ISSN: 0975-833X

Available online at http://www.journalcra.com

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

International Journal of Current Research
Vol. 2, pp. 082-084, March, 2010

RESEARCH ARTICLE

HISTOLOGY OF MALE RATS INDUCED WITH RHIZOMES OF *CURCULIGO ORCHIOIDES* GAERTN

Rajalakshmi, S*., Krishnamoorthy, G. and Sasikala, S.

Department of Zoology, K.M. Centre for Post Graduate Studies, Lawspet, Puducherry (U.T.) – 605 008, INDIA.

ARTICLE INFO

Article History:
Received 5th January, 2010
Received in revised form
15th February, 2010
Accepted 28th February, 2010
Published online 19th March, 2010

Key words:
Curculigo orchioides
Histology
Sperm
Male
Rats

ABSTRACT

The rhizomes of *Curculigo orchioides* have been traditionally acclaimed as aphrodisiac. The ethanolic extract of rhizome was evaluated for its effect on spermatogenesis in ethanol induced albino rats. The increased spermatogenesis in treated group was confirmed by change in histoarchitecture as evidenced by an increase in number of spermatocyte and spermatids. These findings support the folk use of this plant as aphrodisiac.

INTRODUCTION

Alcohol abuse in men causes impaired testosterone production; shrinkage of the testes; reduced sperm counts; abnormal sperm shapes and altered sperm motility. Chronic ethanol abuse causes testicular atrophy and male infertility in alcoholic men (Maneesh *et al.,* 2005). The rhizomes of *Curculigo orchioides,* (Amaryllidaceae) are described in Ayurveda as a Vajikarana rasayana. Rasayana is a unique concept of Ayurveda, which means vital nourishment (Rasa + Ayana) representing a holistic approach, responsible for preventive aspects against ageing as well as curative aspects against diseases. The plant is reported to possess estrogenic, pendiculatiory, hepatoprotective, immunostimulant and antioxidant activities (Vijayanarayana *et al.,* 2007). The present investigation was undertaken with a view to explain the effect of extract on spermatogenesis.

MATERIALS AND METHODS

Animal Stock

The protocol for experimentation was approved by the Institutional Animal Ethical Committee (IAEC Ref. No.845/ac/04/2004 CPCSEA) of Kanchi Mamunivar Centre for Post Graduate Studies, Pondicherry University, Puducherry, India. Albino rats of male sex weighing 130 – 160 g were fed on standard diet of Amrut Laboratory rat pellets (Nav Maharastra Chakan Oil Mills Ltd., Pune) and water *ad libitum*. The animals were housed at room temperature (24± 2^0 C) on a reversed day-night cycle (06:00 hrs to 18:00 hrs).

Plant material

Rhizomes of *Curculigo orchioides* Gaertn were collected at Botanical garden, Puducherry, and taxonomically identified at the Department of Plant Science, Kanchi Mamunivar Centre for Post Graduate Studies, Pondicherry. The dried powdered rhizomes were defatted by extraction with petroleum ether (60-80^0 C). The deffated plant material was then extracted with ethanol (95%), and dried under vacuum (4.08%w/v).

Animal treatment

The animals were divided into three groups, each consisting of five animals depending upon the treatment. *Group I* (Control) was administered once with 0.5 ml of 5% sucrose per kg BW per day (Isocaloric) orally for 60 days. *Group II* was treated once with 25% of 0.5 ml ethanol /kg/BW/day along with 0.5 ml of *Curculigo orchioides* extract (80mg/kg BW/day) orally once for 60 days. *Group III* received once with 0.5 ml of 25% ethanol/kg/BW/day for 60 days orally.

Effect of spermatogenesis

The method reported by Saksena and Dixit (1987) as modified by Chauhan & Dixit (2008) was used. After 60 days of treatment the body weights of animals were taken after which that the animals of control as well as treated groups were killed by rapid decapitation. Testes were removed and cut into small pieces, fixed in Bouin's fixative, dehydrated with varying percentage of ethanol for histological studies. Sections were cut (6μ), stained

*Corresponding author: *srajalakkshmii@gmail.com*
Tel. +91 09710886951

Table 1. Effect of ethanolic extract of *Curculigo orchioides* on body weight and testes weight

Group	Body weight (g.wet.tissue)	Testes weight (g/100g body weight)
Control	158±14.6	2±0.102
Ethanol alone treated	142±8.98	2.2±0.052
Ethanol + *C. orchioides*	172±7.04	2.3±0.066

Table 2. Effect of ethanolic extract of *Curculigo orchioides* on spermatogenic element in male rats

Group	Size of seminiferous tubules (µm)		Number of spermatogenic elements	
	Length	**Breadth**	**Spermatogonia**	**Spermatocyte**
Control	245.1±42.9	112.4±26.4	18.52±3.76	75.25±3.35
Ethanol alone treated	242.2±40.5	107.0±22.1	10.62±2.66	105.52±4.21
Ethanol + *C. orchioides*	303.8±41.7	110.0±28.9	13.72±2.99	116.12±5.51

with eosin and analyzed microscopically. Histometric measurements such as diameter of testes, seminiferous tubules and leydigs cell nucleus were made by random selections of 30 circular sections by using ocular and stage micrometers. The numbers of different spermatogenic elements were also determined.

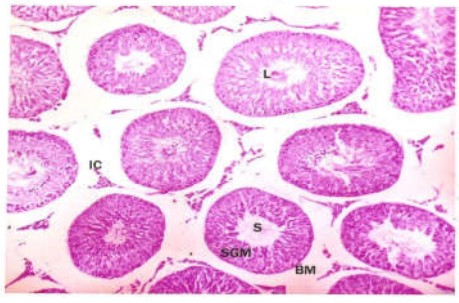
Statistical analysis

Results are expressed as mean ± SD. The significance of the data was evaluated using student t-test.

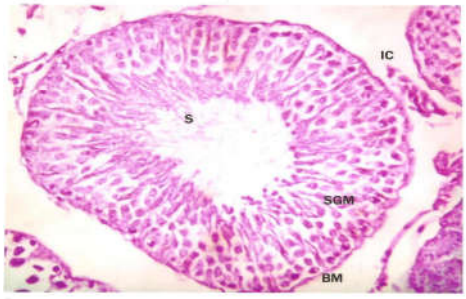
RESULTS

Ethanol exposed rats showed significant decrease in testicular weight than control. However, extract treated group showed slight increase in testes weight (Table 1). The testes section of control group animals showed normal histological texture. The diameter of seminiferous tubules varied within a range. The tubules having maximum diameter, were not abundant and well within range. Sertoli cells had many cytoplasmic processes which were normal in size. Leydig cells had normal nuclear size. Spermatozoa with long tail with small distinct head were more visible (Table 2 and Figure 1).

Plate -1: Light Microscopy Of Adult Rat Testis (Eosin-Red Stain).



A: Control X200



B: Control X450

The histoarchitecture of ethanol alone treated group showed decreased number of spermatogenesis was evident by low number of spermatozoa in seminiferous tubules as compared to control group (Table 2 and Figure 2). The rhizome extract treated group animals showed pronounced effects in terms of testis weight and histological alterations. Since, the weight and size of the testis was greater in extract treated groups almost all seminiferous tubules showed greater diameter. Large numbers of different cells at different stages of spermatogenesis were evident. Sertoli cells were enlarged highly processed and rich nutrients as evidenced by highly granulated cytoplasm. This was the normal response of the Sertoli cells when they were in readiness for providing nutritionally supplementation to large number of spermatozoa (Majumdar, 1995). Leydig cells showed almost enlarged nucleus with darkly stained cytoplasm. Increment in the volume of cells and nucleus were strongly suggestive of the phytochemicals of *C. orchioides*. Almost all tubules were overcrowded with sperm bundles. In some tubules, spermatids were found scattered amidst spermatozoa (Table 2 and Figure 3).

DISCUSSION

The present investigation brings forth the spermatogenic activity of rhizomes of *C. orchioides* on ethanol induced albino rats. Chronic ethanol abuse causes testicular atrophy and male infertility in alcoholic men. The testis has been shown to be highly susceptible to ethanol as it crosses blood testis barrier and depresses spermatogenesis (Maneesh *et al*., 2005). The reduction in testicular weight of ethanol treated rats may be due to reduced tubule size, spermatogenic arrest and inhibition of steroid biosynthesis of Leydig cells (Nandi et al., 1999).

Rhizome has been demonstrated to have antioxidant properties *in-vitro* and *in-vivo* (Venukumar and Latha, 2002; Tang *et al.,* 2004; Bafna and Mishra, 2005). The phenol and phenolic glycoside shows antioxidative property of the rhizome (Wu *et al*., 2005). This antioxidant defense system are of major importance because peroxidative damage is currently regarded as the single most important cause of impaired testicular function underpinning the pathological consequences of wide range of conditions from testicular torsion to alcoholism (Aitken and Roman, 2007). In a normal situation, the antioxidant mechanisms present in the reproductive tissues and their secretions are likely to quench these reactive oxygen species (ROS) and protect

against oxidative damage to gonadal cells and mature spermatozoa (Sikka, 2001).

Plate -2: Light Microscopy Of Adult Rat Testis (Eosin-Red Stain).

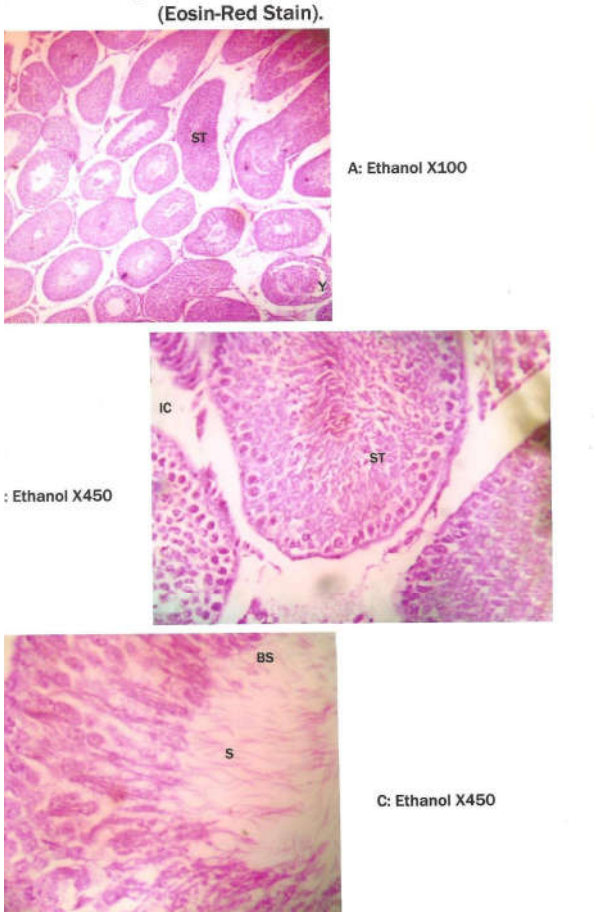
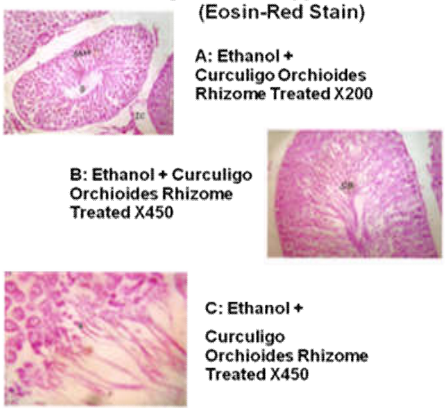


Plate -3: Light Microscopy of Adult Rat Testis (Eosin-Red Stain)



Reduced number of spermatozoa, mal formed spermatozoa or their reduced or insufficient motility are the leading causes of disturbed fertility or infertility in alcoholic men. The drug may thus provide and alternative for management of infertility due to reduced spermatogenesis. Further studies are necessary to elucidate the compounds of the ethanolic extract of *C.orchioides* is responsible for enhancing spermatogenesis in rats.

ACKNOWLEDGEMENT

Authors gratefully acknowledge Prof. V. Anandan, HOD and the Director, KMCPGS, Puducherry, for providing necessary facilities.

REFERENCES

Aitken R.J. and Roman Shaun D. 2007. Antioxidant systems and oxidative stress in the testes. In: Cheng CY, editor. Molecular mechanism in spermatogenesis. *Landes Bioscience* p.1.

Bafna A.R. and Mishra S.H. 2005. In vitro antioxidant activity of methanol extract of rhizomes of *Curculigo orchioides* Gaertn. *Ars Pharm*. 46**(**2): 125-138.

Chauhan N.S and Dixit V.K. 2008. Spermatogenic activity of rhizomes of *Curculigo orchioides* Gaertn in male rats. *Intern. J. Applied Res. Natural Products*. **1**(2):26-31.

Maneesh M., Jayalekshmi H., Sanjiba Dutta, Amit Chakrabarti and Vasudevan D.M. 2005. Role of oxidative stress in ethanol induced germ cell apoptosis – an experimental study in rats. *I.J. Clinic.Biochem*. 20(2): 62-67.

Nandi S., Banerjee P.P. and Zirkin B.R. 1999. Germ cell apoptosis in the testes of Sprague Dawley rats following testosterone withdrawal by ethane 1,2, dimethanesulfonate administration: Relationship of Fas? *Biol. Reprod*. 61: 70-75.

Saksena S. and Dixit V.K. 1987. Role of total alkaloids of Mucuna pruriens Baker in spermatogenesis in albino rats. *Indian J. Nat. Prod.* 2:3-7.

Sikka S.C. 2001. Relative impact of oxidative stress on male reproductive function. *Curr. Medici. Chem*. 8:851-862.

Tang S. Y., Whiteman M., Peng Z.F., Jenner A., Yong E.L. and Halliwell B. 2004. Characterization of antioxidant and antiglycation properties and isolation of active ingredients from traditional Chinese medicines. *Free Radical Biology and Medicine*. 36:1575-1587.

Vijayanarayana K., Rodrigues R.S., Chandrashekhar K.S., Subrahmanyam E.V.S. 2007. Evaluation of estrogeneic acitivity of alcoholic extract of rhizomes of *Curculigo orchioideds*. *J. Ethanopharmacol*. 114(2): 241-245.

Wu Q., Fu D.X., Hou A.J., Lei G.Q., Liu Z.J., Chen J.K. and Zhou T.S. 2005. Antioxidative phenols and phenolic glycosides from *Curculigo orchioides*. *Chem. Pharm. Bull.* 53(8): 1065-7.

****