Original Article

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by IJRPS Journal

Home Page: https://ijrps.com/

Targeting calcium dependant/cAMP/PKA/CREB pathway and GABAergic transmission by drotaverine and hesperidin for amelioration of tardive dyskinesia

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Article History

Received on: 16 may 2024
Revised on: 05 Aug 2024
Accepted on: 08 Aug 2024

Keywords

Drotaverine;
Hesperidin;
Psychotic Anxiety;
Reserpine

Abstract

Antipsychotics, commonly used for anxiety and psychosis, are linked to severe side effects like fatal ventricular arrhythmias, sudden cardiac death, and movement disorders, limiting their therapeutic use. This research aimed to explore novel mechanisms to treat psychotic anxiety while minimizing these risks. Drotaverine activates the cAMP/PKA/CREB pathway, while Hesperidin inhibits dopaminergic hyperactivity. Using a reserpine-induced orofacial dyskinesia model, which mimics tardive dyskinesia linked to oxidative stress, reserpine (1 mg/kg, s.c) was administered for three days. Rats were pre-treated with drotaverine (8 mg/kg, p.o) and hesperidin (50 mg/kg, p.o) for five days. Statistical analysis via one-way ANOVA and Dunnett's test showed that reserpine significantly increased vacuous chewing movements (VCMs), tongue protrusions (TPs), and reduced locomotion and exploration. Pre-treatment with drotaverine and hesperidin reduced VCMs and TPs. Reserpine-treated rats had lower catalase and higher lipid peroxidation (LPO) levels, indicators of oxidative stress, which were reversed by the drugs. Additionally, drotaverine improved cognition by modulating the cAMP/PKA/CREB pathway, and Hesperidin offered neuroprotection through GABAergic transmission. Both drugs, alone or in combination, demonstrate potential as alternative therapies for psychotic anxiety with neuroprotective benefits.

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eISSN: 0975-7538
DOI: https://doi.org/10.26452/ijrps.v15i3.4700

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1. INTRODUCTION

Psychosis is a frequent and functionally disruptive symptom of many psychiatric, neurodevelopmental, neurologic, and medical diseases [1]. In addition to the conventional core positive and negative symptom dimensions, anxiety disorders and symptoms are prevalent in schizophrenia and can manifest at any stage of the illness [2]. Although it has long been understood that anxiety plays a significant role in the psychopathology of schizophrenia, there is a dearth of information on the incidence of anxiety symptoms or syndromes in schizophrenia [3].

