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, lespiride, 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].
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 fogliness 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, hepatoprotective 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) °C 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 °C 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 administrated 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 sulphiride (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/ sulphiride[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 °C 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 was 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 administered i.p. once daily (8:00 to 10:00 am) for 16 continuous days, whereas METC was administered 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 at 400 mg/kg/day. Sulpiride was administered i.p. once daily (8 am to 10 am) for 28 continuous days, whereas METC was administered 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 chloride. The dopamine assay was then performed with spectrophotometer at 330-375 nm[26].

2.11. Assay of superoxide dismutase

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 °C. Thereafter supernatant was collected and stored at 80 °C 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 H2O2, 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 tetracetic 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](image1.png)

**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 I receive haloperidol at 5 mg/kg/d, Group B receive sulpiride at 20 mg/kg/d, Group II receive haloperidol at 5 mg/kg/d + methanolic extract of *T. cordifolia* at 200 mg/kg/d, Group III receive haloperidol at 5 mg/kg/d + methanolic extract of *T. cordifolia* at 400 mg/kg/d, Group IV receive sulpiride at 20 mg/kg/d + methanolic extract of *T. cordifolia* at 200 mg/kg/d, and Group V 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 I and Group B), #P<0.05 (Group II, Group B vs. Group III, Group IV, Group C, and Group D).

![Figure 2](image2.png)

**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) μg/mg protein and (9.860±0.040) μg/mg protein respectively, and also increased catalase level to (0.940±0.007) μg/mg protein and (1.150±0.007) μg/mg protein respectively. In experiment number two (sulpiride induced hyperprolactinemia in rats), when METC at 200 mg/kg/day and METC at 400 mg/kg/day were administrated, superoxide dismutase level was increased to (7.930±0.007) μg/mg protein and (8.540±0.010) μg/mg protein respectively and catalase level was increased to (0.980±0.004) μg/mg protein and (1.220±0.008) μg/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](image)

**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 I, and Group B), *P<0.05 (Group I, Group B vs. Group II, 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 (<i>10<sup>6</sup></i>/μL) | Total leukocyte count (<i>10<sup>9</sup></i>/μL) | Hemoglobin (g/dL) | Packed cell volume (%) | Platelet (<i>10<sup>12</sup></i>/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 I | 6.190 ± 0.008<sup>a</sup> | 6.920 ± 0.006<sup>a</sup> | 12.620 ± 0.006<sup>a</sup> | 44.830 ± 0.008<sup>a</sup> | 2.130 ± 0.006<sup>a</sup> | 61.780 ± 0.006<sup>a</sup> | 0.870 ± 0.006<sup>a</sup> |
| Group B | 6.240 ± 0.008<sup>a</sup> | 6.850 ± 0.006<sup>a</sup> | 12.660 ± 0.008<sup>a</sup> | 44.720 ± 0.006<sup>a</sup> | 2.070 ± 0.008<sup>a</sup> | 61.630 ± 0.008<sup>a</sup> | 0.850 ± 0.004<sup>a</sup> |
| Group II | 6.790 ± 0.004<sup>a</sup> | 4.990 ± 0.008<sup>a</sup> | 13.400 ± 0.008<sup>a</sup> | 44.170 ± 0.008<sup>a</sup> | 1.670 ± 0.008<sup>a</sup> | 60.510 ± 0.008<sup>a</sup> | 0.817 ± 0.004<sup>a</sup> |
| Group IV | 7.110 ± 0.004<sup>a</sup> | 4.670 ± 0.008<sup>a</sup> | 13.950 ± 0.008<sup>a</sup> | 43.770 ± 0.008<sup>a</sup> | 1.500 ± 0.008<sup>a</sup> | 59.810 ± 0.008<sup>a</sup> | 0.784 ± 0.006<sup>a</sup> |
| Group C | 6.870 ± 0.008<sup>a</sup> | 5.120 ± 0.006<sup>a</sup> | 13.370 ± 0.008<sup>a</sup> | 44.270 ± 0.008<sup>a</sup> | 1.650 ± 0.001<sup>a</sup> | 59.660 ± 0.008<sup>a</sup> | 0.812 ± 0.008<sup>a</sup> |
| Group D | 7.110 ± 0.008<sup>a</sup> | 4.880 ± 0.008<sup>a</sup> | 13.930 ± 0.008<sup>a</sup> | 43.900 ± 0.008<sup>a</sup> | 1.470 ± 0.001<sup>a</sup> | 59.410 ± 0.008<sup>a</sup> | 0.637 ± 0.004<sup>a</sup> |

Data are expressed as mean ± SD; n=6; *P<0.05 (the control vs. Group I and Group B); **P<0.05 (Group I, Group B vs. Group II, 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.
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 incidents 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 the tuberoinfundibular 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 incidents 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.

![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.](image-url)
Conflict of interest statement

The authors declare no conflict of interest in any form.

