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Effects of lactational exposure of olanzapine and risperidone on hematology and lymphoid organs histopathology: A comparative study in mice neonates

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ABSTRACT

Body weight gain, sexual/reproductive dysfunction and hematological abnormalities are serious consequences of atypical antipsychotics treatment. No attempts however have been made preclinically to elucidate the adverse hematological impacts. Presently, effects of lactational exposure of olanzapine (4, 8 and 10 mg/kg) and risperidone (1 and 2 mg/kg) on hematology as well as lymphoid organ histopathology of mice neonates were investigated. Both olanzapine and risperidone transfers through milk and make the neonates susceptible to their adverse side effects. Corticosterone elevation tendency of both the drugs further enhance the susceptibility for immune dysfunction. Analysis of total and differential leukocytes counts revealed neutropenia with all the doses of olanzapine but only with risperidone 2 mg/kg. Weight analysis and histopathology of thymus and spleen indicated a state of suppression; less in the risperidone-exposed groups. Significant plasma corticosterone elevation occurred on 4 and 8 mg/kg olanzapine exposures but not with 10 mg/kg as well as with both the risperidone doses. Elevation of plasma prolactin levels occurred dose-dependently for both the drugs. Hematological toxicity (neutropenia) might be the direct toxic effects of the drugs/unstable metabolites on circulating neutrophils and/or on the bone marrow hemopoietic cells. Direct toxicity of the drugs might also have suppressed the lymphoid organs thymus and spleen. Further, it could be associated to hormonal imbalance induced by adverse pharmacological effects of the drugs on the endocrine system. Suppression of lymphoid organs in olanzapine groups might have resulted because of corticosteronemia and hyperprolactinemia, while in risperidone it could be mediated by pronounced hyperprolactinemic effect alone.

1. Introduction

Antipsychotics mediated hematological abnormalities and related pathology has currently become matters of great concern. Olanzapine, a widely prescribed atypical antipsychotic drug, is generally considered hematologically safe (Bhana et al., 2001; Dernovsek and Tavear, 2000). Recent clinical studies, however, reported olanzapine-induced hematological abnormalities like leukopenia, granulocytopenia, neutropenia, thrombocytopenia and anemia (Cadario, 2000; Gajwani and Tesar, 2000; Oyesanmi et al., 1999; Stergiou et al., 2005; Sayin and Cosar, 2006; Tu and Yang, 2002). Delieu and co-workers reported abnormal immature neutrophil leukocytes in schizophrenic patients treated with olanzapine (Delieu et al., 2006, 2009). Similar adverse hematological effects were reported on treatment with typical antipsychotics such as chlorpromazine and haloperidol (Abdullah et al., 2003; Delieu et al., 2009) and atypical antipsychotics clozapine (Lieberman and Tasman, 2006) and risperidone (Finkel et al., 1998). The neutrophil pathology and leukopenia thus reported on olanzapine and other antipsychotic are the outcome of clinical studies only. No attempts have been made so far to demonstrate this preclinically. To identify the probable cause of antipsychotic drug-mediated hematological adverse effects and related pathology, assessment of these drugs on animal models is essential. Furthermore, reports on hematological abnormalities of atypical antipsychotic drugs are restricted to the adults only. Studies on neonates are lacking, who are more susceptible through their lactating mothers. Transfer of various atypical antipsychotic drugs (olanzapine, risperidone and others) to the infants through milk is reported (Gardiner et al., 2003; Goldstein et al., 2000, Ilett et al., 2004). The present investigation was therefore undertaken for the evaluation of adverse impacts of lactational olanzapine and risperidone exposure on hematological parameters of mice neonates. The hematological abnormalities are well correlated to immune dysfunctioning (Raanani and Ben-Bassat, 2002; Ruzek et al., 2005; Williams et al., 2000). The atypical antipsychotic-induced hematological abnormalities has been linked to immune dysfunctioning as well (Uetrecht, 1992; Williams et al., 2000; Yunis et al., 1995). The effects of the drug on the immune/lymphoid organs thymus and spleen were assessed in the respect of weight analysis and histopathology for better understanding of the mechanism of hematological abnormalities. Furthermore, the

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immune system functioning is under the neuroendocrine regulation and *vice-versa* (Besedovsky and Rey, 2007; Gerez et al., 2007; Webster et al., 2002). Hyperprolactinemia is a widely reported endocrine dysregulation on the treatment of conventional antipsychotics, also reported for some atypical antipsychotic drugs (Cutler, 2003; Knegtering et al., 2003; Konarzewska et al., 2009). Though generally considered prolactin sparing, olanzapine-induced hyperprolactinemia was reported in clinics (Kapur et al., 1998, Konarzewska et al., 2009). Recently, we reported hyperprolactinemia in mice neonates lactationally exposed to olanzapine and risperidone (Mishra and Mohanty, 2009, in press). Olanzapine and risperidone-induced corticosterone elevation in rodents also have been reported (Assié et al., 2008; Baptista et al., 2002; Marx et al., 2003). Involvement of corticosterone and prolactin in regulation of immune functioning has been elucidated (Besedovsky and Rey, 2007; Webster et al., 2002). Plasma measures of these two hormones were also assayed to correlate atypical antipsychotic drug-induced endocrine dysregulations to the adverse impact on immune organs as well as on hematological parameters.