Pathological anxiety is among the most prevalent comorbid conditions in psychiatric disorders) [4]. Up to 65% of patients with schizophrenia may experience anxiety symptoms, and these symptoms may be severe enough to warrant a diagnosis of one or more concomitant anxiety disorders, such as obsessive-compulsive disorder (OCD) and post-traumatic stress disorder (PTSD) [5]. Patients with both schizophrenia and anxiety features experience more severe limitations in the areas of employment and social life as well as more widespread functional impairment, which suggests that anxiety features in these patients further deteriorate their already poor quality of life [6]. Atypical antipsychotics like quetiapine, aripiprazole, olanzapine, and risperidone have been used to treat a variety of mood and anxiety disorders since it was discovered that they were effective in addressing a variety of anxiety and depressive symptoms in people with schizophrenia and schizoaffective disorders. Antipsychotics have long been considered a viable treatment for anxiety disorders, but studies have been restricted by the potential of long-lasting adverse effects [7]. Antipsychotics therapy show a wide range of adverse effects, including cardiac arrhythmias, sudden cardiac death, tardive dyskinesia, akathisia, and other conditions [8][9]. Long-term use of antipsychotics produces involuntary muscle movements, also known as tardive dyskinesia, can range from a mild tremor to an uncontrollable movement of the entire body. Tardive dyskinesia appears months or years after beginning of antipsychotic medication, and their interaction with other drugs are much more complicated. It is well established that reserpine contributes to the emergence of tardive dyskinesia and it provides the animal model of TD [10],[11]. While increased oxidative stress with cumulative free radical damage is a well-known aspect of brain ageing, the hypothesis that tardive dyskinesia may be caused by the neurotoxic effects of free radical byproducts from catecholamine metabolism in the basal ganglia has received an abundance of interest [12]. Reserpine also depletes brain catecholamines, which results in an akinetic state in test animals [13]. Orofacial dyskinesia (OD) is characterised by increased vacuous chewing action, tongue protrusion, orofacial burst, and cataleptic behaviour in rats treated with this monoamine-depleting chemical [14]. Drotaverine is an antispasmodic drug [15]. Drotaverine has the potential to block L-type voltage-operated calcium channels (L-VOCC) [16]. It inhibits the phosphodiesterase 4 which is involve in cAMP/PKA/CREB pathway [17]. It works by increasing cAMP (cyclic adenosine monophosphate) levels, which led to the production of the cAMP adenyl cyclase enzyme. cAMP turns on the protein kinase A, which in turn phosphorylates the cAMP response element binding protein (CREB) [18]. Further studies suggested that the Ca^{2+}-dependent signal transduction pathways can also cause CREB phosphorylation, that was previously assumed to be only mediated by the cAMP/protein kinase A (PKA) pathway. In addition, Ca^{2+} influx through L-type voltage-sensitive Ca^{2+} channels or α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors and the release of Ca^{2+} from intracellular stores, induced by the stimulation of growth factors receptors, activate the extracellular signal-related protein kinase (ERK)/mitogen-activated protein kinase pathway and induce CREB phosphorylation through the ribosomal S6 kinase 2 in PC12 cells in primary neuronal cultures and in brain slices [19]. From the literature survey, experiments on animals and patients have suggested that intracellular calcium ions within the brain's cells may be involved in controlling the activity of dopamine and choline, which motivated to evaluate the effect of drotaverine in the treatment of TD by blocking L-type of calcium channel [20]. Hesperidin (Hesp), a bioflavonoid which possesses significant neuroprotective, anti-inflammatory, analgesic, antibacterial, antifungal, antiviral, antihypercholesterolemic, and anticancer effects [21]. Hesperidin improves brain function by restoring the mitochondrial function, increasing GABAergic activity and cholinergic transmission. The increased GABAergic transmission demonstrated an inhibitory control of dopaminergic hyperactivity and prevents psychosis as well as anxiety. The primary structural component of flavonoids involved in the treatment of psychosis is sites produced by hydroxyl groups for the complexing of calcium (Ca^{2+}) and restoring the activity of NMDA-Rs, hence reducing psychosis. Hesperidin work as a potent antioxidant against superoxide, singlet oxygen, and hydroxyl radicals and make a major contribution to the intracellular antioxidant defence system as a whole. Current literature survey indicated that hesperidin exerts neuroprotective effect by

reducing excitotoxicity or neurotoxicity [22]. Hence the present study investigated the neuroprotective potential of drotaverine and hesperidin in reserpine induced orofacial dyskinesia model of psychotic anxiety by measuring VCMs, TP frequency and locomotor activity. Also, resolved its mechanism of action with respect to biochemical imbalances.

2. METHODS:

2.1. Chemicals:

Drotaverine (Aarti pharmaceuticals India, Ltd.), Hesperidin (Yucca Enterprises, Mumbai, India), Reserpine (Research Lab (Mumbai, Maharashtra, India). All the chemicals used were of analytical grade and purchased from standard manufacturers.

2.2. Experimental Animals

Wistar strain rats (180-200 g) of either sex were used for the study. Animals were procured and kept in polypropylene cages and maintained under the standard laboratory environmental conditions; temperature 25 ± 2ºC, 12: 12 h L: D cycle and 50 ± 5% relative humidity with free access to food and water *ad libitum*. Before the test, the animals were acclimated to the laboratory environment. During the light period, all experimental work was done (08:00-16:00 h). The research was carried out in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), based in New Delhi's (India). The study protocol (IAEC/2023/01) was approved by the Institutional Animal Ethical Committee of M.V.P.S College of Pharmacy in Nashik.

2.3. Experimental design for Reserpine-induced orofacial dyskinesia

Animals were randomly divided into six groups (n=6 in each group).Group I-(control),treated with 0.1% Acetic acid (vehicle for reserpine), Group II-Reserpine (1mg/kg s.c), Group III - Drotaverine (8 mg/kg, p.o) + Reserpine (1 mg/kg s.c), Group IV - Hesperidin (50 mg/kg, p.o) + Reserpine (1 mg/kg s.c), Group V - Drotaverine (8 mg/kg, p.o) + Hesperidin (50 mg/kg, p.o) + Reserpine (1 mg/kg s.c), Group VI – Risperidone (5 mg/kg, p.o) + Reserpine (1 mg/kg s.c).Risperidone used as a standard drug.