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References

[1] Tiwari P, Chandy A, Kumar K, Mishra R, Ahirwar D. Neuroleptic drug induced hyperprolactinemia: Pathophysiology, safety and acceptability. Asian Pac J Reprod 2013; 2(1): 69-75.

[2] Tiwari P, Panik R, Bhattacharya A, Ahirwar D, Chandy A. Evidences of possible side effects of neuroleptic drugs: A systematic review. Asian Pac J Reprod 2012; 1(4): 330-336.

[3] Besnard I, Auclair V, Callery G, Gabriel-Bordeneuve C, Roberge C. Antipsychotic-drug-induced hyperprolactinemia: Physiopathology, clinical features and guidance. Encephale 2014; 40(1): 86-94.

[4] Molitch ME. Pharmacologic resistance in prolactinoma patients. Asian Pac J Reprod 2013; 2(1): 69-75.

[5] Bostwick JR, Guthrie SK, Ellingrod VL. Antipsychotic-induced hyperprolactinemia. Pharmacother J Hum Pharmacol Drug Ther 2009; 29(1): 64-73.

[6] Serri O, Chik CL, Ur E, Ezzat S. Diagnosis and management of hyperprolactinemia. GMAJ 2003; 169(6): 575-581.

[7] Cohen LG, Biederman J. Treatment of risperidone-induced hyperprolactinemia with a dopamine agonist in children. J Child Adolesc Psychopharm 2001; 11(4): 435-440.

[8] Mohan Kumar SM, Kasturi BS, Shin AC, Balasubramanian P, Gilbreath ET, Subramanian M, et al. Chronic estradiol exposure induces oxidative stress in the hypothalamus to decrease hypothalamic dopamine and cause hyperprolactinemia. Am J Physiol Regul Integr Comp Physiol 2010; 300(3): R693-699.

[9] Martins MR, Petronilho FC, Gomes KM, Dal-Pizzol F, Streck EL, Quevedo J. Antipsychotic-induced oxidative stress in rat brain. Neurotox Res 2008; 13(1): 63-69.

[10] Wang D, Wong HK, Sze CW, Feng Y, Zhang Z. Dopamine D2 receptors involved in Peony-Glycyrrhiza decoction, an herbal preparation against antipsychotic-associated hyperprolactinemia. Prog Neuropsychopharmacol Biol Psychiatry 2012; 39: 332-338.

[11] Melmed S, Casanueva FF, Hoffman AR, Kleinberg DL, Montori VM, Schlechte JA, et al. Diagnosis and treatment of hyperprolactinemia: An Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2011; 96(2): 273-288.

[12] Shimon I, Benbassat C, Hadani M. Effectiveness of long-term cabergoline treatment for giant prolactinoma: Study of 12 men. Eur J Endocrinol 2007; 156(2): 225-231.

[13] Hattori N. Appropriate dosing of pergolide in monotherapy and adjunctive therapy in Parkinson’s disease. Curr Opin Neurol 2003; 16: S21-25.

[14] Weintraub D, Siderowf AD, Potenza MN, Goveas J, Morales KH, Duda JE, et al. Association of dopamine agonist use with impulse control disorders in Parkinson disease. Arch Neurol 2006; 63(7): 969-973.

[15] Kumar GP, Anilakumar KR, Naveen S. Phytochemicals having neuroprotective properties from dietary sources and medicinal herbs. J Pharmacogn Phytochem 2015; 7(1): 1-17.

[16] Luqman S, Kaushik S, Srivastava S, Kumar R, Bawankule DU, Pal A, et al. Protective effect of medicinal plant extracts on biomarkers of oxidative stress in erythrocytes. Pharm Biol 2009; 47(6): 483-490.

[17] Mousavi SM, Niazmand S, Hosseini M, Hassanzadeh Z, Sadeghnia HR, Vafeae F, et al. Beneficial effects of Teucrium polium and metformin on diabetes-induced memory impairments and brain tissue oxidative damage in rats. Int J Alzheimers Dis 2015; 2015: 493729.

[18] Tiwari P, Nayak P, Prusty SK, Sahu PK. Phytochemistry and pharmacology of Tinospora cordifolia: A review. System Res Pharm 2018; 9(1): 70-78.

[19] Singh S, Birla H, Rai S, Zahra W, Singh S. Neuroprotective role of Tinospora cordifolia in MPTP induced Parkinsonian mouse model. Mov Disord 2018; 38: S11-S11.

[20] Kosaraju J, Chinni S, Roy PD, Kannan E, Antony AS, Kumar MS. Neuroprotective effect of Tinospora cordifolia ethanol extract on 6-hydroxy dopamine induced Parkinsonism. Indian J Pharmacol 2014; 46(2): 176.

