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RESEARCH ARTICLE

PROTECTIVE EFFECTS OF *MUCUNA PRURIENS* LINN. SEED EXTRACT ON ETHANOL INDUCED REPRODUCTIVE TOXICITY IN MALE RATS

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ABSTRACT

The effect of ethanol on the biochemical changes of total lipid and total phospholipid fractions were analysed using TLC technology. A noticeable change due to ethanol was observed in eight significant lipid fractions such as phosphatidyl inositol, phosphatidyl serine, sphingomyelin, phosphatidyl choline, lyso phosphatidyl choline, phosphatidyl ethanolamine, cardiolipin and phosphatidyl acid . However, restoration to normal values has been attributed by the treatment of *Mucuna pruriens* seed extract along with ethanol. This may be one mechanism by which the seed extract increases the production of testosterone to lipid desaturates towards the maturation of spermatozoa.

INTRODUCTION

Mucuna pruriens (MP) is a twinning herb found in the tropics and well known for producing itching (Rajeshwar et al., 2005). This property is attributed to the presence of 5-hydroxytryptamine (5-HT) in the hair on the pods (Amstrong et al., 1953). MP seeds are herbaceous forage and food legumes that have for a long time found widespread usage as rotation crops for management of various pests and weeds control (Buckles, 1995; Duke, 1981). It is little known and used for human food and animal feed in Nigeria (Emenalon et al., 2004). The seeds have been reported to be anti-diabetic (Dhawan et al., 1980). Use of the bean in livestock feeding is one of the best ways of exploiting its agronomic and nutritional potentials as the bean contains relatively high protein and energy contents (Emenalom and Udedibie, 1998; Udedibie and Carlini, 1998; Pugalenthi et al., 2006). The beans are known to produce the unusual non-protein amino acid, L-dopa, a potent neurotransmitter precursor that is believed, in part, to be responsible for the toxicity of the Mucuna seeds (Lorenzetti et al., 1998). Alcohol abuse is a major health problem worldwide. Alcohol has numerous deleterious effects on health both in young and older individuals (Speckens *et al.,* 1991). Chronic ethanol abuse in males results in decreased testosterone production, reduced sperm output, and testis atrophy (Lieber, 1995; Armstrong *et al*., 1999). Lipids are major biochemical components of the testes. It plays a vital role in spermatogenesis and steroidogenesis (Johnson, 1970). Drugs derived from plants are known to play a vital role in the management of reproductive diseases. There is a need to develop medicinal plants useful in the treatment of reproductive problems.

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This agent is used in the treatment of Parkinson's disease (Bell and Janzen, 1971; Daxembichler et al., 1971; Hussain et al., 1997). The anti-epileptic and anti-neoplastic activity of methanol extract of *M. pruriens* has been reported (Gupta et al., 1997). Rajeshwar et al. (2005) have revealed that the methanol extract of MP seeds showed significant *in – vitro* anti-oxidant activity while it has also been indicated that the methanol extract of *M. pruriens* can be a potential source of natural anti-oxidant and anti-microbial agent (Rajeshwar et al., 2005). It restores antioxidant levels and reduces lipid peroxide content (Shukla et al., 2007).

All parts of *M. pruriens* possess valuable medicinal properties (Caius, 1989) and it has been studied for various activities like anti-diabetic (Akhtar et al., 1990); aphrodisiac, anti-neoplastic, anti-epileptic, antimicrobial activities (Sathiyanarayanan et al., 2007). Infact, its learning and memory enhancement has been detailed by Poornachandra et al. (2005) just as its aphrodisiac and antivenom activities have been detailed respectively by Rajendran et al. (1997), Shukla et al. (2007), Guerranti et al. (2002) and Fattepur et al. (2008). Its antihelmintic activity has been demonstrated by Jalalpore (2007). The study has been aimed at evaluating the protective effect of the seed extract of *M. pruriens* on alcohol induced reproductive toxicity in male albino rats.

MATERIAL AND METHODS

The protocol for the conduct of experiments was approved by the Institutional Animal Ethical Committee (IAEC) and 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, affiliated to Pondicherry University, Pudhucherry, India.

Plant Material

The dried *Mucuna pruriens* seeds were obtained and validated at the Indian Medicinal Co-operative Practioner's Society (IMCOPS), India. The seeds were washed with water, shade-dried and the seeds were pulverized with a mechanical grinder. The powdered drug was administered to the rats based on kg/ body weight.

Reproductive Toxin

Absolute ethanol (99.9%), was used to prepare alcohol solution, made in China by Jaingsu Hugzi international trade.

Preparation of aqueous extract

15 g of dry fine powder of *Mucuna pruriens* seed was suspended in 500 ml of distilled water and stirred magnetically overnight (12hrs) at 37^0C.