2.4. Induction of orofacial dyskinesia:

The vehicle-treated group received 0.2% PEG in distilled water was administered orally for five days and 0.1% acetic acid solution subcutaneously every other day for three days (the vehicle for reserpine), 24 h after the administration of PEG, the first injection of acetic acid was administered. The reserpine group received 1 mg/kg reserpine every other day for three days. The drotaverine plus reserpine group, hesperidin plus reserpine and combination groups received oral dose of 8 mg/kg drotaverine and 50 mg/kg hesperidin over the course of 5 days, alternated with 3 days of s.c. reserpine at 1 mg/kg. Drotaverine, hesperidin or PEG was administered 24 hrs before reserpine. Reserpine was delivered 30 minutes after drotaverine and hesperidin. Similar procedures were taken with the group of animals who had received risperidone treatments [23].

3. Behavioural tests

3.1. Quantification of dyskinesia

Rats were placed separately in a small Plexiglas observation cage (30 x20 x 20 cm) to score vacuous chewing movements (VCMs) and tongue protrusion frequency on the test day to quantify the development of oral dyskinesia. Before performing behavioural assessments, the animals were allowed 10 minutes to acclimate to the observation cage.

Mirrors were set under the floor and behind the back wall of the cage to permit observation of oral dyskinesia when the animal was faced away from the observer. A single vertical mouth opening not oriented towards physical substance and visible tongue extension outside of mouth were characterised as VCMs and tongue protrusions respectively. VCMs and tongue protrusions that occurred during grooming were not included. For a period of 15 minutes, the frequency of these oral movements was recorded [24].

3.2. Locomotor activity

The astrophotometer's activity has been assessed for alertness. The digital actophotometer was made of a square metallic chamber (30 cm x 30 cm) with six built-in photosensors and digital counters. The light beam falling on the photocell is interrupted as the animal moves, and a count is digitally recorded as a result.

Actophotometer counts correspond to animal movement. As a result, the quantity of counts was utilised to quantify the subject animal's locomotor activity.

All of the animals were placed in the actophotometer for 10 minutes, and the locomotor activity was measured every 10 minutes [23].

3.3. Hole and Board test

The hole board test was used to assess animal behaviour such as curiosity and exploration. The hole-board is made up of a wooden box (40x40 cm) with 16 holes (diameter 3 cm) spaced evenly on the floor. The animal's tendency to insert its head into the holes was observed and recorded. The board was elevated so that the rats poking their heads into the hole couldn't see the bottom. The animals were allowed to explore the holes in the board for 5min, and the total number of pokes per 5 min was measured by visual observation for each rat [25].

3.4. Dissection and homogenization

The animals were sacrificed using a euthanasia chamber on the last day, 1 h after all behavioural tests, and their brains were removed. The brains were separated and weighed after being rinsed in an isotonic saline solution. To make tissue homogenate (10 % w/v), 0.1M phosphate buffer (pH 7.4) was used. Centrifugation of the homogenate (10% w/v) at 1000 g for 20 minutes at 4 °C after nuclear fractionation for catalase assay (Remi - C30, Remi Industries Ltd. Mumbai, India). Centrifugation of homogenate at 12000 g for 60 minutes at 4°C after nuclear fraction for other enzyme assays. Following biochemical parameters were performed using an ElicoTM BL 200 bio spectrophotometer [12].

4. Biochemical parameters

4.1. Catalase activity (CAT)

The Luck Method was used to assess catalase activity. H_2O_2 breakdown is assessed using this technique at 240 nm. The necessary assay mixture contained 3 ml of 0.01 M H_2O_2- phosphate buffer (pH 7) and 0.05 ml of tissue homogenate supernatant (10% w/v) At 240 nm, the change in absorbance was seen after 1 minute. In order to determine enzyme activity, the millimolar extinction coefficient of H_2O_2 (0.071) was utilised. The results were represented as milligrams of protein per minute of H_2O_2 decomposition [26].