2. Materials and methods

2.1. Animals

Adult parkes mice were procured from Central Drug Research Institute (CDRI), Lucknow, India and housed under standard laboratory conditions in 12:12 h light/dark cycle (lights on between 07:00 and 19:00) in a temperature (21 ± 2 °C) and humidity (55 ± 5%) controlled room. They had free access to laboratory mice food (pelleted mice food) and water. Twenty eight post-partum female mice (Age 2-3 months) weighing 35 ± 5 g and their pups were used in this study. They were maintained in seven groups (Group I to Group VII); four dams with their pups (29-34 pups)/group. Each dam along with their pups was housed singly in PVC cages ($290 \times 320 \times 390$ mm). Group I (11 male pups) was maintained as control, while Group II (12 male pups), Group III (14 male pups) and Group IV (12 male pups) were exposed to olanzapine. For risperidone, Group V (13 male pups) was control, Group VI (14 male pups) and Group VII (13 male pups) were the experimentals.

2.2. Drugs and experimental design

Olanzapine (Olanex, Ranbaxy, India) was dissolved in a minimum quantity of acetic acid, made the final volume with distilled water and the pH adjusted to 5.5-6.0 with 0.1 M NaOH. Risperidone (Respidon, Torrent, India) was dissolved in distilled water. The drug doses were calculated as the base equivalent weight and administered via subcutaneous route in a volume of 10 ml/kg. Atypical antipsychotic drugs or their vehicles were administered to post-partum mothers over a period of 21 days, from day one of delivery till the separation of pups at weaning. A single daily injection was given between 12:00 hr to14:00 hr. The drug doses were decided following the study reports on rodents (Kapur et al., 2003); 4-6 times higher doses of olanzapine (4, 8 and 10 mg/kg) and risperidone (1 and 2 mg/kg) were administered to get the clinically comparable D_2 receptor occupancy (70-80%). Group I mothers (olanzapine control) received vehicle (distilled water with minimum amount of acetic acid), Group II to Group IV were injected olanzapine doses of 4, 8, and 10 mg/kg respectively. Group V mothers were treated with distilled water (risperidone control), while Group VI and VII received risperidone doses of 1 and 2 mg/kg respectively. On completion of the experiments, cardiac blood was sucked from ether anaesthetized pups using heparinized micro syringe and collected in microcentrifuge tubes for further hematological and hormonal analysis. After blood sampling, the pups were decapitated, thymus and spleen were immediately dissected out, freed from the adjacent tissues, blotted and weighed (Studies on female pups are not included in the present work). The tissues were then fixed in Bouin's fluid for further histological study. The experimental protocol was approved by the Institutional Animal Ethical Committee of the University according to the guidelines of the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA), Ministry of Environment and Forests, Government of India.

2.3. Hematological analysis

The total leukocyte count was obtained by hemocytometer using Turk's solution (glacial acetic acid, 1 ml; 1% aqueous gentian violet, 1 ml and distilled water to make 100 ml) to haemolyse the red blood cells. For differential leukocyte count, the lymphocyte and neutrophil number/percentage was obtained by microscopic examination of peripheral blood smears stained by general Leishmann stain.

2.4. Bone marrow leukocyte count

Femur bones of both the legs were dissected out and bone marrow strip was flushed into a sterilized test tube with the help of syringe using phosphate buffer. Bone marrow strip was then well agitated to make a homogenous cell suspension. A small drop of the suspension was put on the clean slide; a thin film was prepared and dried. The slide was stained with Leishmann stain and observed under microscope for differential leukocyte count.

2.5. Histology

Thymus and spleen tissues were fixed overnight, washed thoroughly, dehydrated in graded series of alcohol and cleared in xylene prior to paraffin embedding. Sections of 5-7 μm thickness were stretched on glass slides, deparaffinized and stained with hematoxylin and eosin for basic histopathological analysis under light microscope.

2.6. Morphometric studies

For quantitative evaluation of size of thymocytes and splenocytes of different experimental groups as well as of control, the diameters were measured. Measurements were done in randomly selected cross sections using the Leica IM 50 software.

2.7. Hormone assay

Blood samples were centrifuged at 3000 r.p.m. for 10 min. The plasma supernatant was collected and stored at –20 °C until hormonal assay. Plasma levels of corticosterone and prolactin were determined in duplicate by using corticosterone-ELISA kit (*NEOGEN*, USA) and prolactin-ELISA kit (Eliscan, RFCL Ltd., India). For both the hormone assays, both the intraassay and interassay coefficients of variations were less than 10%.

