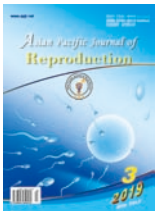

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Tinospora cordifolia attenuates antipsychotic drug induced hyperprolactinemia in Wistar rats

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ABSTRACT

Objective: To evaluate the anti-hyperprolactinemic effect of methanolic extract of *Tinospora cordifolia* against antipsychotic/neuroleptic drug induced hyperprolactinemia.

Methods: A total of 48 Wistar albino rats were chosen in the study. To induce hyperprolactinemia, haloperidol at 5 mg/kg/day was intraperitoneally administered for 16 continuous days and sulpiride at 20 mg/kg/day was administered intraperitoneally for 28 continuous days. Methanolic extract of *Tinospora cordifolia* at 200 mg/kg/day and 400 mg/kg/day were administered orally 30 min before administration of haloperidol and sulpiride for 16 and 28 days, respectively. Then, we had evaluated prolactin, dopamine and antioxidant status in the treatment group as compared to haloperidol and sulpiride.

Results: There was a significant (P<0.05) increase in serum prolactin level and decrease in dopamine level in the haloperidol and sulpiride treated animals. However, methanolic extract of *Tinospora cordifolia* significantly (P<0.05) decreased serum prolactin level and increased brain dopamine level. Further, superoxide dismutase and catalase level were also decreased significantly in the haloperidol and sulpiride treated groups as compared to those of the control group and the antioxidant status was restored significantly on treatment with methanolic extract of *Tinospora cordifolia*. Furthermore, methanolic extract of *Tinospora cordifolia* also reduced total leukocyte count, and increased red blood cell count and hemoglobin concentration. In addition, the spleen did not show signs of infection or inflammation in the experiments.

Conclusions: Methanolic extract of *Tinospora cordifolia* has a significant anti-hyperprolactinemic effect which may be attributed to neuroprotective and antioxidant effects of its signature constituents like stepharanine.

1. Introduction

Hyperprolactinemia is a much over-looked, undesirable side effect which comes into action with the use of typical antipsychotic drugs such as haloperidol, chlorpromazine, loxapine, thioridazine, and fluphenazine as well as with the usage of atypical antipsychotic drugs such as clozapine, olanzapine, amisulpride, lesuride, sulpiride, aripiprazole, and risperidone[1-5]. Apart from the pituitary disorder, hypothyroidism also contributes towards development of hyperprolactinemia[6,7]. It has also been noticed that prolonged exposure of estradiol decreases dopamine resulting in oxidative stress which leads to development of hyperprolactinemia[8,9].

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In hyperprolactinemia, dopamine agonist like bromocriptin is considered to be the drug of choice which considerably reduces the amount of prolactin released. It basically acts by decreasing the size of lactotroph cells of the anterior pituitary gland. Similarly, cabergoline is another drug of preference for the treatment of hyperprolactinemia[10–12]. However, usage of dopamine receptor agonist has a grey side as it manifests to development of mental fogginess and postural hypotension which limits their therapeutic utility which cannot be ignored or subsided[13,14]. Thus, there is a high need of development of suitable drug therapy which can minimize the adverse incident associated with anti-psychotic drugs.

In the recent years, many researchers have been making series of efforts towards identifying and developing suitable phytomedicines for neuroprotection. Indeed, our mighty nature comes with a bunch of medicinal plants that are documented to be a good source of potential antioxidants that can protect brain from oxidative stress[15–17]. Hence, it's high time we should start unfolding these new drug therapies to counter hyperprolactinemia. *Tinospora cordifolia* (*T. cordifolia*) is an important medicinal plant of Indian system of medicine, which possesses anti-diabetic, anti-asthmatic, anti-toxin, anti-gout and anti-diarrhoeal, antileprotic, anticancer, antioxidant, antimicrobial, immuno modulatory, anti-osteoprotic, hepato-protective and neuroprotective activity[18]. Previous findings on *T. cordifolia* showed that in addition to providing neuroprotection, it also works towards providing protection against neurodegeneration of dopaminergic neurons[19,20]. There are limited literature on anti-hyperprolactinemia effect of *T. cordifolia*. So, an effort has been made to explore the effect of *T. cordifolia* in the management of antipsychotic-drug induced hyperprolactinemia.