4.2. Lipid peroxidation (LPO)

The method developed by Wills (1966) was used to measure the amount of lipid peroxidation in the brain quantitatively. By reacting with thiobarbituric acid at 532 nm, this method evaluated the amount of malondialdehyde (MDA) produced. 1.5 ml of 20% acetic acid, 0.1 ml of tissue homogenate, 0.2 ml of 8% sodium lauryl sulphate (SLS), and 1.5 ml of a 0.8% thiobarbituric acid (TBA) solution make up the reaction mixture. Then, this combination was cooked for one hour on a water bath at 95 0C. It was then given a 15:1 n-butanol and pyridine mixture in the amount of 5ml. After a vigorous shaking, the mixture was centrifuged at 2200 g for 5 min. The organic layer's upper layer's absorbance was measured at 532 nm. The molar extension coefficient of the chromophore was used to convert the values into nM of MDA per milligrams of protein. (1.56 × 105 M-1cm-1) [27],[28].

5. Histopathological Studies

Brain tissue was removed by sacrificing and preserved in 10% formalin solution. Fixation was carried out in paraffin wax blocks. Sections of tissue were cut at 40 µm by using the digital microtome. For qualitative histopathological analysis haematoxylin and eosin (H&E) stain was used. 100X magnification power were used for the histopathological analysis of the cutting sections of brain [18].

6. Statistical analysis

Results were expressed as mean ± standard error of the mean Data were subjected to one-way analysis of variance (ANOVA) followed by Dunnett's test. Graph Pad Prism 9.5.1 software (GraphPad®, San Diego, CA, USA).

7. RESULTS:

7.1. Reserpine -induced orofacial dyskinesia:

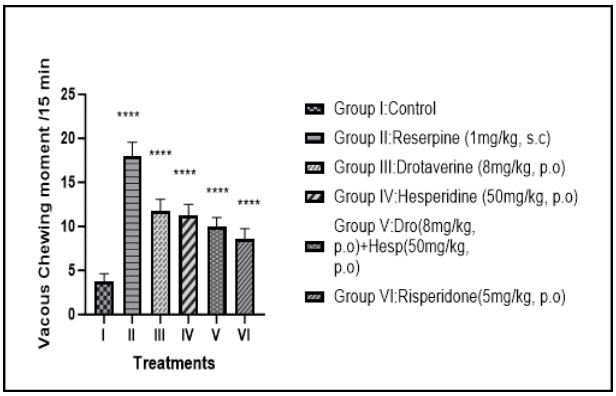

Figure 1 Effect of drotaverine and hesperidin on vacuous chewing moments by reserpine induced orofacial dyskinesia

All values expressed as mean ± S.E.M. Group II compared with group I. Groups III, IV, V and VI, compared with group II. One-Way ANOVA followed by Dunnett's test.

ns – Non significant, *, # p < 0.05, **, ## p < 0.01, ***, ### p < 0.001 and ****, #### p<0.0001.

Animal treated with reserpine for 3 days produced VCMs and TP in experimental animals, indicating orofacial dyskinesia. Reserpine group showed significant (p<0.0001) increase in VCMs and TP frequency compared to control group. Treatment with drotaverine (8 mg/kg), hesperidin (50 mg/kg) and their combination significantly reduced VCMs and TPs as compared to reserpine treated group. Risperidone (5 mg/kg) decreased VCMs and TP frequency as compared to reserpine group (p<0.0001)

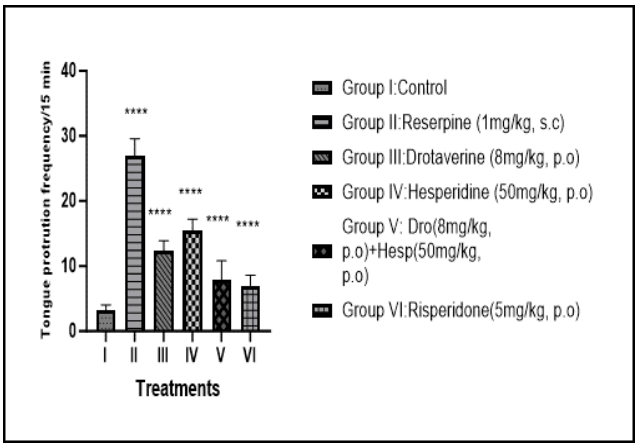

Figure 2 Effect of drotaverine and hesperidin on tongue protrusions by reserpine induced orofacial dyskinesia

All values expressed as mean ± S.E.M. Group II compared with group I. Groups III, IV, V and VI, compared with group II. One-Way ANOVA followed by Dunnett's test.

ns – Non significant, *, # p < 0.05, **, ## p < 0.01, ***, ### p < 0.001 and ****, #### p<0.0001.

