Global Veterinaria 12 (2): 165-170, 2014
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2014.12.02.76196

Investigation Lithium Chloride Role in Improving the Infertility from Methylphenidate (Ritalin) in Wistar Male Rats

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Abstract: Methylphenidate Hydrochloride (MPH) the more for treatment Attention Deficit/Hyperactivity Disorder (ADHD) had prescribed. Lithium salts using frequently for treatment madness (mania), bipolar disorders. Since increase in MPH consumption in the last years undoubtedly, is surveillance logical so that prevent possibility drug overuse and its detrimental consequence. Forty-eight Wistar rats were randomly distributed into eight experimental groups of 6 rats each. Methylphenidate (1.25; 2.5 and 5 mg/kg b.w ip) in 0.9% saline and Lithium Chloride (1 mg/kg b.w SC) in distilled water solution dissolved. Significant difference in the interaction Ritalin with litium in body weight, testis weight,relative testis/body weight, motility, viability and sperm concentration was observed. The present study showed that, the efficacy of low dose Lithium in preventing the toxic effects of MPH in the rat testes. Therefore, lithium may reduce the side effect of MPH.

Key words: Methylphenidate Hydrochloride · Lithium · Body Weight · Testis Weight · Relative Testis/Body Weight · Motility · Viability · Concentration

INTRODUCTION

Methylphenidate Hydrochloride (MPH) with brand name Ritalin the more for treatment Attention Deficit/ Hyperactivity Disorder (ADHD) has prescribed [1-4]. MPH via preventing of reuptake norepinephrine and dopamine causes increasing the catecholamines concentration in synaptic space [5-7]. According to researches, children with ADHD have to treat during several years (4-9) with Ritalin [3, 8-13].The Ritalin abuse in non-medical situations among youth and adolescents frequently reported in scientific publications [10,11,14]. The MPH also by many university students had used for improve focus [15]. According to investigations, more customers of Ritalin in 18-25 age range have been seen [16]. Apparently, the people believe that Ritalin consumption is best way to maximize performance with least trouble! Drug Enforcement Agency (DAE) U.S, has been reported that Methylphenidate Hydrochloride production level had increased of 16 thousand kilograms pounds in 1999 to 50 million kilograms pounds in 2009, also somehow unbelievable between 2004-2009 MPH production had increased three times [17]. The United States had been consuming eighty-five percent of the world's Methylphenidate [18].

Lithium is an alkali metal, its salts frequently for treatment madness (mania), bipolar disorders, hypothyroidism [19], prevalence of diabetes [20], the function of adrenocortical hormones has prescribed [21]. It has been reported that decrease in norepinephrine secretion by Lithium cause reducing serum level of cortisol [22]. Patients with ADHD often suffering from the mania and depression disorders, therefore, Lithium furthermore Ritalin has prescribed for them [23]. Medical knowledge is poor about the interaction between Lithium Chloride with Methylphenidate Hydrochloride on the reproductive system. Above all, regarding to the widespread abuse of Ritalin for cognitive enhancement by students and entertainment purposes among adolescents and young adults, undoubtedly inhibition or reduce the destructive effects of MPH on testicular function is logical of seem. The survey results in future as basic knowledge for countries development have used by experts.

MATERIALS AND METHODS

For surgery, the animals completely unconscious and accordance with the European Communities Council Directive 22 September 2010

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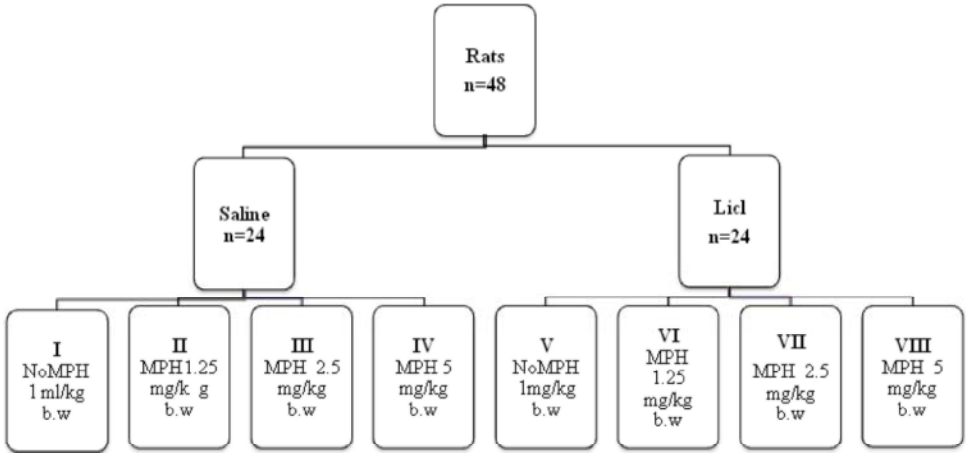

Fig. 1: Summarize of experimental design.