Animal stock

Adult male albino rats (Wistar strain) weighing 140 – 200 g were used in this study. All the animals were housed in a cross-ventilated room (22± 25 ^{0}C), 12 hours light 12 hours dark cycle). The animals were fed with standard Amrut Laboratory rat pellets (Nav Maharastra Chakan Oil Mills Ltd., Pune) and water *ad libitum*.

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* received once with 0.5 ml of 25% ethanol/kg/BW/day for 60 days orally. *Group III* was treated once with 25% of 0.5 ml ethanol /kg/BW/day along with 0.5 ml of *Mucuna pruriens* aqueous extract (15mg/kg BW/day) orally for 60 days.

Collection of organ

Animals from all the groups were sacrificed 24 hours after the 60 days of treatment. The animals were perfused transcardially with 0.9% physiological saline for about an hour. After complete perfusion, testes were removed, rinsed in saline, blotted and weighed accurately on a microbalance and frozen at -20°C until further biochemical analysis.

Lipid was analyzed by the method of Frings *et al*., and phospholipid analyzed by the method of Fiske and Subbarow (1925) as per Marinetti (1962). Eight lipid fractions such as phosphatidyl inositol (PI), phosphatidyl serine (PS), sphingomyelin (SPH), phosphatidyl choline (PC), lyso phosphatidyl choline (LPC), phosphatidyl ethanolamine (PE), cardiolipin (CL) and phosphatidyl acid (PA) were analyzed

Statistical Analysis

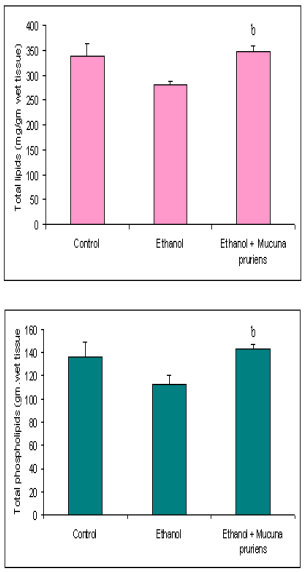
Results were presented as mean standard deviation (Mean ± S.D. for all values). Student 't' test was used for the test of significance (Hill, 1971).

RESULTS

Ethanol induced changes in the total testis cellular lipid and phospholipid classes are presented in figure1. The data obtained in the present study reveals the concentration of total lipids and total phospholipids were significantly reduced in the testis of adult rats treated with ethanol. *Mucuna pruriens* seed extract given along with ethanol prevents the reduction in testicular lipids significantly ($p<0.01$). However ethanol + *Mucuna pruriens* treated revealed restoration of normal pattern of testis lipids (346.91mg/g wet.tissue). Similarly, phospholipids also reduced in ethanol treated rat (112.636 g wet tissue) and restoration with *Mucuna pruriens* treated (142.739 g wet tissue) revealed significant change ($P < 0.01$) than the ethanol treated alone. Consequently, *Mucuna pruriens* showed significant changes in the levels of phospholipids when compared to control.

The changes in phospholipid fractions (Fig.2) are due to the administration of ethanol. However, eight

Fig. 1: Effect of ethanol and *Mucuna pruriens* seed on total lipids and phospholipids in male albino rats



b = p<0.01: ethanol treated vs *Mucuna pruriens* + ethanol treated groups

significant lipid fractions such as phosphatidyl inositol (PI), phosphatidyl serine (PS), sphingomyelin (SPH), phosphatidyl choline (PC), lyso phosphatidyl choline (LPC), phosphatidyl ethanolamine (PE), cardiolipin (CL) and phosphatidyl acid (PA) were obtained. SPH and PC reveled significant changes ($p <0.01$) between ethanol and ethanol + *Mucuna pruriens* treated. Similarly, LPC and PE revealed significant changes ($p <0.001$) between ethanol and ethanol + *Mucuna pruriens* treated. However, PC and PE showed significant changes ($p<0.05$) between control and other groups. Similarly, LPC was the only fraction to be affected more significantly ($p<0.001$) between control and other groups.

DISCUSSION

In the present study the biochemical changes of total lipid and total phospholipid fractions were significantly altered. Alcoholics often have fertility disturbance with low sperm count and impaired sperm motility (Srikanth *et al*., 1999). The alcohol abuse is responsible for impotence that is seen commonly in chronic alcoholic men. The ethanol also inhibits the function of hypothalamus and pituitary. Zhu *et al*., (1997) reported that chronic alcohol administration to male animals is associated with testicular atrophy and gonadal failure.