7.2. Effect on locomotor activity

Reserpine treated groups showed significant (p<0.01) \decrease in locomotor activity as compared to control group. Treatment with drotaverine (8 mg/kg), Hesperidin (50 mg/kg) and their combination significantly increased total locomotor activity as compared to reserpine treated group. Risperidone treated group also showed significantly (p<0.01) increase in the locomotor activity . All values expressed as mean ± S.E.M. Group II compared with group I. Groups III, IV, V and VI, compared with group II. One-Way ANOVA followed by Dunnett's test.

ns – Non significant, *, # p < 0.05, **, ## p < 0.01, ***, ### p < 0.001 and ****, #### p<0.0001.

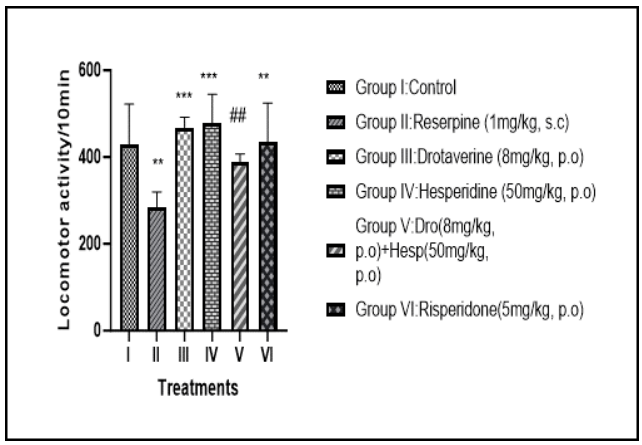

Figure 3 Effect of drotaverine and hesperidin on locomotor activity by reserpine induced orofacial dyskinesia

7.3. Effect on number of poking in Hole and Board

Reserpine treated group showed significant (p<0.0001) decrease in nose poking as compared to control animals. Treatment with drotaverine (8 mg/kg), hesperidin (50 mg/kg) and their combination treatment significantly increased nose poking as compared to reserpine group. Risperidone treated group also showed significantly (p<0.001) increase in nose poking .

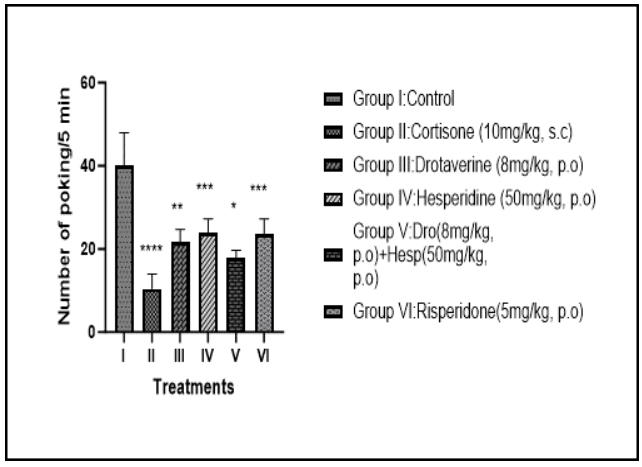

Figure 4 Effect of drotaverine and hesperidin on hole and board test by reserpine induced orofacial dyskinesia

All values expressed as mean ± S.E.M. Group II compared with group I. Groups III, IV, V and VI, compared with group II. One-Way ANOVA followed by Dunnett's test.

ns – Non significant, *, # p < 0.05, **, ## p < 0.01, ***, ### p < 0.001 and ****, #### p<0.0001.

7.4. Biochemical parameters

7.4.1. Catalase activity (CAT)

Protective antioxidant enzyme CAT was significantly (p<0.0001) reduced in reserpine treated group as compared to control group, While treatment with drotaverine (8 mg/kg), hesperidin (50 mg/kg) and their combination significantly restored CAT levels to normal. Risperidone also significantly restored CAT levels in animals (p<0.0001) .