Abbreviations, the drugs dose for each group include:1ml/kg b.w Saline (Group I); 1.25 mg/kg b.w MPH (Group II); 2.5 mg/kg b.w MPH (Group III); 5 mg/kg b.w MPH (Group IV);1mg/kg b.w Licl (Group V);1.25 mg/kg b.w MPH +Licl (Group VI); 2.5 mg/kg b.w MPH +Licl (Group VII); 5 mg/kg b.w MPH+Licl (Group VIII), n = 6 per group.

(2010/63/EU) to performed [24]. In addition the protocol was approved by the ethics committee of our institution.

Animal: Animals were rats with Post Natal Days (PND) 50-53 and between 130-170 gram weight. The rats have purchased from Pasteur Institute Tehran in Iran and were keeping in temperature 22± 2°C, humidity %30-%40, an alternating light period (starting lighting 7 AM and began darkness 7 PM) and in cages with their length, width and height, respectively, 45 × 30 × 15 cm in university animal room. After adaptation, all animals have divided into 8 groups (n=6) randomly.

Experimental Procedure and Dosing: Methylphenidate Hydrochloride and Lithium Chloride from Sigma-Aldrich (St. Louis, USA) has purchased. Ritalin (1.25; 2.5 and 5 mg/kg b.w IP) dissolved in 0.9% saline solution, these doses were chosen based on past resource [25-29]. Lithium Chloride (1 mg/kg b.w SC) in distilled water solution was dissolved. In addition saline (1ml/ kg b.w SC), was injected in control group animals. All injections with equal volume 1ml, Lithium Chloride and saline (At o'clock 13:00PM) were at an hour before Ritalin [26]. Treatment for 30 days with one day interval was performed (PND 60-90) [30]. In PND 90 in order to determine weight changes, rats were weighted. Then twenty-four hours after injection last by euthanasia method with taking 2.5 ml/kg b.w IV Equithesin, rats anesthetized, then testes removed separately and were weighted with digital balance (Sartorius, Germany) readability three decimal (0.001g). For calculate the effect of drugs on the animals, testis weight (mg/g b.w) measured and eventually, sperm analysis was performed for each the rats.

Preparing of Sperm Suspension: The epididymis caudal was removed and holed in petri dish containing 5 ml of Hepes-buffered modified with mT-H (Tyrode's medium) under air placed, at 37 °C [31].

Tyrode's medium included:131.89 mM NaCl+ 2.68 mM KCl+ 0.49 mM MgCl2.6H2O+ 1.80 mM $CaCl_2.2H_2O$+ 0.36 mM $NaH_2PO4.2H_2O$+ 5.56 mM glucose+ 20 mM Hepes+ 5 µg phenol red/ml+ 50 µg kana-mycin/ml + 4 mg bovine serum albumin/ml (fraction V), its pH by using NaOH set almost 7.55 at 20°C [32].

Sperm motility: Motility was evaluated in first step for sperm analysis. In this case 10 µl of semen was assessed on a slide under a cover glass 22 × 22 mm,with magnification of ×200-400 microscope phase contrast optics [33]. The motility as quantitative parameter between 0-1 has shown, as motile sperm (1) and immotile sperm (0) per unit area [32]. According to World Health Organization (WHO), assess at least 200 spermatozoa was required [34].

Sperm Viability: About 90 µl of % 0.5 Eosin-Nigrosin (Merck, Germany) dissolved in 10 µl of semen fluid (dilution the sample 1/10) and immediately repeated pipetting (up and down) the semen homogenized and thoroughly mixture. Then 20 µl of this liquefaction of the

semen using the hemocytometer (cell counter chamber the frequently used to count cell numbers such as blood count and sperm count) at a magnification of ×100 under oil immersion was counted. Sperms red dead and alive were colorless [30,35]. According to WHO, assess at least 200 spermatozoa is required. [34]

Epididymal Sperm Count: Sperm concentration is not a index of testicular function, but on the whole is, number representative of spermatozoa in the semen fluid [39]. Another thing to keep in mind, is these index for fertility and pregnancy rate which as million/ml of suspension expressed. According to World Health Organization, at least counting two sets of 200 sperm is required [32].