2. Materials and methods

2.1. Animals for experiment

A total of 48 Wistar albino rats, both male and female rats weighing (120±5) g, were selected randomly from animal house, School of Pharmaceutical Sciences, Siksha O Anusandhan University, Bhubaneswar, India. The room temperature was maintained at (22±2) ℃ with food and water *ad libitum*. The animals were transferred to the laboratory at least 1 h prior to performing the experiment. The experiments were performed during day time (08:00-16:00). The study was conducted according to Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines after due approval of the experimental protocol from Institutional Animal Ethics Committee, Siksha O Anusandhan University, India (Reg. No. 1171/PO/Re/S/08/CPCSEA).

2.2. Plant collection

Fresh stem of *T. cordifolia* plants were collected locally from Bilaspur Chhattisgarh, India. The plant was collected during day time.

2.3. Plant authentication

The plants were identified by Dr. Panda PC, principal scientist of Regional Plant Resource Center, Bhubaneswar, Odisha, India (accession field No. PT-01 *T. cordifolia*). The authenticated plant was kept in the herbarium file for record purpose.

2.4. Preparation of extract

The stem of *T. cordifolia* was dried under shade and then ground into a fine powder. The dried powder (250 g) of stem was extracted with methanol in a soxhlet apparatus at 60-70 ℃ each for 10-12 h consecutively. The dried extract was stored in sterile amber color air tight container in refrigerator until its use in experiment[21].

2.5. Preliminary phytochemical screening

The crude extracts of *T. cordifolia* were subjected to different chemical tests for the detection of phytoconstituents such as tannins, alkaloids, saponins, phenolics, flavonoids, glycosides, triterpenoids, steroids, volatile oils using standard method[22].

2.6. Acute toxicity study

Acute oral toxicity was in compliance with the Organization of Economic Cooperation and Development guidelines 420. Sighting study was done using a single fasted female rat per dose to select the dose for main study. After administration of METC [(50, 300, 2 000 mg/kg, orally (*p.o.*)] animals were observed for first 30 min and subsequently for 4 h, and then periodically during the first 24 h. A total of three animals were used in the sighting study. If no signs of toxicity or mortalities observed on rats within the first 24 h, then main study was conducted with the highest safe dose where 4 more rats (2 female rats and 2 male rats) were taken in addition to the animal used in sighting study. A total of seven animals were used in the acute toxicity study. The methanolic extract of *T. cordifolia* (METC) was administred and the animals were observed closely for body weight, signs of toxicity and mortality for next 14 days.

2.7. Drug and treatment

Haloperidol (HiMedia Laboratories Pvt. Ltd, Mumbai, India) at 5 mg/10 mL/kg/day for 16 days and sulpiride (Unimed Technologies Ltd, Panchmahal, India) at 20 mg/10 mL/kg/day for 28 days were administered intraperitoneally (*i.p.*). Plant extracts were administered *p.o.* daily 30 min prior to the administration of haloperidol/sulpiride[23,24].

2.8. Serum collection and storage

Blood samples were collected by cardiac puncture. Serum samples were separated by centrifugation, frozen and stored at -4 ℃ until use. Prolactin hormone was estimated by enzyme immune assay method using rat prolactin kit (Erba Lachema s.r.o., Czech Republic)[25].

2.9. Pharmacological evaluation

No mortality was observed up to a dose of 2 000 mg/kg. So we used two doses 200 mg/kg and 400 mg/kg which wass in agreement with earlier studies. The METC was tested against haloperidol (5 mg/kg) and sulpiride (20 mg/kg) induced hyperprolactinemia.

2.9.1. Haloperidol induced hyperprolactinemia

Twenty four Wistar albino rats were divided into four groups, with six rats (including three male and three female rats) in each group. Group Ⅰ (control group) received saline at 2 mL/kg/day, Group Ⅱ received haloperidol at 5 mg/kg/day, Group Ⅲ received haloperidol at 5 mg/kg/day + METC at 200 mg/kg/day and Group Ⅳ received haloperidol at 5 mg/kg/day + METC at 400 mg/kg/day. The haloperidol was administred *i.p.* once daily (8:00 to 10:00 am) for 16 continuous days, whereas METC was administred *p.o.* once daily respectively[23]. On the 17th day, blood was drawn by cardiac puncture for measurement of prolactin level, and the same animals were sacrificed to isolate brain. The brain homogenate was used to estimate the dopamine and antioxidant status.

