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Effect of Methylphenidate and buspirone-methylphenidate co-administration on biochemical and hematological parameters in rats: Implications for safe and confrontational use

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Abstract: Methylphenidate (MPH) is a psychostimulant, beneficial in attention deficit hyperactivity disorder (ADHD). Previously it has been shown that MPH-induced locomotor sensitization could be attenuate by buspirone co administration however the effect of chronic MPH and co-administration of MPH-buspirone on biochemical and hematological parameters are unknown. This study is designed to investigate these parameters after long term administration of MPH, Buspirone and their combination in rats. 40 male Wister rats were divided in to 4 groups, and treated with saline, MPH (2mg/kg/day), Buspirone (10mg/kg/day) and MPH-Buspirone co-administration (2mg/kg/day +10mg/kg/day; respectively) up to six weeks. Administration of MPH significantly increase blood glucose level in saline treated control rats, however co-administration of MPH-buspirone exhibited less effect on blood glucose levels. Serum creatinine levels significantly decreased in all treated groups as compared to control but highly significant results were seen with combination treatment. Co-administration of MPH-buspirone and buspirone treated rats exhibited increased cholesterol and hemoglobin values. All treated groups showed increased values of hematocrit, MCV, MCH and MCHC compared to control group. RBCs and WBC's count were decreased in all treated groups. The platelet count rose significantly by Buspirone and MPH-buspirone administration, while MPH showed decreased platelet count. Thus, results suggested that prolong co-administration of MPH-buspirone is safe and effective for ADHD patients by preventing adverse effects not only on behavioral but also on biochemical and hematological parameter.

Keywords: Methylphenidate, buspirone, attention deficit hyperactivity disorder, biochemical analysis, hematological assessment.

INTRODUCTION

Methylphenidate (MPH) is extensively use in attention deficit hyperactivity disorder (ADHD) treatment (Froehlich *et al.,* 2018; Froehlich *et al.,* 2013), however, the recreational and miss use of MPH has been increasing (Chai *et al.,* 2012; McCabe *et al.,* 2005). MPH increases norepinephrine and dopaminergic transmission by inhibiting the catecholamine's reuptake from synaptic cleft (Volkow *et al.,* 2001; Marsteller *et al.,* 2002). After (18 mg) dose, about 80% is eliminating out as (α-phenyl-2-piperidine acetic acid) ritalinic acid, its pharmacological activity is negligible (Faraj *et al.,* 1974; McCallum *et al.,* 2019). Endres *et al.,* (2017) demonstrated that anxiety-like behaviors inhibit by MPH exposure (e.g., freezing, hiding) and reduced cortisol in stressed zebra fish (i.e., stress) (Levin, 2011).

MPH- induced behavioral and metabolic effects are influenced by dose, route of administration, age and schedule of administration. Augmented conversion of inactive metabolites after oral MPH doses have seen with decreased bioavailability (Gerasimov *et al*., 2000; Volkow and Swanson, 2003). Similarly other researchers suggested decrease in serum glucose values after methylphenidate initiation (Gontkovsky *et al.,* 2007).

Biochemical and pharmacological actions of MPH are due to its ability to increase the levels of dopamine in striatum region of brain. Buttarelli *et al.,* 2011 reported that stimulation or increase production of dopamine impairs leukocyte production and decreases immunity. Furthermore as Mohamed *et al.,* 2011 reported that MPH by improving RBC count, improves infantile iron deficiency induced cognitive deficits in rodents.

Montagnini (2014) showed that MPH daily administration results altered endocrine function and growth in the developing rats. These results demonstrate MPH metabolic and biochemical effects associated with age

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and dose at dosing initiation with some chronic effects potential for within post-dosing observation window (Gurbuz *et al.,* 2016). For that reason various non-stimulant medications that affect dopaminergic and noradrenergic pathways have showed efficacy in ADHD treatment (Mohammadi & Akhondzadeh, 2011). Buspirone possesses an antidepressant and an anxiolytic action, with high selectivity and affinity for the 5-HT1A serotonin subtype receptor (Fava, 2007). Buspirone induced neurochemical effects represent both its post-synaptic and pre-synaptic actions. It has an affinity for serotonergic 5-HT2 receptors and dopaminergic receptors (Mohammadi *et al.,* 2018). In treating ADHD, buspirone might also be effective because of its dopaminergic effects (Mohammadi & Akhondzadeh, 2011). We have found few clinical trials on the comparative effect of MPH and buspirone on ADHD children that investigated only efficacy of these two stimulants despite of reporting their comparative effect on biochemical and hematological parameters (Mohammadi *et al.,* 2019; Davari-Ashtiani *et al.,* 2010). Therefore current study is design to identify the role of MPH alone and with combination of buspirone on metabolic and blood biochemical parameters to assess the chronic use safety and risk.