Statistical Analysis: Data were compared by ANOVA (Two-way ANOVA) and Post Hoc test of Tukey with group and treatment as main factors (4×2). All tests were calculating as two-tailed with SPSS Version 19 software. In order to further explore the main effects, all values were reported as mean ± SEM. For all the experiments, the significance level was set at P<0.05.

RESULTS

A significant diversity in the body weight [F= 4.075; d_f =3; P<0.013] (Fig. 2), testis weight [F= 2.8770;d_f =3 ; P<0.048] (Fig. 3), relative testis/body weight [F=2.333; d_f=7; P<0.043] (Fig.4), motility [F=4.428; d_f=3; P<0.009] (Fig. 5), viability [F=11.26; d_f=3; P<0.0005] (Fig. 6) and sperm concentration [F=6.970;d_f=3;P<0. 001] (Fig. 7) in the interaction Ritalin with litium was observed.

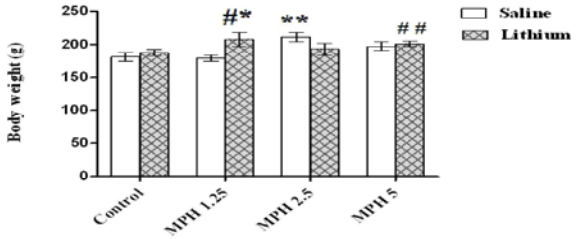

Fig. 2: The effect of MPH &Licl interaction on body weight. A significant difference in rats exposed to MPH 2.5(mg/Kg b.w) showed versus saline (**:P<0.004). MPH treatment 1.25 and MPH 5 (mg/Kg b.w) following lithium significantly increased compared to lithium (#: P<0.04 ; ## : P<0.008).Also MPH treatment 1.25(mg/Kg b.w) following lithium significantly increased compared to MPH 1.25(mg/Kg b.w).(*: P<0.007) n = 6 per group.

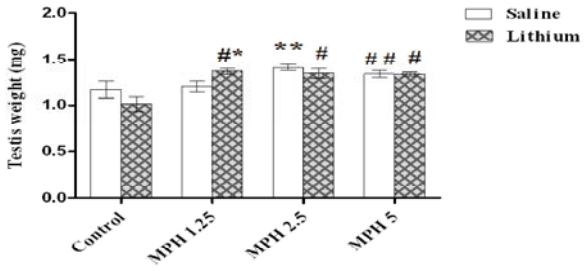

Fig. 3: The effect of MPH &Licl interaction on testis weight. A significant difference in testis weight was detected among the groups. Rats exposed to MPH 2.5 and MPH5 (mg/Kg b.w) showed the significant increase versus Saline.(**:P<0.005; ##:P<0.04). MPH treatment 1.25, MPH 2.5 and MPH 5(mg/Kg b.w) following lithium significantly increased compared to Lithium (#: P<0.0005).MPH treatment following lithium 1.25(mg/Kg b.w) significantly increased compared to MPH 1.25(mg/Kg b.w). (* P< 0. 04) n = 6 per group.

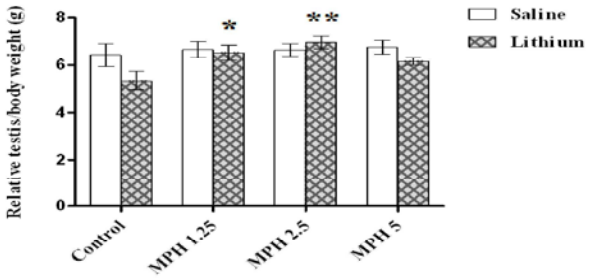

Fig. 4: The effect of MPH & Licl interaction on relative testis/body weight. A significant difference in relative testis/body weight were detected among the groups .MPH treatment 1.25 and MPH 2.5(mg/Kg b.w) following lithium significantly increased compared with lithium. (*: P< 0. 014, **:P< 0.001) n = 6 per group.

DISCUSSION

More efforts have been made to study effects of lasting a long period of time psychostimlant disclosure on reproductive system. Recent results obviously demonstrate that expressed again stimulant administration can make sensitive and thus increase the normal act or process of motivating for sex behavior [21]. This effect was interpreted in terms of clearly drug effects on mesolimbic reward system. However, peripheral effects on gonads and reproductive system of Methylphenidate Hydrochloride and Lithium Chloride interaction not taken

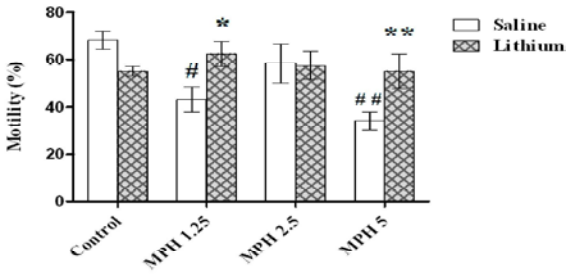

Fig. 5: The effect of MPH &Licl interaction on sperm motility. A significant difference in sperm motility was detected among the groups. Rats exposed to MPH 1.25 and MPH 5 (mg/Kg b.w) showed the significant decreased versus Saline (#: P<0. 003; ##: P<0.0005). MPH 1.25 and MPH 5 (mg/Kg b.w) treatment following lithium the significant increased compared with their counterpart controls. (*: P<0. 01, **P<0.018) n = 6 per group.

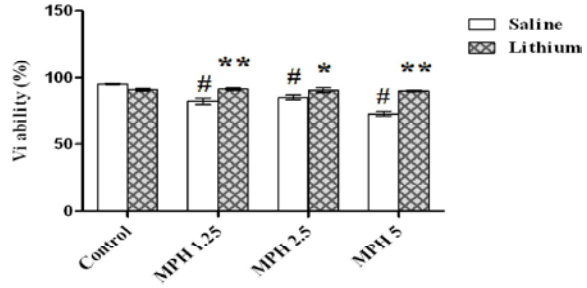

Fig. 6: The effect of MPH & Licl interaction on sperm viability. A significant difference in sperm viability was detected among the groups. MPH treatment significantly decreased sperm viability level in the MPH 1.25,MPH 2.5 and MPH 5(mg/Kg b.w) groups versus Saline(#: P<0.0005).MPH 2.5 and MPH 5 (mg/Kg b.w) treatment following lithium significantly increased compared with their counterpart controls. (** :P<0.0005; *: P<0.024; **: P<0.0005). n = 6 per group.

into account. This research showed the efficacy of low dose Lithium (1mg/kg b.w) in preventing the destructive effects of Ritalin in the rat testes. Although the testicular damage induced by Ritalin is well recognized [37], the precise mechanisms underlying its toxicity to the testes remained unclear. There is evidence that several α- and β-adrenergic receptors are expressed in the rat testis and spermatozoa of rats, mice and humans. These findings seemingly explain the route of MPH effects in the mammalian male reproductive tissues [38], so the effect of

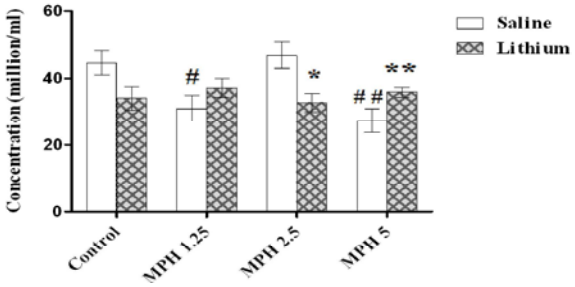

Fig. 7: The effect of MPH & Licl interaction on sperm concentration. A significant difference in sperm concentration was detected among the groups. Rats exposed to MPH 1.25 and MPH 5(mg/Kg b.w) showed the significant decreased versus Saline(# : P< 0.007 ; ## : P< 0.001).MPH 2.5 and MPH 5 (mg/Kg b.w) treatment following lithium showed the significant difference compared with their counterpart controls (*: P<0.016; **:P<0.006). n = 6 per group.

lithium may be via this mechanism. The present study showed that, the efficacy of low dose Lithium in preventing the toxic effects of MPH in the rat testes. Therefore, lithium may reduce the side effect of MPH.

In conclusion, The present study showed that, the efficacy of low dose Lithium in preventing the toxic effects of MPH in the rat testes. Therefore, lithium may reduce the side effect of MPH.we believe that our findings must still be cautiously interpreted and long-term prospective studies on animals with different ages and MPH doses must be conducted as studies on human testicular morphology, sperm development and fertility is somewhat difficult due to ethical factors. Comparison of adults with and without a history of long-term MPH use may disclose interesting results in terms of fertility, offering new approaches to future studies.

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