2.9.2. Sulpiride induced hyperprolactinemia

Similarly, another twenty four Wistar albino rats were divided into four groups, with six rats (including three male and three female rats) in each group. Group A (control) received saline at 2 mL/kg/day, Group B received sulpiride at 20 mg/kg/day, Group C received sulpiride at 20 mg/kg/day+METC at 200 mg/kg/day, and Group D received sulpiride at 20 mg/kg/day+METC 400 mg/kg/day. Sulpiride was administred *i.p.* once daily (8 am to 10 am) for 28 continuous days, whereas METC was administred *p.o.* once daily respectively[24]. On the 29th day, blood was drawn by cardiac puncture for measurement of prolactin level, and the same animals were sacrificed to isolate brain. The brain homogenate was used to estimate the dopamine and antioxidant status.

2.10. Dopamine estimation

The entire brain of a rat was homogenized for about 1 min in hydrogen chloride-butanol (1:10) mixture and subjected to centrifugation at 3 000 rpm for about 10 min. And 1.0 mL of aliquot supernatant phase was removed followed by its addition to centrifuge tube comprising 2.5 mL hexane and 0.3 mL of 0.1 M hydrogen chloridel. The dopamine assay was then performed with spectrofluorimeter at 330-375 nm[26].

2.11. Assessment of antioxidant status

2.11.1. Assay of superoxide dismutase

The whole brain was homogenized by addition of 1 mL 0.01 M sodium phosphate buffer at pH=7.0. The homogenate was centrifuged at 10 000 rotation per minute for 15 min at 4 ℃. Thereafter supernatant was collected and stored at 80 ℃ to estimate superoxide dismutase content. Variation in absorbance was observed at 420 nm by using JASCO (V-630) UV spectrophotometer against reagent blank[27,28] and brain antioxidant status was determined.

2.11.2. Assay of catalase

The whole brain tissue was homogenized and about 20 μL tissue supernatant was added to the 980 μL of the assay mixture consisting of 900 μL of 10 mmol/L of H_2O_2, 50 μL of tris hydrogen chloride buffer (pH-8) and 30 μL of distilled water. The variation in absorbance was then measured at 240 nm. The results were expressed as mmol/min/mg of protein[29].

2.12. Haematological studies

Blood was collected in tubes containing ethylene diamine tetraacetic acid as an anticoagulant and the blood analysis was carried out by hematological autoanalyzer for measurement of red blood cell count, total leukocyte count, hemoglobin, packed cell volume, platelet, and mean corpuscular volume. The weight of spleen was also measured[30,31].

2.13. Isolation of stepharanine

About 20 g of METC was dissolved in chloroform and ethanol (2:8). The solution was then dried and evaporated up to a pale yellow colour viscous residue (1.7 g). Further the residue was placed on a silica gel column and eluted with chloroform and gradually enriched with ethanol to afford three fraction. Optimised fraction was to be eluted with chloroform and ethanol. This process was repeated and subjected to silica gel column chromatography to keep single compound (750 mg)[32,33]. This material was further purified by recrystallization with ethanol to yield small needle shape crystal of stepharanine (99% purity).

2.14. High performance liquid chromatography/mass spectrometry (LC/MS)

LC/MS analysis was carried out using Agilent Technologies (6545 Q-TOF LC/MS) instrument at the Central Instrumentation Facility, Birla Institute of Technology and Science, Pilani, India.

2.15. Histopathological examination of pituitary gland, adrenal gland and spleen

Animals from each group were sacrificed 24 h after last treatment (on 17th or 29th day) following ethical procedure. For histopathological examination, pituitary gland, adrenal gland and spleen were separated and stored in 10% formalin solution. Samples embedded in paraffin wax were used for a serial section at 5 μm and stained with hematoxylin-eosin and mounted on a glass slide for microscopic evaluation[34].

2.16. Statistical analysis

Data were presented as mean ± standard deviation (mean ± SD). One-way analysis of variance followed by *post hoc* Tukey's *t*-test was applied. $P<0.05$ was considered for statistical analysis.

3. Results

3.1. Anti-hyperprolactinemic effect

Prolactin level in the control group was (10.930±0.008) ng/mL which was significantly (P<0.05) increased to (25.140±0.009) ng/mL and (19.820±0.010) ng/mL respectively after administration of haloperidol (5 mg/kg/day for 16 days) and sulpiride (20 mg/kg/day for 28 days). Administration of METC at 200 mg/kg/day and 400 mg/kg/day significantly (P<0.05) decreased prolactin level to (14.610±0.010) ng/mL and (13.820±0.010) ng/mL respectively in haloperidol induced hyperprolactinemic rats and to (14.450±0.010) ng/mL and (12.790±0.008) ng/mL respectively in sulpiride induced hyperprolactinemic rats (Figure 1).