MATERIALS AND METHODS

Animals

Forty locally bred male Wister rats were used weighting 180-200gms, Purchased from the animal house of HEJ University of Karachi, Pakistan. The protocol of the experiment was endorsed by the BASR committee under the BASR/No/0012/Pharm. Animals were handled as per the guidelines provided by the National research council 1996. Mice were accommodated in standard cages where diet and water were provided at libitum. Animals were kept under a cycle of 12hr of dark/light, at room temperature 24+2^{o}C.

Drugs

Methylphenidate Hydrochloride and Buspirone procured from Sigma Aldrich and prepared in saline on daily basis.

Dosing protocol

In this study saline (2.0ml/kg/day) administer to control rats, MPH (2mg/kg/day) to 2nd group, buspirone (10 mg/kg/day) to 3rd group and MPH (2mg/kg/day) buspirone (10mg/kg/day) co administered to 4th group orally 2 times daily for 6 weeks (Aoyama *et al.,* 1990; Natio *et al.,* 2003).

Decapitation and blood sample collection

To avoid the effect of order experiment was performed in such a way that treated and control rats were decapitated by guillotine alternately in a balanced design. After decapitation immediately blood samples were collected in different heparinized tubes for the estimation of blood glucose, creatinine, cholesterol, hemoglobin, RBCs, hematocrit, MCV, MCH, MCHC, WBCs and Platelet count. Helsinki declaration (1964).

Estimation of Glucose

Following the plasma separation, Random blood glucose were estimated during 3 hours of sample collection, by the method of enzymatic colorimetric test GOD/POD/ PAP (Shirwaikar *et al.,* 2005) on Humalyzer 3000 (Semi-automatic chemistry analyzer model # 16780 by Human Germany) using standard kits supplied by company.

Estimation of Creatinine

Serum creatinine were determined by Jaffe's reaction method with the use of commercially available enzyme kit and using an automatic analyzer (Architect c8000 Clinical Chemistry System, USA) (Bartels & Boehme; 1971).

Estimation of Cholesterol

Cholesterol was determined by AU-480 Chemistry Analyzer (Beckman Coulter, USA) (Sblendorio and Palmieri, 2008).

Hematological parameters

Blood samples were collected from rats into heparinized tubes after decapitation. White blood cells (WBC), lymphocyte and monocyte ratio, red blood cells (RBC), hematocrit (Hct), hemoglobin (Hb), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), and platelet count (PLT) were measured on Hematology Analyzer model # 6400/S, Human Germany.

STATISTICAL ANALYSIS

The results were compiled as mean ±S.D. By using SPSS software (version 16.0) statistical analysis was performed. Data of the effect on blood glucose, creatinine, cholesterol, hemoglobin, RBCs, hematocrit, MCV, MCH, MCHC, WBCs and Platelet count in treated and control animals were analyzed by using two-way ANOVA analysis of variance. Tukey's test for Post hoc showed significant if the values lie between $p<0.01$ and $p<0.05$.

RESULTS

Effect on Glucose level

The effect of MPH, buspirone and co-administration of MPH-buspirone on blood glucose levels are shown in fig # 1.Data analysis by 2-way ANOVA exhibited significant effects of MPH ($F=41.544$, $df=1, 24$, $p<0.05$) on blood glucose levels however non-significant effect were seen with buspirone ($F=1.274$, $df=1,24$, $p>0.05$) and buspirone *MPH combination ($F=3.389$, $df=1,24$, $p>0.05$).

Post hoc by tukey's test demonstrated that blood glucose levels significantly increased ($p<0.01$) in MPH treated and co-administration of MPH-buspirone treated rats from control rats and increased ($p<0.01$) in co-administration of MPH-buspirone treated rats from buspirone treated rats.