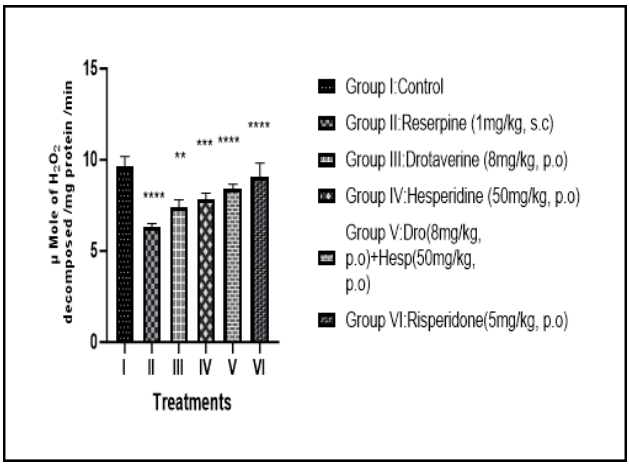

Figure 5 Effect of drotaverine and hesperidin on catalase level by reserpine induced orofacial dyskinesia

All values expressed as mean ± S.E.M. Group II compared with group I. Groups III, IV, V and VI, compared with group II. One-Way ANOVA followed by Dunnett's test. ns – Non significant, *, # p < 0.05, **, ## p < 0.01, ***, ### p < 0.001 and ****, #### p<0.0001.

7.4.2. Lipid peroxidation assay (LPO)

LPO levels significantly (p<0.0001) increased in reserpine treated group as compared to control. Drotaverine (8 mg/kg), hesperidin (50 mg/kg) and their combination significantly (p<0.0001) reduced LPO levels as compared to reserpine treated rats. LPO levels also decrease by Risperidone (p<0.0001) . All values expressed as mean ± S.E.M. Group II compared with group I. Groups III, IV, V and VI, compared with group II. One-Way ANOVA followed by Dunnett's test.

ns – Non significant, *, # p < 0.05, **, ## p < 0.01, ***, ### p < 0.001 and ****, #### p<0.0001.

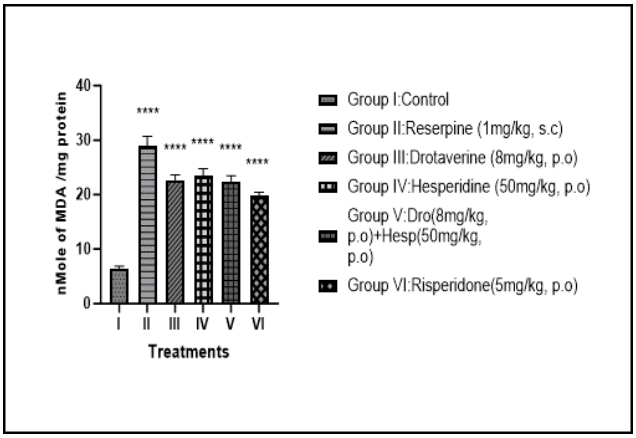

Figure 6 Effect of drotaverine and hesperidin on lipid peroxidation level by reserpine induced orofacial dyskinesia

7.4.3. Histopathology

Histopathological structure of cerebral cortex of control group showed normal architecture while reserpine treated group revealed the presence of ghost cells (G), vacuolated cytoplasm (Vc) indicating damage to cerebral cortex. Treatment with drotaverine (8 mg/kg), hesperidin (50 mg/kg) and their combination showed the presence of ghost cells, vacuolated cytoplasm, however the number of ghost cells and vacuolated cytoplasm is lesser compared to reserpine treated group. Risperidone also showed a smaller number of ghost cells, Vacuolated cytoplasm (**Figure 7**).

8.DISCUSSION

The findings of the present investigation showed that drotaverine and hesperidin are neuroprotective agent against reserpine-induced orofacial dyskinesia. The increase in vacuous chewing movements and tongue protrusions produced by acute reserpine exposure was significantly decreased by drotaverine and hesperidin.Reserpine-induced VCMs and tongue protrusions seem to be an improved model of tardive dyskinesia (TD). Since reserpine has historically been associated with the onset of TD in humans, characteristic features of reserpine-induced oral dyskinesia closely resemble the symptoms of TD [28].As a result, the model was effective in elucidating the role of dopaminergic