3.2. Dopaminergic action

Dopamine level in control rat brain was (38.270±0.011) U/g which significantly (P<0.05) decreased dopamine level to (11.560±0.010) U/g and (14.170±0.010) U/g respectively after administration of haloperidol (5 mg/kg/day for 16 days) and sulpiride (20 mg/kg/day for 28 days). Furthermore, administration of METC at 200 mg/kg/day and 400 mg/kg/day showed significant (P<0.05) increase in dopamine level to (32.170±0.007) U/g and (35.190±0.019) U/g respectively in haloperidol induced hyperprolactinemic rats and to (32.100±0.126) U/g and (34.450±0.010) U/g respectively in sulpiride induced hyperprolactinemic rats (Figure 2).

3.3. Antioxidant status of superoxide dismutase and catalase

Antioxidant status of METC was also evaluated against haloperidol and sulpiride induced hyperprolactinemia. In our study, we have seen that superoxide dismutase level was (11.280±0.011) μ/mg protein and catalase level was (2.080±0.009) μ/mg protein in brain of control rat. Prolonged administration of haloperidol significantly (P<0.05) decreased superoxide dismutase level to (5.280±0.008) μ/mg protein and catalase level to (0.660±0.008) μ/mg protein. Administration of sulpiride also significantly (P<0.05) dropped the level of superoxide dismutase down to (6.320±0.012) μ/mg protein and catalase to (0.850±0.007) μ/mg protein. On the other hand,

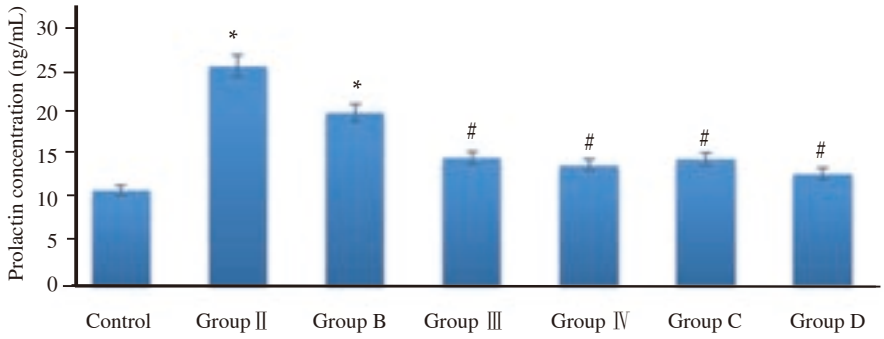

Figure 1. Effect of haloperidol, sulpiride and methanolic extract of *T. cordifolia* on blood serum prolactin level in albino rats. The control group receive saline at 2 mL/kg/d, Group Ⅱ receive haloperidol at 5 mg/kg/d, Group B receive sulpiride at 20 mg/kg/d, Group Ⅲ receive haloperidol at 5 mg/kg/d + methanolic extract of *T. cordifolia* at 200 mg/kg/d, Group Ⅳ receive haloperidol at 5 mg/kg/d + methanolic extract of *T. cordifolia* at 400 mg/kg/d, Group C receive sulpiride at 20 mg/kg/d + methanolic extract of *T. cordifolia* at 200 mg/kg/d, and Group D receive sulpiride at 20 mg/kg/d + methanolic extract of *T. cordifolia* 400 mg/kg/d. Data are expressed as mean ± SD; n=6; *P<0.05 (the control *vs*. Group Ⅱ and Group B), #P<0.05 (Group Ⅱ, Group B *vs*. Group Ⅲ, Group Ⅳ, Group C, and Group D).

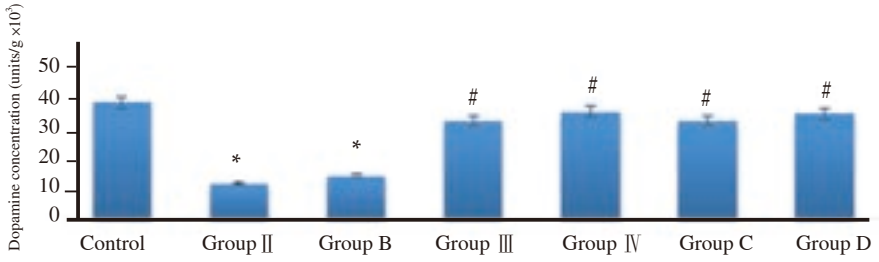

Figure 2. Effect of haloperidol, sulpiride and methanolic extract of *T. cordifolia* on brain neurotransmitter levels (dopamine) in albino rats. Data are expressed as mean ± SD; n=6. One way analysis of variance is followed by *post hoc* Tukey's test. *P<0.05 (the control *vs*. Group Ⅱ and Group B), #P<0.05 (Group Ⅱ, Group B *vs*. Group Ⅲ, Group Ⅳ, Group C, and Group D).