Effect on Creatinine levels

The effect of MPH, buspirone and co-administration of MPH-buspirone on serum creatinine levels are shown in fig. 2. Data analysis by 2-way ANOVA demonstrated statistically significant result of MPH ($F=63.02$, $df=1,24$, $p<0.05$), buspirone ($F=16.76$, $df=1,24$, $p<0.05$) and buspirone *MPH combination ($F=51.96$, $df=1,24$, $p<0.01$) on creatinine levels. Post hoc test by Tukey's showed that Blood creatinine levels significantly decreased ($p<0.01$) in MPH, buspirone and co-administration treated rats from saline treated control rats.

Effect on blood glucose level in methylphenidate, buspirone and their co-administration treated rats

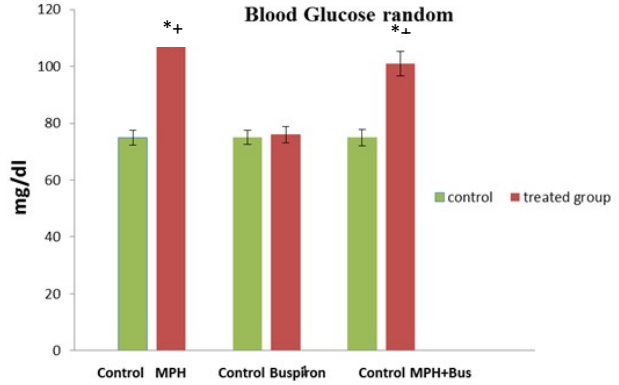

Fig. 1: Blood glucose levels in methylphenidate treated, buspirone treated and co-administration of methylphenidate-buspirone treated rats. Values are means ±SD (n=6). Significant differences by Tukey's test:*$p<0.01$ from saline treated control rats and +$p<0.01$ from buspirone treated rats.

Effect on creatinine levels in methylphenidate, buspirone and their co-administration treated rats

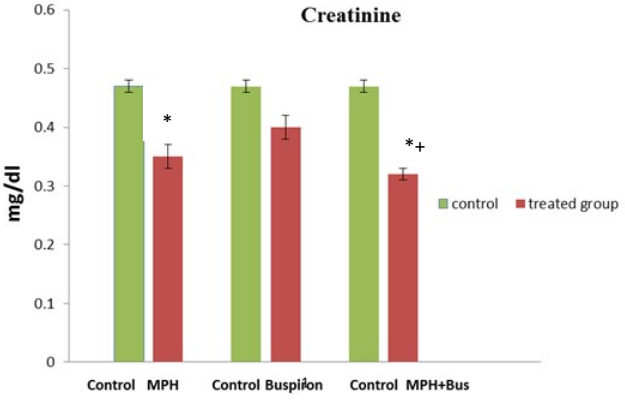

Fig. 2: Effect of on levels of creatinine in methylphenidate treated, buspirone treated and co-administration of methylphenidate-buspirone treated rats. Values are means ±SD (n=6). Significant differences by Tukey's test: *$p<0.01$ from saline treated control rats following two-way ANOVA.

Effect on Cholesterol

The effect of MPH, buspirone and co-administration of MPH-buspirone on total cholesterol are shown in fig. 3. Data analysis by 2-way ANOVA demonstrated significant result of MPH ($F=20.77$, $df=1,24$, $p<0.05$) insignificant effects of buspirone ($F=0.964$, $df=1,24$, $p>0.05$) and statistically significant effect of buspirone*MPH combination ($F=6.9$, $df=1,24$, $p<0.01$) were perceived.

