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RESEARCH ARTICLE

EFFECT OF ETHANOL ON PHOSPHOLIPID OF RAT BRAIN

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ABSTRACT

The effect of *Mucuna pruriens* seed extract on Brain phospholipids was studied in Ethanol treated male pubertal rats. The phospholipid fractions of this rat brain were compared with Ethanol treated and control group rats. The suppressive effect of ethanol on phospholipid fractions was correlated with altered brain function. The application of Mucuna with alcohol relieves the suppressive effect of ethanol, and protects the brain from damage by its anti-oxidants principles. The present study suggests the Mucuna pruriens seed treatment may be beneficial to prevent ethanol-induced toxicity on phospholipids mediated membrane function.

INTRODUCTION

The most sensitive organ to alcohol is the brain (Harshway, 2006). Prolonged use of ethanol results in dependence, and discontinuation of ethanol produces a severe withdrawal syndrome marked by anxiety, ataxia, hyperalgesia, seizures, coma, and even death (Adinoff *et al.,* 1988; Little, 1999). Lipids play both structural and functional roles, and these roles are particularly relevant in the brain, which is the organ with the highest concentration of lipids after adipose tissue (Salvati *et al.,* 2000).

Ethanol increases lipid peroxidation in the liver (Diluzio, 1966; 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), and skeletal muscles (Adachi *et al.,* 2000). Ethanol produces a variety of physiological and behavioral effects in the central nervous system (Gonthier *et al.,* 2004). Acute alcohol intoxication could result in changes in regional brain function, as assessed by changes in glucose metabolism or cerebral blood flow (Volkow *et al.,* 1988; 1990), cognitive performance (Lau *et al.,* 1995; Curtin *et al.,* 2001), motor function (Lemon *et al.,* 1993; Quillian *et al.,* 1999) and behaviour (Giancola, 2000).

Alcohol's effects on the developing brain are particularly complex. Neurons die after alcohol exposure during one stage of development (eg., before neurons migrate to their final location) interferes with subsequent developmental stages (eg., migration or differentiation). Alcohol can also induce apoptosis.

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This has been demonstrated both in animal models of early alcohol exposure (eg., the cranial neural crest in embryos) (Cartwright *et al.,* 1998), and in isolated CNS cells grown in culture, including cells from the hypothalamus (De *et al.,* 1994).

High doses of alcohol modify the brain lipids causing brain cells to produce less protein (Harshway, 2006). Administration of ethanol to rats significantly altered the phospholipids metabolism of the brain (Sun et al., 1987).
L – Dopa is used to make dopamine, an important brain chemical involved in mood, sexuality, and movement. *Mucuna pruriens* supplements increase dopamine content in the cortex of the rat brain. Dopamine is an essential component of our body and it required for proper functioning of brain. Inspite of the tremendous advance made in allopathic medicine, no effective medicine is available to prevent the brain damage by alcohol consumptions. Velvet bean has been gaining popularity over the last few years in the natural products market-especially the sports nutrition industry. It is also showing up as an ingredient in various weight loss, libido, brain/memory, anti-aging and body builder formulas. Traditionally, velvet bean has been used as a nerve tonic for nervous system disorders. Due to the high concentration of L-dopa in the seeds, it has been studied for its possible use in Parkinson's disease management.

Hence, in the present preliminary study, an attempt is made to find out the impact of *Mucuna pruriens* seed powder on the phospholipids classes of brain in ethanol treated male pubertal rat.

MATERIALS AND METHODS

Healthy male albino rats of Wistar strain (60 days old) were used in the present investigation. 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 (Nav Maharastra Chakan Oil Mills Ltd., Pune). Drinking water was made available *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 daily once for 60 days. **Group III** were treated with 25% of 0.5 ml ethanol and 0.5 ml of Mucuna seed powder (15mg/kg body weight) aqueous extract daily once for 60 days.

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, the skull was cut open and the brain was removed, rinsed in saline, blotted and weighed. They were weighed accurately on a microbalance and frozen at -20°C until further analysis. The total lipids were estimated by the method of Fring's *et al.,* (1972) and phospsholipids were separated by thin layer chromatographic method using silica gel-G and the solvent system used was proposed by Narayanan *et al.,* (1985), with slight modification. The modified method of Fiske and Subbarow (1925) as per Marinetti (1962) was employed for phospholipids estimation.

RESULT AND DISCUSSION

The impact of ethanol and Mucuna pruriens seed extract on albino male pubertal rat brain tissue phospholipid classes were assessed in the present study. There was no significant variation in the body weight of Ethanol treated, and Ethanol + Mucuna pruriens seed extract treated rats with control rats.

The wet weight of brain in these three groups shows a variation. The wet weight of brain was marginally lower in ethanol treated than control, however it is not significant. The combined treatment (Ethanol + Mucuna pruriens seed) had a stimulatory effect and increased the weight of brain tissue. The brain weight of this group was statistically significant compared to control (P<0.01) and Ethanol treated (P<0.01) group rats.

Administration of Ethanol to the rats for 60 days, decreased the total lipids in the brain tissue, but this influence is not significant. A profound increase in the total lipid concentration in the brain tissue was evident, after the administration of Ethanol + *Mucuna pruriens* seed 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 lipid are in par with the control animals.

Table 1. Influence of Ethanol and Mucuna pruriens seed on Total Lipid (mg/gm wet tissue)and Total Phospholipids (mg/gm wet tissue) of Rat Brain

Group	Body Weight gms	Brain Weight gm/100gm body weight	Total Lipid	Total Phospholipid
Control (C)	250.366 ± 16.02	0.350 ± 0.02	415.11 ± 14.31	142.19 ± 3.92
Ethanol treated (E)	243.996 ± 11.89	0.314 ± 0.02	385.04 ± 13.19	*** 115.18 ± 3.06
Ethanol + Mucuna seed extract (EM)	243.242 ± 10.63	b ** 0.454 ± 0.02	b 433.27 ± 7.85	c 147.07 ± 4.42

**=p<0.01, ***=p<0.001 Control Vs other groups
B=p<0.01, c=p<0.001 Ethanol treated Vs Ethanol + *Mucuna* treated Group

The total phospholipid concentrations showed a decrease (P<0.001) after Ethanol treatment to the male rats. Table I depicts the changes in phospholipid classes in the brain tissues, in relation to the administration of Ethanol alone and Ethanol + *Mucuna pruriens* seed extract combined to the rats. Significant reductions of phosphatidyl choline (PC) and phosphatidyl ethanol amine (PE) were seen after Ethanol alone treatment to the rats. Contrary to PC and PE a significant increase in lysophosphatidyl choline (LPC) was observed in Ethanol alone given rat brain tissue. The concentration of PC and PE was restored to normalcy by this combined treatment of *Mucuna pruriens* seed extract. The concentration of LPC was brought down to the control level by the Ethanol + Mucuna *pruriens seed extract* treatment.The concentration of the phospholipid fractions like

Table 2. Influence of Ethanol and Mucuna pruriens Seed on Rat Brain Phospholipid classes

Group	Phosphatidyl inositol	Phosphatidyl serine	Sphingo-myelin	Phosphatidyl Ethanol-amine	Lyso-Phosphatidyl choline	Phosphatidyl choline	Cardio-lipin	Phosphatidic acid
Control (C)	12.3 ± 0.65	12.8 ± 0.57	14.11 ± 1.49	41.17 ± 1.48	12.97 ± 0.97	36.76 ± 1.12	5.21 ± 0.79	4.34 ± 0.62
Ethanol Treated (E)	11.9 ± 0.59	11.9 ± 0.75	12.99 ± 0.66	*** 22.87 ± 1.80	** 18.07 ± 1.26	*** 22.10 ± 1.84	5.07 ± 0.59	6.84 ± 0.99
Ethanol + Mucuna Seed Extract (EM)	12.5 ± 0.72	12.8 ± 0.88	15.73 ± 1.77	c 44.55 ± 0.70	b 11.58 ± 1.09	c 38.32 ± 1.27	5.67 ± 0.44	4.31 ± 0.58