[21] Tiwari P, Kumar K, Panik R, Pandey A, Pandey A, Sahu PK. Hepatoprotective potentials of Butea monosperma stem bark extract against carbon tetrachloride induced hepatotoxicity in albino rats. Int J Med Sci 2011; 8(3): 252-255.

[22] Kokate CK, Purohit AP, Gokhale SB. (eds). Pharmacognosy. 1st ed. New Delhi: Niralri Prakashan; 1990. p. 178-181.

[23] Kumar SK, Abhishek P, Kumar SP, Prashant T. Study of oestrus cycle periodicity and oogenesis of adult albino rats: Response to antipsychotic-associated hyperprolactinemia. Int J Fertil Steril 2011; 3(8): 252-255.

[24] Mostafapour S, Zare S, Sadrkhianlou RA, Ahmaedi A, Razi M. Sulpiride-induced hyperprolactinemia in mature female rats: Evidence for alterations in the reproductive system, pituitary and ovarian hormones. Int J Fertil Steril 2014; 8(2): 193.

[25] Fahie-Wilson MN, John R, Ellis AR. Macroprolactin; high molecular mass forms of circulating prolactin. Ann Clin Biochem 2005; 42(3): 175-192.
Prusty SK, Pati AK, Subudhi BB, Sahu PK. Chronic forced swimming induced stress alters behavioural, histological and anti-oxidant status. *Indian Drugs* 2017; 54(06): 58-64.

Das MK, Tiwari P, Prusty SK, Sahu PK. Neuroprotective potential of metformin against forced swimming induced neurodegeneration Wistar albino rats. *Asian J Biol Sci* 2018; 11(2): 89-97.

Da Costa Júnior JS, de Almeida AA, Costa JP, das Gracas Lopes Cito AM, Saffi J, de Freitas RM. Superoxide dismutase and catalase activities in rat hippocampus pretreated with garcinelliptone FC from *Platonia insignis*. *Pharm Biol* 2012; 50(4): 453-457.

Mishra AC, Mohanty B. Effects of lactational exposure of olanzapine and risperidone on alcohol induced anxiety in Wistar albino rats. *Indian Drugs* 2012; 50(4): 453-457.

Elalfy MM, Aboumosalam MS, Ali FR. Biochemical, hematological and pathological effects of bispypribac sodium in female albino rats. *J Vet Sci Technol* 2017; 8: 467.

Maurya R, Gupta P, Chand K, Kumar M, Dixin P, Singh N, et al. Constituents of *Tinospora sinensis* and their antileishmanial activity against *Leishmania donovani*. *Nat Prod Res* 2009; 23(12): 1134-1143.

Zhang Y, Shi Q, Shi P, Zhang W, Cheng Y. Characterization of isoquinoline alkaloids, diterpenoids and steroids in the Chinese herb *Tinospora sagittata* and *Tinospora capillipes* by high-performance liquid chromatography/electrospray ionization with multisstage mass spectrometry. *Rapid Commun Mass Spectrom* 2006; 20(15): 2328-2342.

Mahmood BH. Anatomical and histological study of pituitary gland of the rats in Iraq. *J Krb Univ* 2014; 12(3): 221-228.

Valenti O, Cifelli P, Gilli KM, Grace AA. Antipsychotic drugs rapidly induce dopamine neuron depolarization block in a developmental rat model of schizophrenia. *J Neurosci* 2011; 31(34): 12330-12338.

Richelson E, Souder T. Binding of antipsychotic drugs to human brain receptors: Focus on newer generation compounds. *Life Sci* 2000; 68(1): 29-39.

Nadig PD, Revankar RR, Dethe SM, Narayanswamy SB, Aliyar MA. Effect of *Tinospora cordifolia* on experimental diabetic neuropathy. *Indian J Pharmacol* 2012; 44(5): 580.

Mutalik M, Mutalik M. *Tinospora cordifolia*: Role in depression, cognition and memory. *Austral J Med Herb* 2011; 23(4): 168.

Kosaraju J, Chimni S, Roy PD, Kannan E, Antony AS, Kumar MS. Neuroprotective effect of *Tinospora cordifolia* ethanol extract on 6-hydroxy dopamine induced Parkinsonism. *Indian J Pharmacol* 2014; 46(2): 176.

Janhunen S, Ahtee L. Differential nicotinic regulation of the nigrostriatal and mesolimbic dopaminergic pathways: Implications for drug development. *Neurosci Biobehav Rev* 2007; 31(3): 287-314.

Valenti O, Cifelli P, Gill KM, Grace AA. Antipsychotic drugs rapidly induce dopamine neuron depolarization block in a developmental rat model of schizophrenia. *J Neurosci* 2011; 31(34): 12330-12338.

The current page contains a list of references, likely related to pharmacology and related fields, with emphasis on antipsychotics and their effects. The listed articles cover a range of topics from biochemical and pharmacological effects to behavioral and histological studies in animal models.