Effect on cholesterol level in methylphenidate, buspirone and their co-administration treated rats

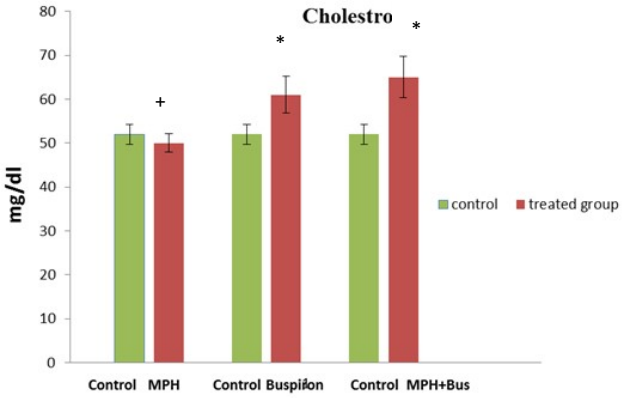

Fig. 3: Effect on levels of cholesterol in methylphenidate treated, buspirone treated and co-administration of methylphenidate-buspirone treated rats. Values are means ±SD (n=6). Significant differences by Tukey's test: **$p<0.01$ and *$p<0.05$ from saline treated control rats and ++$p<0.01$ and +$p<0.05$ from methylphenidate treated rats following two-way ANOVA.

Effect on hemoglobin in methylphenidate, buspirone and their co-administration treated rats

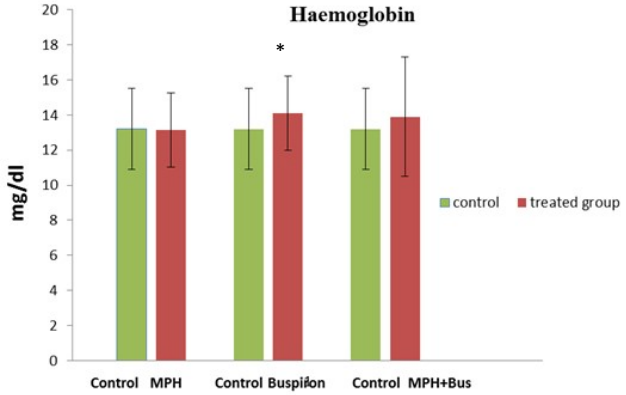

Fig. 4: Effect on hemoglobin in methylphenidate treated, buspirone treated and co-administration of methylphenidate-buspirone treated rats. Values are means ±SD (n=6). Significant differences by Tukey's test:

*p<0.05 from saline treated control rats and +p<0.05 from methylphenidate treated rats following two-way ANOVA.

Post hoc test by Tukey's confirmed that Cholesterol levels significantly rose (p<0.01) in co-administration treated rats and (p<0.05) in buspirone treated rats from MPH and saline treated control rats.

Effect on RBCs in methylphenidate, buspirone and their co-administration treated rats

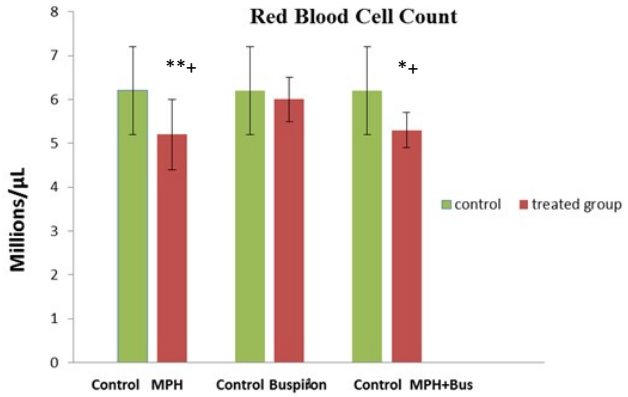

Fig. 5: Effect on red blood cells (RBCS) in methylphenidate treated, buspirone treated and co-administration of methylphenidate-buspirone treated rats. Values are means ±SD (n=6). Significant differences by Tukey's test: *p<0.05 from saline treated control rats following two-way ANOVA.

Effect on hematocrit in methylphenidate, buspirone and their co-administration treated rats

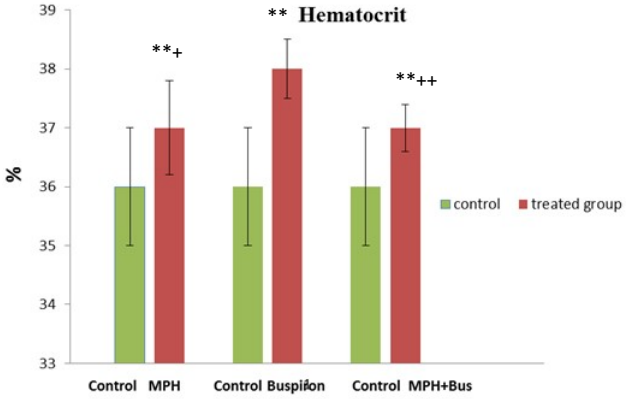

Fig. 6: Effect on hematocrit in methylphenidate treated, buspirone treated and co-administration of methylphenidate-buspirone treated rats. Values are means ±SD (n=6). Significant differences by Tukey's test: **p<0.01 and *p<0.05 from saline treated control rats following two-way ANOVA.