The present study reveals that ethanol acts as a direct toxicant through biochemical changes, which induce sexual, functional impairment and fertility problems. The results are in agreement with Calleja *et al.*, (1997). Ethanol contributes directly (or) indirectly to variety of

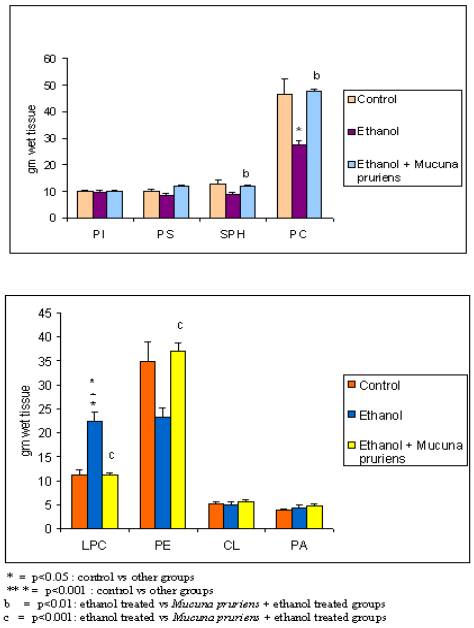
serious disorders in different body organs. It is known to act primarily within the lipid bilayer of biological membrane affecting many of its physio-chemical structural properties (Smith *et al*., 1973; Gilani *et al.,* 1986).

The reduced values are restored to normal by the treatment of ethanol + *Mucuna pruriens*. The differences in the lipid classes could be due to the ethanol treatment. In addition, differences in the chemical composition of testes could be responsible for differences in the response to ethanol (Marco *et al.*, 1986, Sanchez Amate *et al.*, 1992). Phospholipids form the integral part of the sperm membrane along with cholesterol (Davis *et al.,* 1978). 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. This could possibly be the case in the present study since an inverse proportion of lipid and phospholipid were recorded in the control and treated with *Mucuna pruriens* rats.

The lipid content and distribution of mammalian germ cell change in an ordered fashion from the first spermatogonial cell division, through spermatogenesis and epididymal maturation, to capacitation and fuse with the zygotes in the female genital tract as reviewed by Jonas (1998) and Flesch and Gadella (2000). During these changes, the important requirements of the germ cells are to be met; preservation of the genome integrity and acquisition of fusibility. In the progression of germ cell differentiation from spermatogonium to condensing spermatid in mice, the relative amount of phospholipids increases with a decrease in lipid fractions. Besides, essential fatty acids, lipid compositions are essential in morphology of the spermatozoa (Saether *et al.,* 2003).

During sperm maturation in the epididymis, the anterior head membrane undergoes a well-defined series of chemical changes. These include an enrichment of highly unsaturated phospholipids, which leads to a decrease in general membrane stability. This increase is correlated with a selective loss of sperm phospholipids through out the epididymis, resulting in an elevated proportion of choline plasmalogen. Similarly, in the present study the *Mucuna pruriens* treatment enhances the levels of phosphatidyl choline and lysophosphatidyl choline when compared to that of the ethanol treated rats. Consequently, the morphological changes also occur during spermatogenesis. Such alterations during maturation are caused by the distortion of lipids and phospholipids by ethanol (Nolan and Hammerstedt, 1997). However, ethanol induces the antioxidant defense strategies of spermatozoa and enhanced lipid peroxidation that results in sperm immobilization, reduced acrosomal reaction and membrane fluidity (Amstrong *et al.,* 1999). The possibility of utilizing velvet beans as a commercial source of L. dopa was used in the treatment of Parkinson's disease. *Mucuna pruriens* possess significantly higher anti-Parkinson activity compared with levodopa in the 6, hydroxydopamine (6, OHDA) lesion rat model of Parkinson's disease (Manyam *et al.,* 2004).

Fig. 2: Effect of ethanol and *Mucuna pruriens* seed on phospholipid fractions in male albino rats



* = p<0.05 : control vs other groups
** = p<0.001 : control vs other groups
b = p<0.01: ethanol treated vs *Mucuna pruriens* + ethanol treated groups
c = p<0.001: ethanol treated vs *Mucuna pruriens* + ethanol treated groups

L–Dopa (Levodopa) turns into dopamine after it enters the blood stream. The blood carries the dopamine into the brain, where it naturally increases HGH production from the pituitary gland. The increased dopamine levels also optimize the production of other hormones, including testosterone. The increased testosterone leads to increased sex drive and improved sexual performance for both men and women. L.Dopa is one of the few substances that cross the blood brain barrier and converts into Dopamine. Dopamine is a very powerful neurotransmitter. Increased L.Dopa levels have a strong aphrodisiac affect (Capo-chichi *et al.,* 2002).

In the present work, a general decrease in the levels of lipids and phospholipid classes were recorded in the rats treated with ethanol. However, restoration to normal values has been attributed by the treatment of *Mucuna pruriens* seed extract along with ethanol.

In conclusion, the data obtained in the present study, suggest that the *Mucuna pruriens* may interfere the lipid metabolism in testes of mammalian animals. The seed extract also highlights the activities of lipid desaturate revealing their important role in the maturation of mammalian spermatozoa.

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