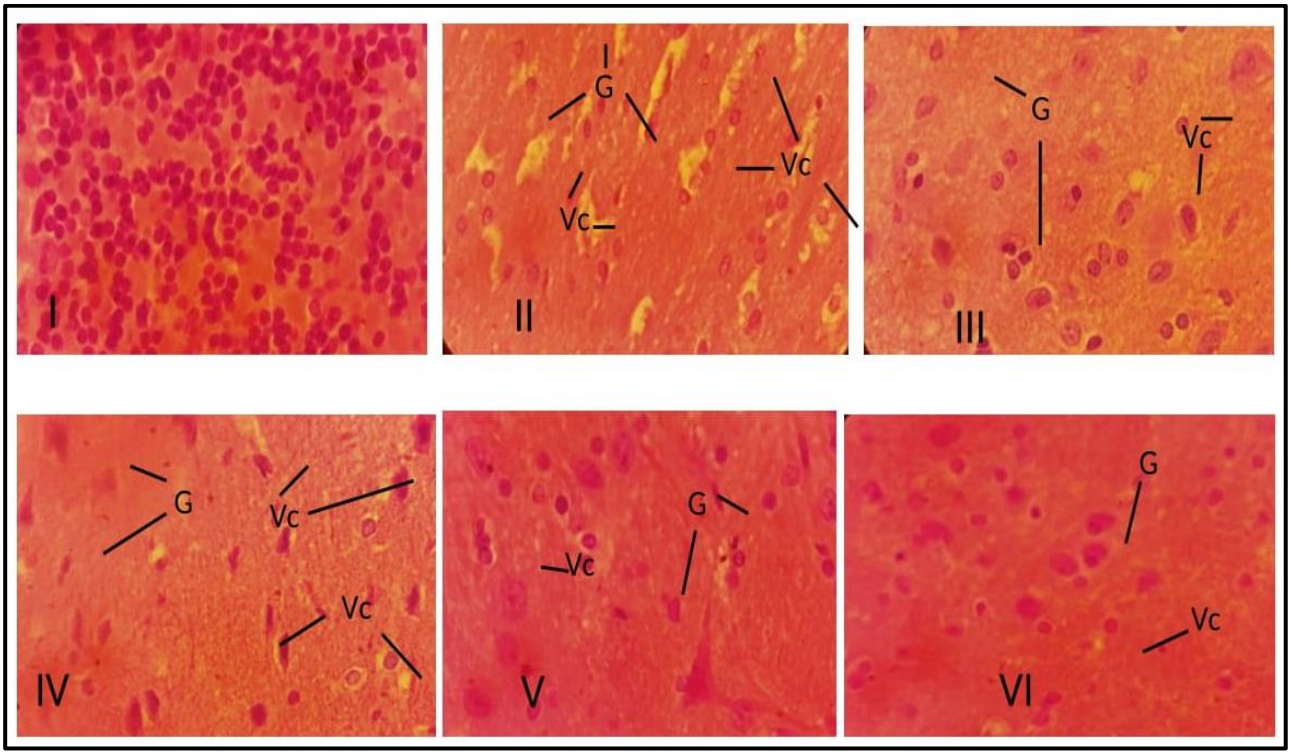

Figure 7 Histopathological changes of cerebral cortex of rats in different groups.
I: Control group, histopathological structure of cerebral cortex showing normal architecture.
II: Reserpine treated revealed the presence of ghost cells (G), Vacuolated cytoplasm (Vc) indicating damage to cerebral cortex.
III: Drotaverine (8 mg/kg, p.o.), IV: Hesperidin (50 mg/kg, p. o), V: Drotaverine (8 mg/kg, p. o) + Hesperidin (50 mg/kg, p. o), VI: Risperidone (5 mg/kg, p. o). These groups also showed the presence of ghost cells (G), Vacuolated cytoplasm (Vc), however the number of ghost cells and vacuolated cytoplasm is lesser compared to reserpine group (Group II).

neurotransmission in motor control as well as screening for potential medications to treat psychotic anxiety. Reserpine induces oral dyskinesia,akinesia, hypokinesia by blocking vesicular monoamine transferase VMAT. Reserpine induced orofacial dyskinesia is closely associated with oxidative stress and free radicals are highly involved in development of orofacial dyskinesia in rats. In present study, administration of reserpine (1 mg/kg) significantly increased the frequency of VCMs and tongue protrusions indicating neurotoxicity produced by reserpine. Pre-treatment with drotaverine (8 mg/kg) and hesperidin (50 mg/kg) significantly reduced increase in the frequency of VCMs and tongue protrusions. Total locomotor activity and number of poking was significantly reduced in reserpine treated group as compared to vehicle group. