermatozoa with head abnormality ( $2.11 \pm 0.62$ ) when compared with rats in each of the experimental groups (Group II and III) i.e. 30 mg, 60 mg and 120 mg. The percentage of head abnormality in the rats of group II in 30 mg AST administered rats (+99.52%), in 60 mg (+228.43%) and in 120 mg (+327.96%). was higher than the normal control rats ( $2.11 \pm 0.62$ ). The differences of the means were significant ( $P < 0.05$ ) for both groups (Group II and III). Toxic effect of AST was decreased by aqueous extract of *Euphorbia hirta* Linn. Hence, the elevated levels of abnormality in head of sperm were restored near to the normal level in control group in group III rats [-16.62% in 60 mg (Table 1), -30.01% in 120 mg (Table 2) and -58.36% in 240 mg /kg body weight. (Table 3)].

#### Tail abnormality

The effect of AST was reduced by aqueous extract of *Euphorbia hirta* Linn. So, the elevated levels of tail abnormality in group II ( $3.98 \pm 2.19$ ,  $6.34 \pm 3.71$  and  $8.93 \pm 1.51$ ) were brought back near to the normal control ( $2.27 \pm 0.72$ ) group [-18.09% in 60

mg (Table 1), -45.11% in 120 mg (Table 2) and - 64.83% in 240 mg/kg body wt., (Table 3)].

**Table 1: Effect of aqueous extract of *Euphorbia hirta*(Eh)on 30 mg of tobacco extract intoxicated rats treated with 60 mg extract for 45 days: Total sperm count, Volume, Motility, Abnormal Head and Tail count**

Groups	Sperm Parameters				
	Count	Volume (cm3)	Motility (%)	Sperm abnormality	
				Head	Tail
Normal control (Group-I)	87.25±2.32	5.15±2.89	92.33±3.46	2.11±0.62	2.27±0.72
AEST – 30 mg (Group II)	72.38±2.54*	4.02±3.15*	81.23±2.84*	4.21±3.69 *	3.98±2.19*
% of changes (Normal vs ST)	-17.04	-21.94	-12.02	+99.52	+75.33
Eh-60 mg (Group III)	79.34±1.78*	4.65±4.21*	88.37±1.96*	3.51±2.58*	3.26±2.41*
% of changes (AEST vsEh)	+9.61	+15.67	+8.78	-16.62	-18.09

Values are mean of six individual observations in each group Mean ±SD. “P” denotes statistical Significance P<0.05.

**Table 2: Effect of aqueous extract of *Euphorbia hirta*(Eh)on 60 mg of tobacco extract intoxicated rats treated with 120 mg extract for 45 days: Total sperm count, Volume, Motility, Abnormal Head and Tail count**

Groups	Sperm Parameters				
	Count	Volume (cm3)	Motility (%)	Sperm abnormality	
				Head	Tail
Normal control (Group-I)	87.25±2.32	5.15±2.89	92.33±3.46	2.11±0.62	2.27±0.72
AEST - 60 mg (Group II)	63.46±2.54*	3.15±2.76*	69.74±3.65*	6.93±2.37*	6.34±3.71*
% of changes (Normal vs AEST)	-27.26	-38.83	-24.46	+228.43	+179.29
Eh-120 mg (Group III)	76.12±3.51*	4.32±2.04*	82.91±2.51*	4.85±3.45*	3.48±1.98*
% of changes (ST vsEh)	19.94	37.14	18.88	-30.01	-45.11

Values are mean of six individual observations in each group Mean ±SD. “P” denotes statistical Significance P<0.05

**Table 3: Effect of aqueous extract of *Euphorbia hirta*(Eh)on 120 mg of tobacco extract intoxicated rats treated with 240 mg extract for 45 days: Total sperm count, Volume, Motility, Abnormal Head and Tail count**