2.8. Statistical analysis

All values were represented as means ± SEM. Further analysis was carried by a one-way ANOVA followed by Dunnett's *t* test (SPSS 10 statistical software package, SPSS Inc.). Significance was determined at $P<0.05$.

3. Results

3.1. Effect of atypical antipsychotic drugs on hematological parameters

Peripheral leukocyte count in male neonates exposed to all doses of olanzapine decreased (51.2-58.6%) significantly ($P<0.01$ to $P<0.001$) in comparison to control. Differential leukocyte count revealed a significant reduction in peripheral neutrophil (13.5-

Table 1
Effects of olanzapine and risperidone exposures on hematological parameters of mice neonates. (n = 11-14 pups/group).

Parameters	Experimental groups					
	Control	4 mg/kg Olanzapine	8 mg/kg Olanzapine	10 mg/kg Olanzapine	1 mg/kg Risperidone	2 mg/kg Risperidone
In peripheral blood						
TLC [10^3 mm^{-3}]	11.28 ± 0.67	5.04 ± 0.45 [c] (-55.3)	4.67 ± 0.14 [c] (-58.6)	5.51 ± 0.31 [b] (-51.2)	11.75 ± 0.34	10.89 ± 0.34
DLC (% change from control)						
NEUTROPHIL	39.3 ± 2.74	34.0 ± 2.96 [a] (-13.5)	30.2 ± 2.06 [b] (-23.2)	25.2 ± 1.32 [b] (-35.9)	41.2 ± 1.24	28.8 ± 2.15 [a] (-26.7)
LYMPHOCYTE	48.4 ± 2.06	57.8 ± 3.51 [a] (+19.4)	58.6 ± 2.56 [b] (+20.7)	64.0 ± 2.49 [b] (+32.2)	53.0 ± 2.90	59.5 ± 1.99 [a] (+23)
N/L Ratio	0.824 ± 0.090	0.598 ± 0.088 [a]	0.538 ± 0.050 [a]	0.398 ± 0.035 [b]	0.791 ± 0.034	0.508 ± 0.064 [a]
In bone marrow						
DLC (% change from control)						
NEUTROPHIL	61.00 ± 1.00	55.00 ± 2.08 [a] (-9.8)	54.50 ± 1.82 [a] (-10.7)	53.80 ± 1.31 [b] (-11.8)	58.60 ± 4.08	55.33 ± 4.41
LYMPHOCYTE	36.33 ± 1.66	39.00 ± 3.21	42.25 ± 2.05 [b]	44.50 ± 2.96 [a]	38.40 ± 3.21	42.20 ± 4.96

[a], [b] and [c] showing significance from control at $P<0.05$, $P<0.01$ and $P<0.001$ respectively.
TLC Total Leukocyte Count; DLC Differential Leukocyte Count; N/L Neutrophil: Lymphocyte ratio.

35.9%; $P<0.05$ to $P<0.01$) in olanzapine-exposed groups (Table 1). A dose-dependent significant drop in neutrophil/lymphocyte ratio ($P<0.05$ to $P<0.01$) was observed. In contrast to this, no significant differences in the total leukocyte counts were observed in male neonates exposed to both 1 and 2 mg/kg risperidone (Table 1). A significant decrease ($P<0.05$) in neutrophil percentage and neutrophil/lymphocyte ratio occurred in neonates exposed to 2 mg/kg risperidone. Bone marrow differential counts of leukocytes showed a dose-dependent decrease in the neutrophils in olanzapine-exposed groups (Table 1). Bone marrow leukocyte counts of risperidone-exposed neonates (both 1 and 2 mg/kg) was comparable to control (Table 1). Olanzapine and risperidone treatment did not affect the percentage count of eosinophils, basophils and monocytes (Data not shown).

3.2. Effect of atypical antipsychotic drugs on lymphoid organs

3.2.1. Weight analysis

The percentage thymus weight was reduced significantly in a dose-dependent manner in olanzapine-exposed groups; highest with 10 mg/kg exposure (Table 2). On the contrary, the percentage spleen weight increased; highest with 4 mg/kg followed by 8 and 10 mg/kg in that order (Table 2). Significant reduction in both thymus and spleen weight occurred with 2 mg/kg risperidone-exposure. Group exposed to 1 mg/kg risperidone showed reduction in the relative weight of thymus but increase in the spleen weight, both insignificant.

3.2.2. Lymphoid organ histopathology

3.2.2.1. Thymus. Histopathology of thymus of olanzapine and risperidone-exposed pups revealed distinct morphological alterations showing characteristics of regression (Fig. 1A-R). Cortex to medulla ratio was decreased with all the doses of olanzapine (Fig. 1D, G and J). Demarcation between cortex and medulla became less distinct in groups exposed to 8 and 10 mg/kg olanzapine due to reduced cellularity of cortex (Fig. 1G and J). Many darkly stained apoptotic lymphocytes were observed in the cortex of these two exposed groups (Fig. 1H and K). Cortical necrosis with loose capsular wall (Fig. 1G and J) and degenerated thymocytes (Fig. 1H) was also seen. Cellularity of medulla was also reduced dose-dependently (Fig. 1F, I and L; Table 2). Alterations in the thymus histology were noted only in the 2 mg/kg risperidone-exposed group (Fig. 1M-R). Cellularity of cortex and medulla was reduced along with significant decrease in sizes of cortical and medullary thymocytes (Fig. 1Q and R; Table 2).

3.2.2.2. Spleen. Histology of spleen of control group showed normal arrangement of red and white pulp (Fig. 2A). Follicles in white pulp were well organized with distinct marginal zones and germinal centers (Fig. 2A). A dose-dependent decrease in follicle number was observed in olanzapine-exposed groups. Follicular size was reduced to various degrees with reduced germinal center (Fig. 2D, G and J); the marginal zone became almost indistinct in 8 and 10 mg/kg groups. Density of splenocytes was reduced in white pulp with darkly stained apoptotic lymphocytes (Fig. 2E, H and K). Size of splenocytes of both white pulp and red pulp was significantly reduced (Table 2). Area of red pulp was increased with more number of splenic sinuses and hematopoietic cells; more prominent in group exposed to 4 mg/kg olanzapine followed by 8 and 10 mg/kg groups (Fig. 2F, I and L). In contrast to olanzapine, histopathological alteration of spleen was less in risperidone-exposed groups (Fig. 2M-R). Number of follicles was decreased with reduced germinal centers in group exposed to 2 mg/kg risperidone (Fig. 2P-R). Follicle size was also reduced with decreased cell sizes of splenocytes (Table 2).

Table 2
Effects of olanzapine and risperidone exposures on lymphoid organs of mice neonates. (n = 11-14 pups/group).

Parameters	Experimental Groups					
	Control	4 mg/kg Olanzapine	8 mg/kg Olanzapine	10 mg/kg Olanzapine	1 mg/kg Risperidone	2 mg/kg Risperidone
Thymus Weight (mg/100 g)	522.77 ± 10.90	445.06 ± 4.63[a]	430.05 ± 4.53 [b]	423.53 ± 5.44 [b]	518.07 ± 19.9 [NS]	477.85 ± 14.5 [a]
Thymocyte size						
Cortex (μm)	3.67 ± 0.138	3.51 ± 0.123 [NS]	3.32 ± 0.142 [a]	2.85 ± 0.16 [b]	3.54 ± 0.188 [NS]	3.31 ± 0.115 [a]
Medulla (μm)	3.63 ± 0.225	3.54 ± 0.127 [NS]	3.29 ± 0.086 [a]	3.04 ± 0.10 [b]	3.48 ± 0.164 [NS]	3.28 ± 0.106 [NS]
Spleen Weight (mg/100 g)	521.70 ± 21.48	691.71 ± 12.48 [c]	650.27 ± 5.80 [c]	569.51 ± 13.48 [a]	550.12 ± 12.50 [NS]	486.50 ± 10.70 [a]
Splenocyte Size						
Follicle (μm)	4.05 ± 0.127	3.43 ± 0.142 [b]	3.37 ± 0.138 [b]	3.22 ± 0.101 [b]	3.77 ± 0.158 [a]	3.64 ± 0.112 [b]
Red pulp (μm)	4.01 ± 0.142	3.37 ± 0.101 [c]	3.26 ± 0.142 [c]	2.74 ± 0.161 [c]	3.41 ± 0.138 [c]	3.22 ± 0.141 [c]

[a], [b] and [c] showing significance from control at $P<0.05$, $P<0.01$ and $P<0.001$ respectively. NS Non significant.
(A single set of control data was given as there were no significant differences between the control data of both the drugs).

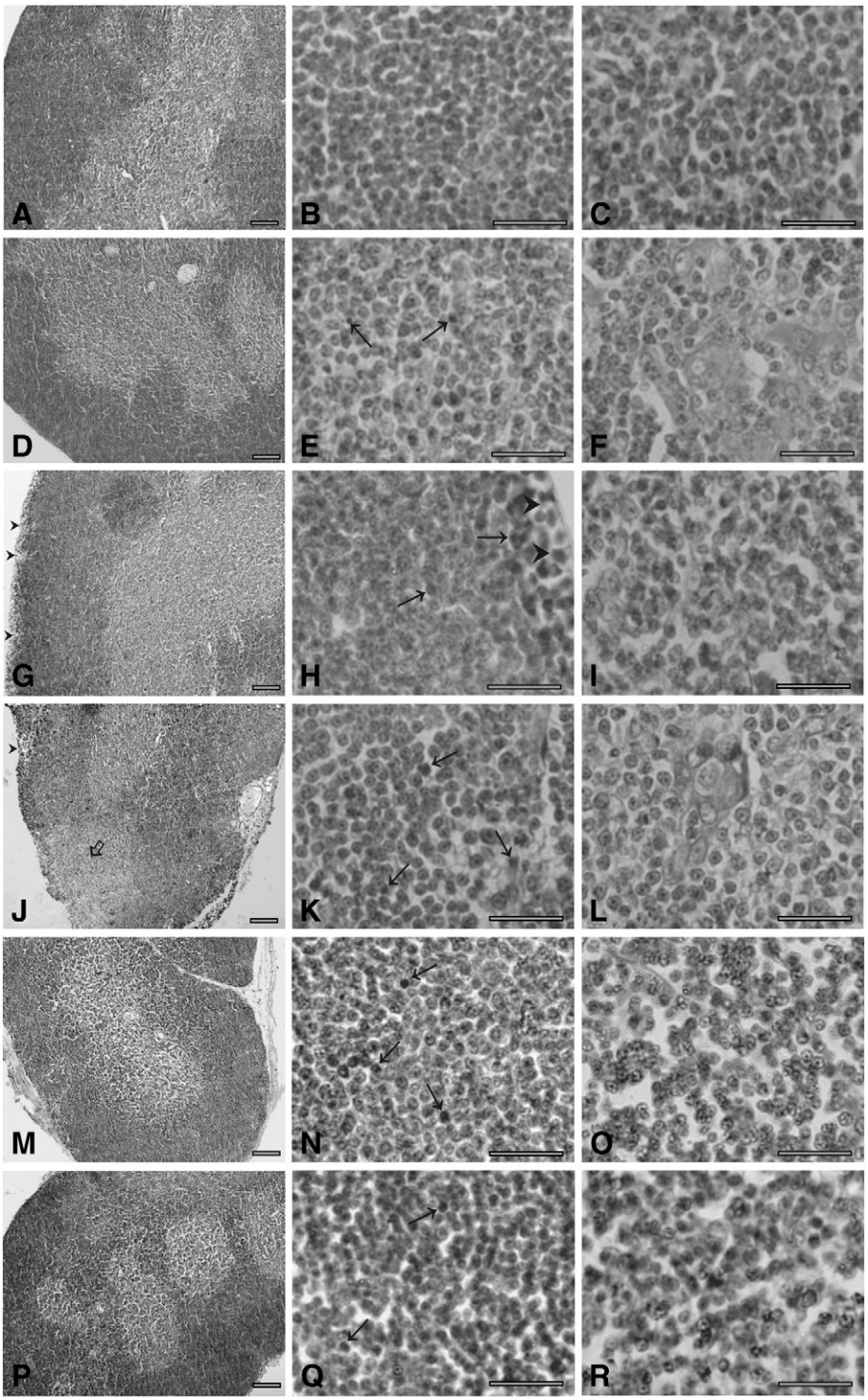

Fig. 1. Histopathology of thymus of olanzapine (4, 8 and 10 mg/kg) and risperidone-exposed (1 and 2 mg/kg) mice neonates. A. Cross section of thymus of control mice showing distinct demarcation between the cortex and medulla. Cortico-medullary junction diminished in a dose-dependent manner (Olanzapine, D: 4 mg/kg; G: 8 mg/kg; J: 10 mg/kg; Risperidone, M: 1 mg/kg; P: 2 mg/kg). Note loosening of capsular wall (arrowheads) and cortical necrosis (open arrow) in 8 and 10 mg/kg olanzapine groups. *Bar* 50 μm. Higher magnification of cortex and medulla showing the drug effect on thymocytes. Apoptotic cells (arrows) are more in cortex of exposed groups (Olanzapine, E: 4 mg/kg; H: 8 mg/kg; K: 10 mg/kg; Risperidone, N: 1 mg/kg; Q: 2 mg/kg) in comparison to control (B). *Bar* 20 μm.

3.3. Effect of atypical antipsychotic drugs on plasma corticosterone and prolactin

As no significant variation was found between different vehicle groups, data of these were pooled. Plasma corticosterone levels altered variably among the different experimental groups (Fig. 3A). Elevation in corticosterone level was significant ($P<0.05$ to $P<0.001$) both with 4 mg/kg (148 ± 12.63 ng/ml) and 8 mg/kg olanzapine (320 ± 28.5 ng/ml), about 3 folds from that of the control (110 ± 7.81 ng/ml) in the latter. On the contrary, plasma corticosterone level was less in group exposed to 10 mg/kg olanzapine (98 ± 14.0 ng/ml) as compared to control, though insignificant. Alteration in plasma corticosterone level at either of the risperidone doses (1 and 2 mg/kg) was not significant (Fig. 3A). Dose-dependent significant increase ($P<0.05$ to $P<0.001$) in

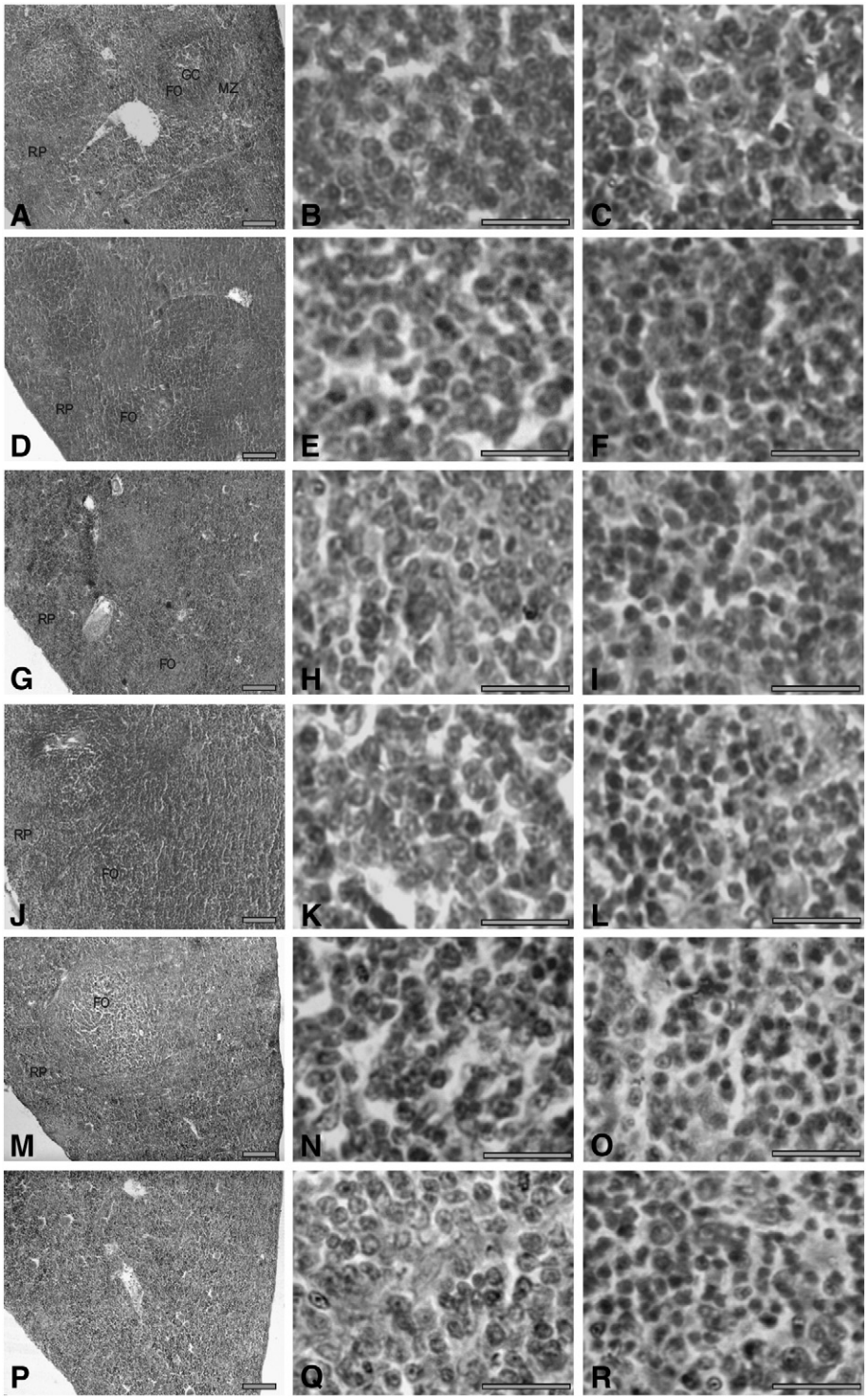

Fig. 2. Histopathology of spleen of olanzapine (4, 8 and 10 mg/kg) and risperidone-exposed (1 and 2 mg/kg) mice neonates. A. Cross section of spleen of control mice showing red pulp (RP) and well developed follicles (FO) with germinal centers (GC) and marginal zones (MZ) in white pulp (WP). Spleen of exposed groups showing reduction in follicular sizes due to reduced germinal centers and marginal zones (Olanzapine, D: 4 mg/kg; G: 8 mg/kg; J: 10 mg/kg; Risperidone, M: 1 mg/kg; P: 2 mg/kg). *Bar* 50 μm. Higher magnification of WP showing reduction in the density and size of splenocytes (Olanzapine, E: 4 mg/kg; H: 8 mg/kg; K: 10 mg/kg; Risperidone, N: 1 mg/kg; Q: 2 mg/kg) as compared to control (B). Note also the reduced size of splenocytes in RP of exposed groups with many aggregated erythropoietic cells (Olanzapine, F: 4 mg/kg; I: 8 mg/kg; L: 10 mg/kg; Risperidone, O: 1 mg/kg; R: 2 mg/kg). *Bar* 20 μm.

circulating prolactin levels were observed in the groups exposed to both olanzapine and risperidone (Fig. 3B). Plasma prolactin elevations of risperidone-exposed neonates (1 and 2 mg/kg) were more as compared to control ($P<0.001$) as well as from that of the groups exposed to 4 and 8 mg/kg olanzapine.

4. Discussion

A precise pathophysiologic understanding of the hematological side effects of antipsychotic drug is lacking; different possible mechanisms have been postulated (Oyesanmi et al., 1999; Pessina et al., 2006). The

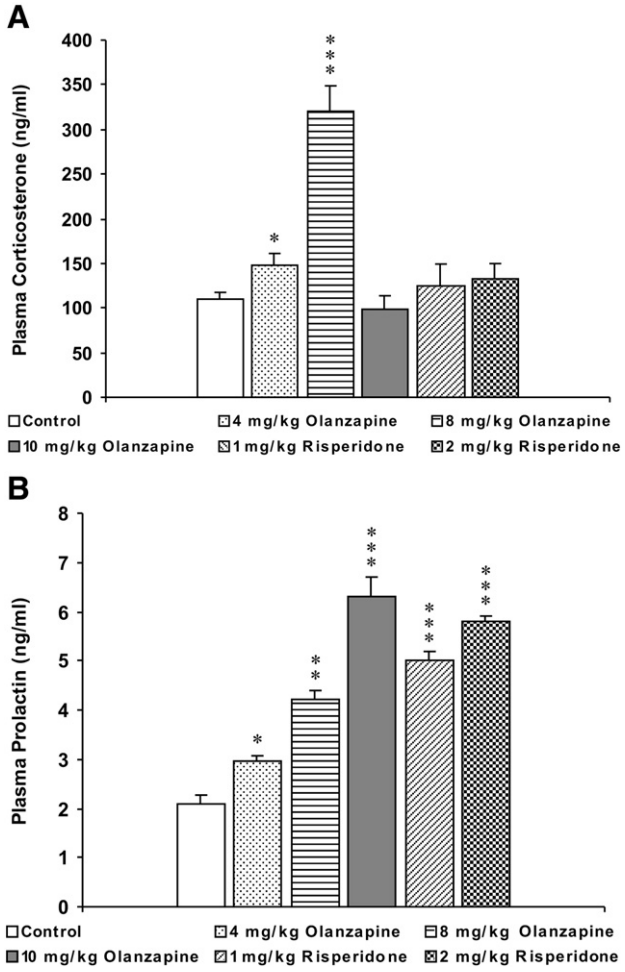

Fig. 3. Effect on plasma corticosterone (A) and prolactin (B) levels in mice neonates exposed to olanzapine (4, 8 and 10 mg/kg) and risperidone (1 and 2 mg/kg). Data are shown as Mean ± SEM of hormone content, n = 11-14 per group. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ significantly different compared to vehicle group.

present study demonstrates distinct hematological abnormalities in mice neonates exposed to atypical antipsychotics olanzapine and risperidone, to a lesser extent in the latter. In olanzapine-exposed male mice neonates a significant drop in white blood cell count was observed. Differential count of leukocytes explained this drop to significant reduction of the neutrophil percentage and neutrophil to lymphocyte ratio in peripheral blood. Significant decrease in neutrophil percentage and neutrophil/lymphocyte ratio also occurred in neonates exposed to 2 mg/kg risperidone. Bone marrow differential leukocyte count also revealed a dose-dependent decrease of the neutrophil percentage on olanzapine exposure, but not in either of the risperidone-exposed groups. There are reports of neutropenia and agranulocytosis mediated by atypical antipsychotic drugs including olanzapine and risperidone (Abdullah et al., 2003; Cadario, 2000; Finkel et al., 1998; Gajwani and Tesar, 2000, Lieberman and Tasman, 2006). As suggested by different workers, decrease in neutrophil count may be due to reduced production because of direct toxic effect of the drug on bone marrow suppression and/or due to increased peripheral destruction (Oyesanmi et al., 1999; Pessina et al., 2006). Some earlier studies on clozapine, chemically much similar to olanzapine (dibenzodiazepine), showed the influence of its unstable metabolites on agranulocytosis by inducing oxidative stress (Robinson, 2006; Williams et al., 1997). Both stable and unstable intracellular metabolites of atypical antipsychotic drugs, especially of clozapine and olanzapine, are reported to be generated in various cell types including granulocytes, bone marrow and liver cells (Maggs et al., 1995; Pirmohamed and Park, 1997). The neutropenia may also be due to the drug-mediated adverse impact on the immune system (Corzo et al., 1995; Turbay et al., 1997; Uetrecht, 1992; Williams et al., 2000; Yunis et al., 1995). Clozapine has been shown to cause agranulocytosis by affecting the expression of certain genes in major histocompatibility complex such as HLA (human leukocyte antigen), HSP 70 (heat shock protein) and TNF (tumor necrosis factor) genes (Corzo et al., 1995; Turbay et al., 1997; Uetrecht, 1992; Yunis et al., 1995).

The weight analysis and histopathology of thymus and spleen clearly demonstrated immunosuppressive effect of olanzapine on post-natal mice; the effects were less profound with risperidone. Significant reduction in thymus weight of olanzapine-exposed groups was reflected in the reduced cortex-medulla ratio and cortical hypocellularity. Hypocellularity of cortex might be due to the apoptosis in the cortical thymocytes as was reported earlier for dexamethasone and other drugs (Eichhorst et al., 2001; Elmore, 2006). Risperidone exposure induced decrease in thymus weight and reduced cellularity only at its higher dose (2 mg/kg). Relative spleen weight was increased in olanzapine-exposed groups, highest with lowest dose (4 mg/kg). The reduction in follicular size of white pulp along with reduced germinal centers as well as reduced splenocyte size indicated a state of immunosuppression as germinal centers of follicles are the sites of B-lymphocyte maturation. In contrast, the red pulp area was increased and characterized by the presence of more sinuses and much aggregated erythropoietic cells; more prominent in group exposed to 4 mg/kg followed by 8 and 10 mg/kg. Olanzapine-induced decrease of peripheral leukocytes might have stimulated hematopoiesis in the red pulp area and may be the reason of splenomegaly, although the size of splenocytes decreased significantly. With increase of the doses (8 mg/kg and 10 mg/kg) the response was compromised. Unlike olanzapine groups, the relative spleen weight was significantly reduced in neonates exposed to 2 mg/kg risperidone though an insignificant increase occurred with 1 mg/kg. The size of splenocytes also reduced in both the risperidone-exposed groups. The exact mechanism of drug-induced negative impact on lymphoid organs thymus and spleen is difficult to predict at this level of the investigation. This may be due to direct toxic impact of the drugs on the immune system as discussed before with regard to drug-mediated neutropenia (Corzo et al., 1995; Turbay et al., 1997; Uetrecht, 1992; Williams et al., 2000; Yunis et al., 1995). In addition, it might be due to drug-induced indirect pharmacologic effect on endocrine system. Immunosuppression could be well correlated to hypercorticosteronemia in olanzapine-exposed groups as corticosterone level increased significantly at two of its lower doses (4 and 8 mg/kg). The adrenal glucocorticoid, corticosterone, is invariably being associated with immunosuppression (Besedovsky and Rey, 2007; Webster et al., 2002). There are reports of elevation of serum corticosterone on olanzapine and risperidone exposure, more with the former than latter (Assié et al., 2008; Baptista et al., 2002; Marx et al., 2003). Marx and co-workers (Marx et al., 2003) reported elevation of serum corticosterone levels upto 10 and 3.7 folds after acute treatment with equivalent doses of olanzapine (10 mg/kg) and risperidone (1 mg/kg) used in the present study. Blood corticosterone elevation by atypical antipsychotic drugs is attributed to their binding affinity towards serotonin (5HT) receptors (Contesse et al., 2000). Olanzapine shows comparatively higher affinity for 5-HT_{1A} receptors than risperidone (Bubeníková et al., 2005), may be a possible reason behind significant elevation in corticosterone level with olanzapine (4 and 8 mg/kg) not with risperidone. The plasma corticosterone levels with 10 mg/kg of olanzapine remained unchanged (an insignificant decrease). The long term exposure to the high dose of olanzapine might have compromised the secretory response of hypothalamic-pituitary-adrenal axis. Immunosuppressiveness observed in 10 mg/kg olanzapine group and both the risperidone groups (1 and 2 mg/kg) could not be linked to corticosterone. Negative impact of these drug doses in the lymphoid organs might be to some extent due to drug-induced hyperprolactinemic effect in addition to the direct toxicity. Among atypical antipsychotic drugs, risperidone is most

hyperprolactinemic (Byerlya et al., 2006; Cutler, 2003; Jayaram et al., 2007; Kinon et al., 2006; Konarzewska et al., 2009) due to its high affinity for D_2 receptors at tuberoinfundibular dopaminergic pathway (Schotte et al., 1996). Though olanzapine generally considered as a prolactin sparing drug, our earlier studies and some others reported PRL elevation (Kapur et al., 1998; Mishra and Mohanty, 2009, in press; Rourke et al., 2006; van Bruggen et al., 2009; Wudarsky et al., 1999). It was suggested that physiological levels of circulating prolactin is essential to maintain normal immunocompetence and both hypo- and hyperprolactinemia are immunosuppressive (Gerli et al., 1987; Matera, 1997; Vidaller et al., 1986, 1992). Hyperprolactinemia-induced negative impact on various haematopoietic cells such as T-cell dysregulation and inhibition of natural killer cells (Gerli et al., 1987; Vidaller et al., 1986, 1992) are reported. Other studies are also there where hyperprolactinemia was linked to autoimmune diseases (Mcmurray, 2001; Orbach and Shoenfeld, 2007). Adverse impact on thymus and spleen in groups exposed to high dose of olanzapine and both the risperidone doses may therefore be induced by pronounced hyperprolactinemic effect of the drugs, if not for corticosterone.

Thus, the olanzapine and risperidone-induced hematological abnormalities and adverse impact on lymphoid organs of mice neonates might be the result of direct toxic impacts on the respective components and/or the indirect effects mediated by endocrine dysregulations. More preclinical investigations are needed for better understanding of the mechanisms.

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