in experiment number one (haloperidol induced hyperprolactinemia in rats), administration of METC at 200 mg/kg/day and 400 mg/kg/day significantly (P<0.05) increased superoxide dismutase level to (8.540±0.010) μ/mg protein and (9.860±0.040) μ/mg protein respectively, and also increased catalase level to (0.940±0.007) μ/mg protein and (1.150±0.007) μ/mg protein respectively. In experiment number two (sulpiride induced hyperprolactinemia in rats), when METC at 200 mg/kg/day and 400 mg/kg/day were administratred, superoxide dismutase level was increased to (7.930±0.007) μ/mg protein and (8.960±0.008) μ/mg protein respectively and catalase level was increased to (0.980±0.004) μ/mg protein and (1.220±0.008) μ/mg protein respectively (Figure 3).

3.4. Hematological evaluation

Haloperidol and sulpiride induced groups showed significant decrease in red blood cell count and hemoglobin count whereas administration of METC at 200 mg/kg/day and 400 mg/kg/day showed significant increase in red blood cell count and hemoglobin count as compared to those of haloperidol and sulpiride induced groups. Further, haloperidol and sulpiride groups showed a significant increase in total leukocyte count, packed cell volume, platelet count and mean corpuscular volume. On the other hand, METC at 200 mg/kg/day and METC at 400 mg/kg/day showed a significant drop in total leukocyte count, packed cell volume, platelet, and mean corpuscular volume as compared to those of haloperidol and sulpiride induced groups (Table 1).

3.5. Phytochemistry

Alkaloid was found to be present in METC after phytochemical screening. LC/MS of crude extract was also done and then isolation was performed. Isolated compound was subjected to LC/MS revealed presence of stepharanine. The molecular weight of stepharanine was 324.35.

3.6. Acute toxicity study

The animals behaved normally during and after the observation period of 14 days. There were no signs of abnormality and toxicity even after administration of a single dose of 2 000 mg/kg METC during the observation period.

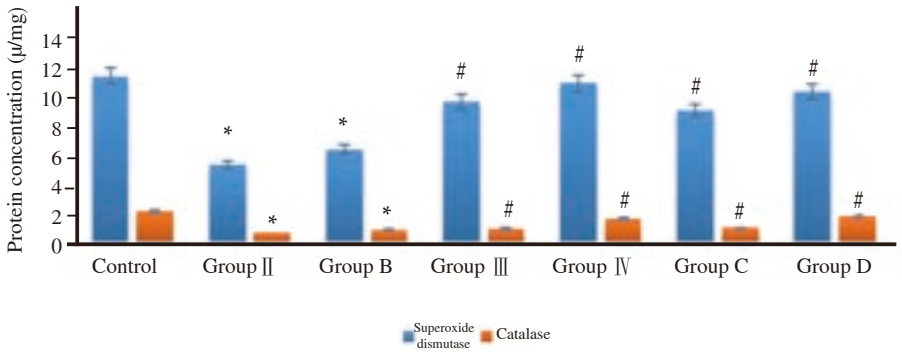

Figure 3. Effect of haloperidol, sulpiride and methanolic extract of *T. cordifolia* on brain antioxidant status in albino rats. Data are expressed as mean ± SD; n=6. One way analysis of variance is followed by *post hoc* Tukey's test. *P<0.05 (the control *vs*. Group Ⅱ and Group B), #P<0.05 (Group Ⅱ, Group B *vs*. Group Ⅲ, Group Ⅳ, Group C, and Group D).

Table 1. Effect of methanolic extract of *T. cordifolia* on hematological parameters in haloperidol and sulpiride treated albino rat.

