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RESEARCH ARTICLE

PROTECTIVE EFFECT OF *Curculigo orchoides* ON RAT TESTES

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ABSTRACT

The effect of *Curculigo orchoides* Gaertn rhizome extract was studies in ethanol treated male pubertal rat testes. Total lipids, phospholipids andlysosomal enzyme-acid phosphatase, alkaline phosphatase and ATPases were compared with ethanol treated and control group rats. The suppressive effect of ethanol on lipid and ATPases were correlated with the altered testicular functions. Administration of *Curculigo orchoides* with alcohol is capable of quenching the oxidative stress and protects the male reproductive organ from ethanol toxicity.The present also study reveals the bioactive principles of *C. orchioides*found to reverse most of the ethanol induced adverse effect on the organ studied.

INTRODUCTION

Testes are a multihormonal target organ (Hall, 1970). Several nucleotide metabolizing enzymes are required for the maturation and function of germ cells in the testes and epididymis (Oligate*et al*., 1984). Ethanol increases lipid peroxidation in the liver (Shaw *et al*., 1983). Reactive oxygen species (ROS) and free radicals are generated during ethanol metabolism, causing oxidative stress and lipid peroxidation in liver (Kurose *et al*., 1996), brain (Calabrese *et al.,* 1998), heart (Nordmann*et al.*, 1992, skeletal muscles (Adachi *et al.*, 2000) and testes (Maneesh*et al*., 2005). Acute ethanol use is associated with low testosterone and altered levels of additional reproduction hormones (Dharma, 2006). Ethanol suppresses reproduction in humans, monkeys, and rodents by inhibiting the release of luteinizing hormone (Rettori and McCann, 1997). Chronic alcohol feeding in rats revealed an increase in testicular levels of oxidatively damaged poly unsaturated fatty acids. Alcoholics often have fertility disturbances with low sperm count and impaired sperm motility (Srikanth*et al*., 1999). Chronic alcoholics are often associated with the impotence, loss of libido, premature or delayed ejaculation, sterility, testicular atrophy and gynecomastia (Boyden and Pampenter, 1983). Semen samples of alcoholics shows decreased sperm count, impaired forward motility of morphologically normal spermatozoa and increased number of teratozoospermia (Villata*et al*., 1997). A direct correlation was found between testicular atrophy and loss of an important anti-oxidant reduced glutathione (Gamal and Sokkary, 1999). Acid and alkaline phosphatase is widely distributed in the testis and is important in the physiology of the sperm. Changes in the activity of the acid and alkaline phosphate arewidely correlated in the physiology of spermatogenic suppression and or suppression of exchange of materials between germinal and sertoli cells (Breton *et al.*, 1966). In a preliminary pharmacological study ethonolic extract of *Curculigo orchioides* revealed increased sexual behavior and mating performance in male rats (Chauhan*et al*., 2007). *C. orchioides* is popularly known as black musali in India. The rhizomes of *C. orchioides* Gaertn (Amaryllidaceae) are described in ayurveda as a vajikaranarasayana which confer upon a man sexual power similar to that of a stallion (Chunekar and Yadav, 2005). The rhizome, as well as the tuberous roots of the plant has been extensively used in indigenous system of medicine in India, Pakistan and China for the treatment of various diseases, including cancer, jaundice, asthma and diarthrosis wound healing (Dhar *et al*., 1968). Therhizomes of the plant are used as tonic, demulcent, diuretic and restorative. The juice extracted from the rhizome has also been used as a tonic to overcome impotency (Chopra *et al.,* 1956). The plant is reported to possess etrogenic (Vijayanarayana *et al*., 2007), pendiculatory (Thankur and Dixit, 2007), hepatoprotective (Rao and Mishra, 1996), and immunostimulant (Bafna and Mishra, 2006). Hence, the present preliminary investigation is undertaken to study the effect of *Curculigo orchioides* rhizome on testicular lipids of male pubertal rats induced with ethanol. The effects of ethanol onlysosomal enzyme-acid phosphatase, alkaline phosphatase and ATPases in the male reproductive system is also considered.

MATERIALS AND METHODS

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The protocol for the conduct of experiments was approved by the Institutional Animal Ethical Committee (IAEC) and the committee for protection and control on safety of experimental animals (Ref.No.845/ac/04/2004 CPCSEA) of Kanchi Mamunivar Centre for Post Graduate Studies, affliated to Pondicherry University, Puducherry, India. Healthy male albino rats of Wistar strain (60 days old) were used in the present study. They were housed in a well-ventilated temperature controlled room with 12 hours light and 12 hours dark schedule. They were fed with standard, balanced pelleted diet (NavMaharastraChakan Oil Mills Ltd., Pune). Drinking water was made *ad libitum.*

The animals were divided into three groups depending upon the treatment. Each group consists of five animals. **Group I** (Control) animals were given 0.5 ml of sucrose (5 %) (Isocaloric) orally daily once for 60 days. **Group II** animals were given orally 0.5 ml of 25 % ethanol of daily once for 60 days. **Group III** were treted with 25 % of 0.5 ml ethanol and 0.5 ml *Curculigo orchoides* rhizome powder (25 mg/kg body weight) aqueous extract daily once for 60 days.

Animals from all the groups were sacrificed 24 hours after 60 days of treatment. The animals were sacrificed by decapitation with 0.9% physiological saline for about an hour. After complete perfusion, the testes were removed from the adjoining tissues, rinsed in saline, blotted and weighed. They were weighed accurately on a microbalance and frozen at -20^{o}C until further analysis. The total lipids were estimated by the method of Fring's *et al*., (1972). The modified method of Fiske and Subbarow (1925) as per Marinetti (1962) was employed for phospholipids estimation. Alkaline phosphatase was assayed following the method of Besse*yet al.,* (1946) and acid phosphatase (Orthophosphoric monoester phosphohydrolase) was estimated following the method of Andersch and Szezypinski (1947) as modified by Tenniswood *et al*., (1976).The calcium, magnesium, sodium and potassium dependent adenosine triphosphatases were estimated according to the method of Takeo and Sakanashi (1985). Results were presented as mean standard deviation (Mean ± S.D. for all values). Student 't' test was usedfor the test of significance (Hill, 1971).

Table 1: Effects of ethanol and *Curculigo orchioides* Gaertn rhizome on total lipids and total phospholipids of rat testes

Group	Body Weight (gms)	Testes weight (gm/100gm body weight)	Total lipids (mg/gm wet tissue)	Total Phospholipid (mg/gm wet tissue)
Control (C)	198 ± 14.6	2.3 ± 0.052	1.298 ± 0.17	0.282 ± 0.17
Ethanol treated (E)	182 ± 8.9	** 2 ± 0.102	1.286 ± 0.10	*** 0.1184 ± 0.008
Ethanol+*C.orchioides* treated (EP)	192 ± 7.0	c ** 2.2 ± 0.066	b 1.894 ± 0.46	b 0.3366 ± 0.08

Each value is Mean ± SEM of five animals. b = p< 0.01: Ethanol treated (E) Vs Ethanol + *C.orchioides* rhizome treated (EP); **=p<0.01, ***=<0.001Control Vs other groups.

Table 2: Effect of ethanol and *Curculigo orchioides* Gaertn rhizome on total protein and ATP ases in male rat testes

Group	Mg ++ ATPase	Ca++ ATPase	Na+k+ATPase
Control (C)	432.6 ± 32.732	491 ± 39.702	512.6 ± 53.543
Ethanol treated (E)	*** 291.42 ±31.234	*** 488.16 ± 59.035	*** 274.84±15.764
Ethanol + *C. orchioides* treated (EP)	c 1037.22±50.230	c ** 1002.32±38.287	c 926.02± 49.771

Each value is Mean ± SEM of five animals. Enzyme activities are expressed as µ moles of pi formed / hr / mg Protein.
* * *= p <0.001 : control vs ethanol (E) treated animals; * * = p < 0.01 : control vs ethanol + *C. orchioides* rhizome treated (EP); c = p <0.001 : ethanol treated (E) vs Ethanol + *C. orchioides* rhizome (EP) treated.

Table 3: Effects of ethanol and *Curculigo orchioides* Gaertn rhizome on phosphomonoesterases in rat testis

Group	Alkaline phosphatase	Acid phosphatase
Control (C)	69.32 ± 0.91	30.08 ± 1.86
Ethanol treated (E)	*** 80.06 ± 1.57	*** 20.14 ± 0.69
Ethanol + *C. orchioides* rhizome (EP)	c 71.66 ± 0.88	c 40.04 ± 0.61

Each value is Mean ± SEM of five animals. Enzyme activities are expressed as µ moles of p-nitrophenol formed /hr/µg protein. * * *= p <0.001: Control vs Ethanol treated (E). c = p <0.001: Ethanol treated + *C. orchioides* rhizome treated (EP).

The impact of ethanol and *Curculigo orchioides* rhizome extract on albino male pubertal rat testes phospholipid and phosphomonoesterases were analyzed in the present study. There was no significant variation in the body weight of ethanol treated, and ethanol + *C. orchioides* rhizome extract treated rats with control rats (Table 1). The wet weight of testes in these groups shows a variation. The wet weight of testes was marginally lower in ethanol treated than control, however it is not significant. The combined treatment (ethanol + *C. orchioides* rhizome) had a stimulatory effect and increases the weight of testes tissue. The testes weight of this group was statistically significant when compared to control ($p< 0.01$) and ethanol treated ($p< 0.01$) group. The testis has been shown to be highly susceptible to ethanol as it crosses blood testes barrier and depresses spermatogenesis. The reduction in the 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). The present study reveals the safe level of testicular protein present in *C. orchioides* that might have activated the spermatogenesis. Administration of ethanol to the rats for 60 days decreased the total lipids in the testes, but this influence is not significant (Table 1). The differences in the lipid composition of testes could be responsible for differences in the response to ethanol (Sanchez Amate *et al*., 1992). A profound increase in the total lipid concentration in the testes was evident after the administration of ethanol + *C. orchioides* rhizome extract. The combined treatment had a stimulatory effect on total lipid concentration ($p<0.01$) than the ethanol treatment alone, and the increased concentration of total lipids are in par with the control animals. The restoration of total lipids from the suppressive action of ethanol, by the *C. orchioides* rhizome suggest the bioactive principles and antioxidant properties of *C. orchioides* would have involved in the maintenance of total testicular lipids. The present finding on the combined effect of ethanol + *C. orchioides* extract treated group of phospholipids of testes reveals the rich source of phospholipids in *C. orchioides*. It is supported that phospholipids form the integral part of the sperm membrane along with cholesterol (Crews, 1982). Phospholipids also supply few fatty acids to be utilized as energy source and for the formation of prostaglandins, which is one of the integral components of the second messenger system (Hirata and Axelrod, 1980). Any changes in the lipid composition of the testis will have a definite impact on male fertility. Interestingly, *C. orchioides*acts as a best source of FSH, LH and testosterone hormones in maintaining the concentration of testes lipids as evidenced by Gambal and Ackerman, (1967). Table 2 depicts the data on testicular alkaline phosphatase (AlP) and acid phosphatase (AcP) activities. The data on alkaline phosphatase and acid phosphatase activities in testes showed an increasing trend in ethanol treated rats than the control. Not much difference was noticed between control and ethanol + *C. orchioides* combined treated rats. The activities of these enzymes were found to be lesser in combined treatment than ethanol alone treatment ($P<0.001$). Acid phosphatase and alkaline phosphatase are known as inducible enzymes whose activity in animal tissue goes up when there is a stress (Leland, 1983). The increased activities of both acid and alkaline phosphatases could be attributed to the destruction of cell membrane and lysosomes which in turn causes testicular damage, as suggested by Saxena and Sarin activities generally showed decreased trend in ethanol alone treated group than the control rats (Table 3). In specific the Mg++ ATPase and Na+ K+ ATPase activities were reduced ($P<0.001$) in ethanol alone treated group. However, when the ethanol was given along with *C. orchioides* rhizome powder enhanced the Mg^{++} ATPase amd Na^{+} K^{+} ATPase activities. On the contrary these enzyme activities in ethanol + *C. orchioides* combined treatment group was comparatively more than ($P<0.001$) ethanol alone treated rats. The activity of Ca^{+} ATPase was comparatively high in ethanol + *C. orchioides* combined treated groups than the control group. However, Ca++ ATPase activity was less in alcohol alone treated group ($P<0.001$).These enzymes play a pivotal role in providing substrate energy forming essential link in the energy generating cycles in sperm metabolism, in fertilization process and in the maintenance of constant osmotic pressure during semen preservation *(*Kamel *et al.,*2009). The phosphatase enzymes in testes plays an important role in transamination and phosphorylation processes in sperm metabolism and thus explain the differences observed in ethanol and ethanol + *C. orchioides* rhizome treated groups. It is suggested that the bioactive principles present in the rhizome of *C. orchioides* would have counteracted the ethanol toxicity at molecular level. In addition, the stimulatory effect of *C. orchioides*on testes ATP ase may also be due to its antioxidant role at cellular level. The increased activities of testes ATP ases after *C. orchioides* treatment may be a basic biochemical mechanism for the improvement of sexual behavior and co-ordination in the ethanol induced animals. These enzymes have a critical role in fertility regulation as evidenced by Mazumder*et al*., (1991). Ca++, Mg++, Na++ and K+ ATP ases have calcium transport activity in spermatozoa. This activity is essential to maintain the required levels of intracellular calcium and to regulate sperm motility and structure (Rita Sikdar*et al*., 1993). Therefore, the study reveals that *C. orchioides* has a protective priniciples similar to as mentioned above.

Further the present study suggests that the *C. orchioides* rhizome treatment may be beneficial to prevent ethanol-induced toxicity on ATP ase mediated to normal transport function. The *C. orchioides* rhizomes may be of useful as a therapeutically principle to prevent the enzymatic changes among alcoholics. However, *C. orchioides* similarly acts as an antioxidant to substantiate the protective mechanism of lipid peroxidation in rats due to ethanol.

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