Effect on Hematological Parameters

Effect on hemoglobin in MPH treated, buspirone treated and co-administration of MPH-buspirone treated rats.

Fig. 4 shows the effect on hemoglobin in repeated MPH, buspirone and co-administration of MPH-buspirone treated rats. Data analysis by 2-way ANOVA demonstrated significant effects of MPH (F=8.68, df=1,24, p<0.05), buspirone (F=6.04, df=1,24, p<0.05) and buspirone*MPH combination (F=11.7, df=1,24, p<0.01).

Tukey's post hoc explained that levels of hemoglobin were raised significantly (p<0.05) in buspirone and in co-administration of MPH- buspirone treated rats too as compared to MPH and control rats.

Effect on MCV in methylphenidate, buspirone and their co-administration treated rats

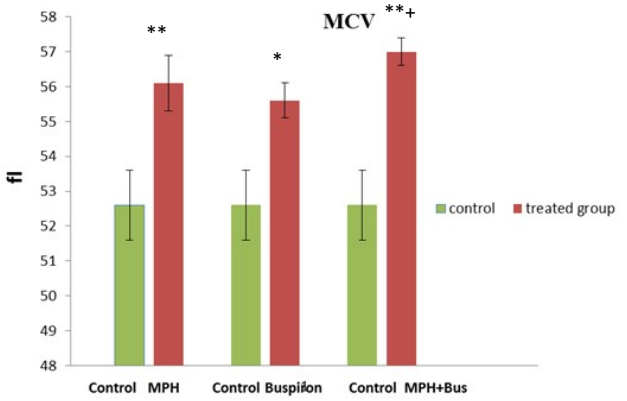

Fig. 7: Effect on MCV in methylphenidate treated, buspirone treated and co-administration of methylphenidate-buspirone treated rats. Values are means ±SD (n=6). Significant differences by Tukey's test: **p<0.01 and *p<0.05 from saline treated control rats following two-way ANOVA.

Effect on MCH in methylphenidate, buspirone and their co-administration treated rats

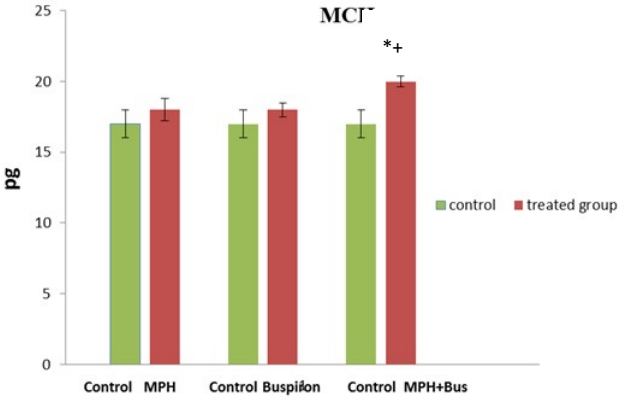

Fig. 8: Effect on MCH in methylphenidate treated, buspirone treated and co-administration of methylphenidate-buspirone treated rats. Values are means ±SD (n=6). Significant differences by Tukey's test: **p<0.01 and *p<0.05 from saline treated control rats following two-way ANOVA.

Effect on RBCs in MPH treated, buspirone treated and co-administration of MPH-buspirone treated rats

Fig. 5 shows the effect on red blood cells in repeated MPH, buspirone and co administration of MPH-buspirone treated rats. Data analysis by 2-way ANOVA demonstrated significant results of MPH ($F=9.56$, $df=1,24$, $p<0.01$), insignificant results of buspirone ($F=0.01$, $df=1,24$, $p>0.05$) and significant decreased levels of RBC's after buspirone*MPH combination treatment ($F=5.06$, $df=1,24$, $p<0.05$). Post hoc test by Tukey's test showed significantly ($p<0.05$) declined red blood cells count in MPH and co administration treated rats compared to control rats.