Groups	Red blood cell count ($\times10^3$cell/μL)	Total leukocyte count ($\times10^3$cell/μL)	Hemoglobin (g/dL)	Packed cell volume (%)	Platelet ($\times10^9$/L)	Mean corpuscular volume (liters/cell)	Spleen weight (g)
Control	7.330 ± 0.008	3.720 ± 0.008	14.360 ± 0.008	43.440 ± 0.008	1.420 ± 0.006	59.330 ± 0.006	0.777 ± 0.006
Group Ⅱ	6.190 ± 0.008*	6.920 ± 0.006*	12.620 ± 0.006*	44.830 ± 0.008*	2.130 ± 0.006*	61.780 ± 0.006*	0.870 ± 0.006*
Group B	6.240 ± 0.008*	6.850 ± 0.006*	12.660 ± 0.008*	44.720 ± 0.006*	2.070 ± 0.008*	61.630 ± 0.008*	0.850 ± 0.004*
Group Ⅲ	6.790 ± 0.004**	4.990 ± 0.008**	13.400 ± 0.008**	44.170 ± 0.008**	1.670 ± 0.008**	60.510 ± 0.008**	0.817 ± 0.004**
Group Ⅳ	7.110 ± 0.004**	4.670 ± 0.008**	13.950 ± 0.008**	43.770 ± 0.008**	1.500 ± 0.008**	59.810 ± 0.008**	0.784 ± 0.006**
Group C	6.870 ± 0.008**	5.120 ± 0.006**	13.370 ± 0.008**	44.270 ± 0.008	1.650 ± 0.001**	59.660 ± 0.008**	0.812 ± 0.008**
Group D	7.110 ± 0.008**	4.680 ± 0.008**	13.930 ± 0.008**	43.900 ± 0.008**	1.470 ± 0.001**	59.410 ± 0.008**	0.637 ± 0.004**

Data are expressed as mean ± SD; n=6; *P<0.05 (the control *vs*. Group Ⅱ and Group B); **P<0.05 (Group Ⅱ, Group B *vs*. Group Ⅲ, Group Ⅳ, Group C, and Group D).

3.7. Histopathological examination

3.7.1. Adrenal gland

METC treated groups were found to have the same cytoarchitecture profile with well-developed cortex and medulla (Figure 4).

3.7.2. Pituitary gland

Section of pituitary tissue showed abnormal brain cytoarchitecture in haloperidol (16 days) and sulpiride (28 days) treated rats. Hydrophilic cells were found normal in control as well as METC treated groups, whereas sulpiride at 20 mg/kg/day treated group showed reversible changes in the hydrophilic cells, and haloperidol at 5 mg/kg/day treated group showed atrophy of the cells which were irreversible (Figure 5). Both haloperidol and sulpiride treated group showed dilated capillary, injured neuronal fibres, irregular acidophil, and basophils cells, and reversible hypertrophic changes in the anterior pituitary gland.

3.7.3. Spleen

The spleen was degraded and damaged with a muddy appearance in haloperidol and sulpiride treated groups. METC at 400 mg/kg/day treated groups showed healthy spleen with brownish reddish appearance. There was moderate to marked increase in the proportion of white pulp (Figure 6).

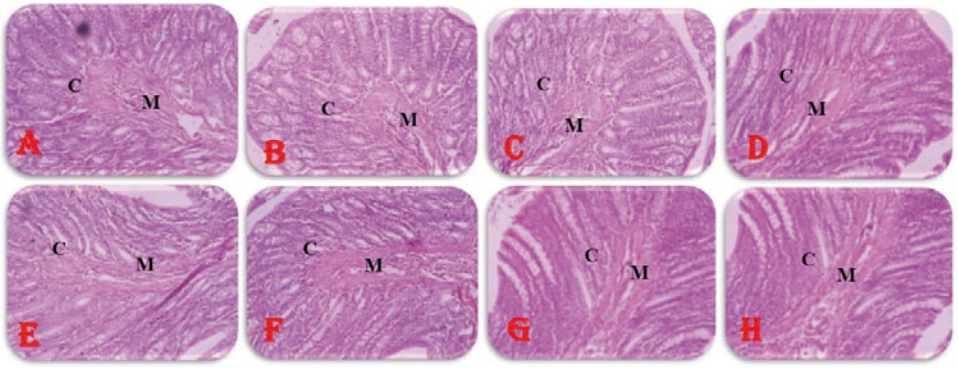

Figure 4. Histopathological examination of adrenal gland (hematoxylin-eosin, 100 ×). A: (the control group) saline at 2 mL/kg/d; B: haloperidol (5 mg/kg/d) for 16 days; C: haloperidol at 5 mg/kg/d + methanolic extract of *T. cordifolia* at 200 mg/kg/d; D: haloperidol at 5 mg/kg/d + methanolic extract of *T. cordifolia* at 400 mg/kg/d; E: (the control group) saline at 2 mL/kg/d; F: sulpiride at 20 mg/kg/d for 28 days; G: sulpiride at 20 mg/kg/d + methanolic extract of *T. cordifolia* at 200 mg/kg/d; H: sulpiride at 20 mg/kg/d + methanolic extract of *T. cordifolia* at 400 mg/kg/d. M = medulla and, C = cortex.

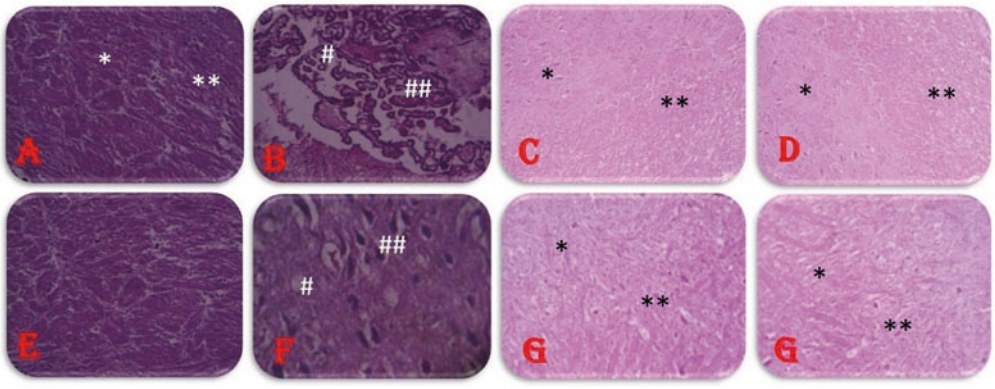

Figure 5. Histopathology of anterior pituitary gland (hematoxylin-eosin, 100 ×). A: (the control group) saline at 2 mL/kg/d; B: haloperidol (5 mg/kg/d) for 16 days; C: haloperidol at 5 mg/kg/d + methanolic extract of *T. cordifolia* at 200 mg/kg/d; D: haloperidol at 5 mg/kg/d + methanolic extract of *T. cordifolia* at 400 mg/kg/d; E: (the control group) saline at 2 mL/kg/d; F: sulpiride at 20 mg/kg/d for 28 days; G: sulpiride at 20 mg/kg/d + methanolic extract of *T. cordifolia* at 200 mg/kg/d; H: sulpiride at 20 mg/kg/d + methanolic extract of *T. cordifolia* at 400 mg/kg/d. *: indicates normal capillary, neuronal fibres and regular acidophil cell; **: indicates basophils cell; #: indicates dilated capillary, injured neuronal fibres, irregular acidophil cell; and ##: indicates minor basophils cells.

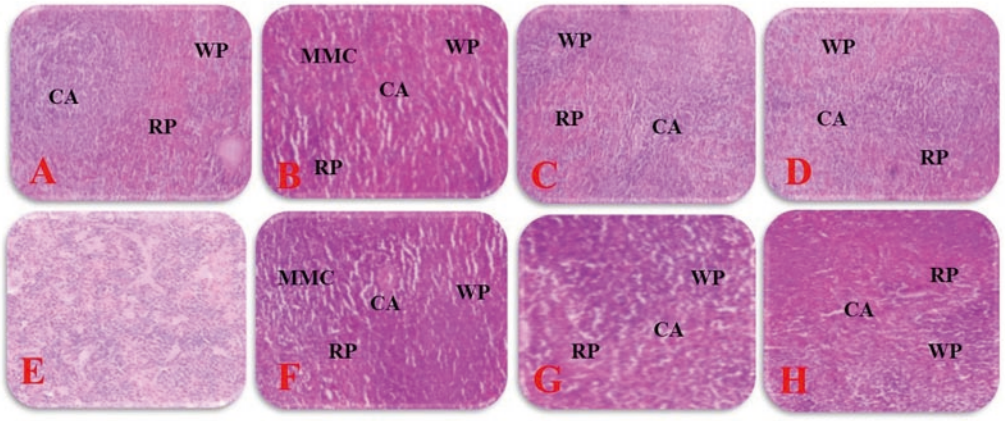

Figure 6. Histopathology of spleen (hematoxylin-eosin, 100 ×). A: (the control group) saline at 2 mL/kg/d; B: haloperidol (5 mg/kg/d) for 16 days; C: haloperidol at 5 mg/kg/d + methanolic extract of *T. cordifolia* at 200 mg/kg/d; D: haloperidol at 5 mg/kg/d + methanolic extract of *T. cordifolia* at 400 mg/kg/d; E: (the control group) saline at 2 mL/kg/d; F: sulpiride at 20 mg/kg/d for 28 days; G: sulpiride at 20 mg/kg/d + methanolic extract of *T. cordifolia* at 200 mg/kg/d; H: sulpiride at 20 mg/kg/d + methanolic extract of *T. cordifolia* at 400 mg/kg/d. RP = red pulp, WP = white pulp, CA = central artery, and MMC = melano macrophage centre.

4. Discussion

Neuroleptic drugs are primarily used for the treatment of psychosis. Repeated administration of antipsychotic drugs inhibits the dopamine synthesis which results in hyperprolactinemia[5,35,36]. Hyperprolactinemia is one of the important adverse incident of neuroleptic-drugs which restricts their therapeutic usefulness. This is the basis of animal models using neuroleptic drugs to induce hyperprolactinemia. Haloperidol at 5 mg/kg/day continuously for 16 days and sulpiride at 20 mg/kg/day continuously for 28 days significantly (P<0.05) increased serum prolactin level which justified our prior work[23,24].

Two doses of METC (200 mg/kg & 400 mg/kg) were selected based on earlier studies. Our acute toxicity study is also supported the selected doses[37]. METC significantly reduced hyperprolactinemia in both the models. However, no dose dependency was seen. METC (200 mg/kg & 400 mg/kg) showed significant increase in the dopamine level in haloperidol (5 mg/kg) and sulpiride (20 mg/kg) treated rats. The increase in dopamine concentration could be due to the active component like stepharanine. Stepharanine present in METC acts as dopamine agonist which inhibited the prolactin release, increased biosynthesis of dopamine or decreased metabolism of dopamine[38,39]. So, METC prevents loss of dopaminergic neurons which may lead to increased dopaminergic activity. However, this effect can also be mediated by neuronal nicotinic acetylcholine receptors which modulate/regulate nigrostriatal and mesolimbic dopaminergic pathways[40]. Antipsychotic drugs block the dopamine release in thetuberoinfundibular pathway which can lead to elevation of blood prolactin. The earlier finding revealed that stepharanine acts on the tuberoinfundibular pathway. It is one of the major dopamine pathway in the brain. So, stepharanine may be increasing dopamine release at this site to regulate prolactin level[41].

Further, previous findings showed that antipsychotic drugs causing prolactin elevation and decreasing dopamine may lead to stimulation of superoxide dismutase, catalase as well as reactive oxygen species level thereby causing oxidative stress[42–44]. Our study is in agreement with this as administration of haloperidol and sulpiride decreases superoxide dismutase and catalase level. METC with antioxidant effects improves neuroprotection in brain of haloperidol and sulpiride induced hyperprolactinemic rats[20,45].

There are certain neuroleptic drugs which are associated with modulation in hematological toxicity (neutropenia, eosinophilia, thrombocytopenia and anemia). These are infrequent but probably life-threatening adverse incident of tranquilizers[46]. Hematological variation might be due to dose dependent and some other immunological factors[47,48]. Hyperprolactinemia may also be associated with central nervous system inflammation[49]. Recent evidence suggested a link between central nervous system inflammation and the autonomic release of pro-inflammatory cytokines by resident macrophages in the spleen. This phenomenon known as brain spleen inflammatory coupling. Again spleen is a major marker of inflammation[50]. So to access inflammatory state of central nervous system and to correlate with haematological changes, histology of spleen was taken into account.

There significant decrease in red blood cell count, hemoglobin and increase in total leukocyte count, packed cell volume, platelet, mean corpuscular volume, and spleen weight as compared to haloperidol and sulpiride treated groups, which may be attributed to damage or infection or inflammation of spleen[51]. Hence, METC may possess remarkable neuro-antiinflammatory effect in haloperidol and sulpiride induced hyperprolactinemia. Stepharanine is used in neuroinflammatory diseases like Alzheimers disease because of its neuro-antiinflammatory property and neuroprotective effect[52]. The anti-hyperprolactinemic effect of METC may be attributed to the neuroprotective action of stepharanine. Further studies are needed to validate the efficacy of *T. cordifolia* in psychiatric patients.

Conflict of interest statement

The authors declare no conflict of interest in any form.

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