Groups	Sperm Parameters				
	Count	Volume (cm3)	Motility (%)	Sperm abnormality	
				Head	Tail
Normal control (Group-I)	87.25±2.32	5.15±2.89	92.33±3.46	2.11±0.62	2.27±0.72
AEST – 120 mg (Group II)	51.74±1.98*	2.04±1.84*	52.20±4.54*	9.03±1.96*	8.93±1.51*
% of changes (Normal vs AEST)	-40.69	-60.38	-43.46	327.96	293.39
Eh-240 mg (Group III)	77.28±2.85*	4.36±2.50*	81.57±2.48*	3.76±3.18*	3.14±3.26*
% of changes (ST vsEh)	49.36	113.72	56.26	-58.36	-64.83

Values are mean of six individual observations in each group Mean ±SD. “P” denotes statistical Significance P<0.05.

#### 4. DISCUSSION

The present studies revealed that the aqueous extract of *Euphorbia hirta* Linn. possessed marked capacity to restore the fertile condition against AEST induced infertility in rats. Due to the absence of reliable drugs to restore the fertile condition and reproductive disorders in the allopathic medicines, many of the plant products were used in therapy of different diseases. Tobacco extract is a complex mixture of hazardous chemicals. It contains nicotine, carbon monoxide, carcinogens and mutagens (Stedman, 1968). Present results show that aqueous extract of smokeless tobacco (AEST)significantly decreases the semen quality including sperm count, volume, motility and morphology. Several studies have been performed on the harmful effects of smoking on the genital system of humans and rats (Stillmanet al., 1986).

The data suggest that the use of smokeless tobacco is as harmful as smoking for reproduction. Sperm parameters like abnormal head and tail of the sperm were significantly

higher in experimental animals (AEST administered) than normal control group. The increase of abnormality of head and tail were dependent on the concentration of tobacco extract. The incidence of AEST was highly significant in the high dose administered group (120 mg/kg) when compared to low dose groups(30 mg, 60 mg).

Experiments were carried out to determine the ability of *Euphorbia hirta* Linn. to reverse the effect of smokeless tobacco on infertility in *albino* rats. In experimental rats infertility was induced by AEST at different doses.i.e 30 mg, 60 mg, 120 mg/kg body weight. Experimental studies suggest the potential toxic effect of aqueous extract of tobacco on male reproduction. AEST induced spermatogenic damage, as shown by a decrease in sperm counts, motility and abnormality of the sperm; which may be due to the impairment of the activity of antioxidant enzymes.

They reported that sperm concentration, percentage of motility, morphology and viability were significantly higher in individuals who

consumed lesser quantity of tobacco than in these who consumed higher quantities (Said *et al.*, 2005). Banerjee *et al.*, 1993, compared the quality of semen samples between different varieties of tobacco addicts (smokers, chewers) with non-addicts (never consumed in any form). The percentage of motility and total count of sperm were significantly low in tobacco chewers.

The plasma membrane of sperm is very responsive to the effect of ROS since it contains abundant poly unsaturated fatty acid. These plentiful poly unsaturated fatty acids create fluidity which is essential for motility of sperm and acrosomal reaction. The unsaturated nature of these molecules predisposes them to ROS attack and lipid peroxidation throughout the plasma membrane of sperm (Abouaet *al.*, 2009). This leads to the damage of sperm with subsequent dysfunction of sperm or cell death. Thus tobacco induced decrease of sperm count and sperm motility and viability may be due to increased oxidative stress.

## CONCLUSION

In conclusion, the administration of aqueous extract of tobacco significantly decreases the fertility in male rats by reducing sperm density, motility, viability and causes abnormality in the head and tail of sperm. Adverse effect on male fertility may be due to decrease in ROS scavenging capacity of tobacco treated rats. These experimental results lend support to the hypothesis that the AEST consumption affects the reproductive function in male rats. The present study was undertaken to evaluate the fertility effect of *Euphorbia hirta* Linn. on tobacco extract caused infertility in male rats. The results confirmed that this plant does indeed have a therapeutic benefit and in restore the normal functions of testes and nearly normal percentage of motility of sperm, sperm count, volume and abnormality of head and tail of the sperm.

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