Effect on MCHC in methylphenidate, buspirone and their co-administration treated rats

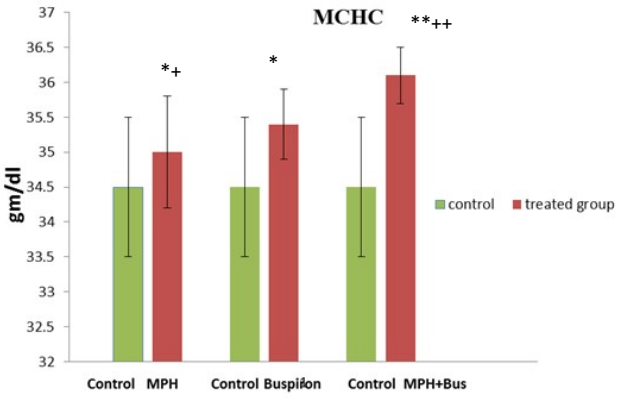

Fig. 9: Effect on MCHC in methylphenidate treated, buspirone treated and co-administration of methylphenidate-buspirone treated rats. Values are means ±SD (n=6). Significant differences by Tukey's test:**p<0.01and *p<0.05 from saline treated control rats; ++p<0.01 from methylphenidate treated rats following two-way ANOVA.

Effect on WBCs in methylphenidate, buspirone and their co-administration treated rats

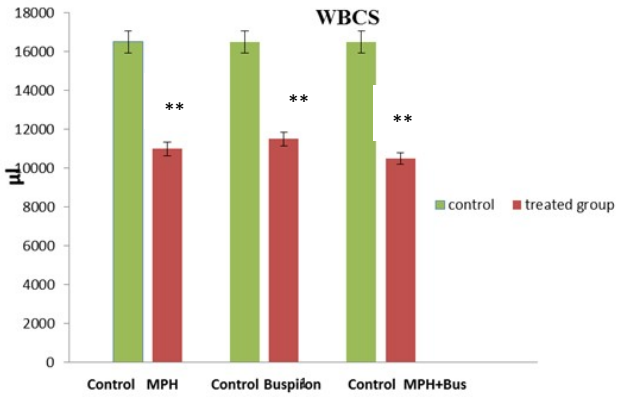

Fig. 10: Effect on white blood cells (WBCS) count /microlitre in methylphenidate treated, buspirone treated and co-administration of methylphenidate-buspirone treated rats. Values are means ±SD (n=6). Significant differences by Tukey's test: *p<0.01 from saline treated control rats following two-way ANOVA.

Effect on Hematocrit in MPH, buspirone treated and co-administration of MPH-buspirone treated rats

Fig. 6 showed the effect on Hematocrit in repeated MPH, buspirone and co-administration of MPH-buspirone treated rats. Data analyzed by 2-way ANOVA demonstrated significant raised levels of hematocrit in MPH ($F=24.62$, $df=1,24$, $p<0.01$) treated group, no change was observed after buspirone administration ($F=3.86$, $df=1,24$, $p>0.05$) however significant raised values were seen after buspirone *MPH combination treatment ($F=54.32$, $df=1,24$, $p<0.05$).

Effect on platelet count in methylphenidate, buspirone and their co-administration treated rats

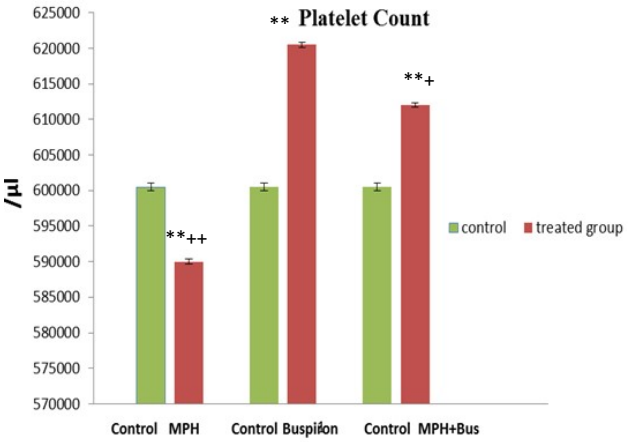

Fig. 11: Effect on platelet count in methylphenidate treated, buspirone treated and co-administration of methylphenidate-buspirone treated rats. Values are means ±SD (n=6). Significant differences by Tukey's test: *p<0.01 from saline treated control rats. following two-way ANOVA.