**=p<0.01, ***=p<0.001 Control Vs other groups
B=p<0.01, c=p<0.001 Ethanol treated Vs Ethanol + Mucuna treated Group

phosphatidyl inositol (PI), phosphatidyl serine (PS), sphingomyelin (SPH), cardiolipin (CL) and phosphatidic acid (PA) appears to be not much of statistical difference between control, Ethanol alone and Ethanol + *Mucuna pruriens* seed extract treated groups.

The results from the present study are in consistent with the recent report, on the reduction of brain weight due to intoxication by chronic alcohol consumption (Jayaraman *et al.,* 2008). Earlier study also demonstrated the loss of mean brain weight in male alcoholic rat than control rat and indicating that alcohol consumption is more important than nutritional deficiency in causing a reduction in brain weight (Harper and Blumbergs, 1982).

A comparable loss of Purkinje cells after ethanol treatment (Jung *et al.,* 2002) could also contribute the reduction of brain weight in ethanol treated groups. The observed loss of brain weight in ethanol groups might be structurally correlated to the reduction of total lipids and total phospholipids due to alcoholism. From these results it is observed that Mucuna pruriens seed containing bioactive principles might have relieved the adverse effect of ethanol on brain weight and increased the brain weight through raising the concentration of total lipids and total phospholipids.

The anti-oxidant agents available in Mucuna pruriens seed might have interacted with pro- oxidant from ethanol and its effect resulted in augmentation of lipid biosynthesis in brain. The recoveries of total phospholipids from the suppressive action of ethanol, by the Mucuna seed suggest that the active principles and antioxidant properties of Mucuna seed would have involved in maintenance of phospholipid in brain.

It has been established that phospholipids are mainly involved in the membrane transport and neurotransmission in the brain (Sun *et al.,* 1971; Sun, 1972). In the normal rat, the PC and PE are the major phospholipids classes in brain (Kreps *et al.,* 1968; Lapetine *et al.,* 1968). Hence, any change in the controlling factors of brain function involving phospholipids may be reflected in these two classes of phospholipids. This may applicable in the present study also.

From this study, it is clear that ethanol has a definite influence on phospholipids in the brain of pubertal male albino rats. The altered phospholipid classes in the brain due to ethanol administration alters the cell permeability, cytotoxicity and possible cell injury. Ethanol induced injury on brain cells are mediated by abnormal formation of free radical species and this may represent a useful approach in the treatment of ethanol related brain disorders (Muscoli *et al.,* 2002). Mucuna pruriens extract is known to enhance mental alertness and improve coordination (Manyam *et al.,* 2004).

Mucuna seeds constitute excellent raw materials for indigenous Ayurvedic drugs and medicines due to the presence of L-Dopa (3,4-dihydroxy-L-phenylalanine) which provides symptomatic relief of Parkinson's disease. Further the Mucuna pruriens has a neurorestorative effect on degenerating neurons by increasing the activity of complex I enzyme and the presence of NADH and coenzyme Q10 (Manyam *et al.,* 2004). The seed of Mucuna contains number of bioactive substance like flavanoid, tryptamine, alkylamines, steroids and L-dopa (Sinha, 1992). Probably these bioactive agents acts on brain cells as a free radical scavengers or inhibitor of lipid peroxidation exert stimulatory effect on brain. Velvet bean has a long history of use in Indian Ayurvedic medicine. The present study suggests the *Mucuna pruriens* seed treatment may be beneficial to prevent ethanol-induced toxicity on phospholipids mediated membrane function.

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