Post hoc by Tukey's test explained significantly increased haematocrit ($p<0.05$) in MPH, co-administration of MPH-buspirone and ($p<0.01$) in buspirone treated rats than control rats.

Effect on MCV in MPH treated, buspirone treated and co-administration of MPH-buspirone treated rats

Fig. 7. Showed the effect on MCV in repeated MPH, buspirone and co-administration of MPH-buspirone treated rats. Data analysis by 2-way ANOVA verified significant effects of MPH and Buspirone on MCV levels ($F=10.43$, $df=1,24$, $p<0.01$), ($F=5.77$, $df=1,24$, $p<0.05$) respectively. However combination of buspirone*MPH ($F=0.13$, $df=1,24$, $p>0.05$) showed insignificant effect.

Post hoc by Tukey's test demonstrated that MCV levels in blood increase significantly ($p<0.01$) in MPH, co-administration of MPH-buspirone ($p<0.05$) in buspirone treated rats than control rats.

Effect on MCH in MPH treated, buspirone treated and co-administration of MPH-buspirone treated rats

Fig. 8 shows the effect on MCH in repeated MPH, buspirone and co-administration of MPH-buspirone treated rats. Data analysis by 2-way ANOVA showed significant effects of buspirone ($F=13.21$, $df=1,24$, $p<0.01$) on MCH. While MPH and buspirone*MPH exhibited in-significant effects on MCH ($F=2.99$, $df=1,24$, $p>0.05$), ($F=0.201$, $df=1,24$, $p>0.05$) after repeated administration.

Post hoc by Tukey's test demonstrated that MCH levels in blood increased significantly ($p<0.01$) in MPH-buspirone co-administration and ($p<0.05$) in MPH treated rats compare to control rats.

Effect on MCHC in MPH treated, buspirone treated and co-administration of MPH-buspirone treated rats

Fig. 9 shows the effect on MCHC (Mean Corpuscular Hemoglobin Concentration) in repeated MPH, buspirone and co-administration of MPH-buspirone treated rats. Data analyzed by2-way ANOVA exhibited significant results after buspirone ($F=38.52$, $df=1,24$, $p<0.01$) administration. While insignificant results were seen after MPH &buspirone*MPH administration on MCHC ($F=0.90$, $df=1,24$, $p>0.05$); ($F=0.33$, $df=1,24$, $p>0.05$) respectively. Post hoc by Tukey's test demonstrated significantly ($p<0.05$) improved MCHC levels in MPH treated and ($p<0.01$) in buspirone treated and in co-administration of MPH-bupirone treated rats.

Effect on WBCS in MPH treated, buspirone treated and co-administration of MPH-buspirone treated rats

Fig. 10 shows the effect on white blood cells in repeated MPH, buspirone and co-administration of MPH-buspirone treated rats. Data analyzed by 2-way ANOVA exhibited that MPH, Buspirone and buspirone*MPH groups significantly decreased WBC's count ($F=83.38$, $DF=1,24$, $p<0.01$), ($F=8.43$, $DF=1,24$, $p<0.05$) and ($F=33.17$, $DF=1,24$, $p<0.01$) respectively. Post hoc by Tukey's revealed highly significant ($p<0.01$) decreased white blood cells count in all drug treated groups as compared to control group.

Effect on platelet count in MPH treated, buspirone treated and co-administration of MPH-buspirone treated rats

Fig. 11 shows the effect on platelet count in repeated MPH, buspirone and co-administration of MPH-buspirone treated rats. Data analyzed by 2-way ANOVA exhibited significant decreased Platelet count after MPH treatment ($F=24.7$, $df=1,24$, $p<0.01$), increased platelet count after buspirone ($F=130.84$, $df=1,24$, $p<0.01$) treatment. While buspirone*MPH ($F=0.409$, $df=1,24$, $p>0.05$) treated group showed insignificant effect.

Post hoc by Tukey's test demonstrated significantly ($p<0.01$) decreased platelet count in MPH treated group and significantly ($p<0.01$) increased platelet count in buspirone and co-administration of MPH–buspirone treated group compared to control group.

DISCUSSION

MPH is among the most widely prescribed medications for ADHD (Evans *et al.,* 2018; Greenhill *et al.,* 2002). CNS stimulants are of therapeutic value despite of many adverse effects like sleep disturbance, appetite suppression, weight loss and reverse tolerance (Banaschewski *et al.,* 2004). Considering the side effects of stimulant and questions regarding the safety of prolong stimulant treatment, also the choice to avoid stimulant medicines, has led most of the parents to search for alternative treatment (Mohammadi *et al.,* 2018). Therefore, concerns exist about the side effects of stimulant medications on blood and biochemical parameters after prolong use (Tehranchi *et al.,* 2018). In current study increased glucose levels were found after treatment of MPH alone however treatment with buspirone & combination (Bus+MPH) exhibited less effect on blood glucose levels. Charach *et al.,* 2020 reported that MPH increases synaptic levels of DA (dopamine) and Norepinephrine, which are responsible for increasing blood pumping from the heart and heart rate results in increases blood pressure and to provide more energy to the body there is break down fat and increase blood sugar levels. On other hand, serotonin appeared to be an inhibitor of insulin and believed to alter the insulin level and blood glucose. (Volkow *et al.,* 2008; Chi *et al.,* 207; Furman and Wilson, 1980). Creatinine levels have decreased in all treated groups (fig. 2) however highly significant results were seen with combination treatment. As Raghunathan *et al.,* (2014) reported that buspirone may be beneficial in the reducing functional and structural changes in a diabetic kidney by decreasing creatinine and urea levels.

Significant decrease were seen in cholesterol levels after MPH administration in comparison to, control as Charach and Collegues 2013 found significantly decreased levels of total triglycerides, cholesterol and lipoproteins in ADHD children. MPH stimulates CNS by increasing synaptic and intracellular norepinephrine and dopamine in rodents (Schiffer *et al.,* 2006; Seeman *et al.,* 2002). Increased concentrations of epinephrine caused an increase hydrolysis of endogenous lipid over the breakdown of glycogen that results hypercholesterolemia (Kunihara & Oshima, 1983; Woudberg *et al*., 2018; Alves-Bezerra & Cohen, 2017). In current study we observed decreased cholesterol levels after MPH but slight increased values have been seen after co-administration of MPH and buspirone treatment in rats. Our observation is in accordance with Charach *et al* (2009) who reported that MPH improves the profile of lipid by decreasing total triglycerides, cholesterol and

LDL levels. Possibly due to Most adverse effects are mild and transient; there is concerns in a clinical setting about the effect of prolong treatment with MPH, buspirone and their co-administration on hematologic variables. In current study, MPH, buspirone and their co-administration after six weeks showed that values of hemoglobin increased in co-administration treated and hematocrit, MCV, MCH and MCHC were greater in MPH and co-administration treated rats compared to control group whereas RBCs decreased in all treated groups suggesting that peripheral iron levels did not differ between drug treated and controls (Menegassi, 2010).

Furthermore we found decreased WBCs count in all drug treated groups so these drugs administration can leads to leucopenia (Wigal *et al.,* 2007, Wiergowsk *et al.,* 2014). Sedky& Lippmann (2006) reported that many psychotropic drugs induced medicinal side-effects. Platelet count was decreased after prolong MPH treatment however increased after both MPH and buspirone treatment. Favrod-Coune *et al.,* (2010) reported increased activation of platelets count after CNS stimulant drugs. Finally current study concluded that in combination of MPH-buspirone treatments the frequency of side effects were ameliorated compare to MPH treatment. Thus this study suggests that in prolong use combination therapy would be best option to reduce side effects in the treatment of ADHD.

CONCLUSION

Our results suggested that prolong co-administration of MPH-buspirone is safe and effective for ADHD patients by preventing adverse effects not only on behavioral but also on biochemical and hematological parameter.

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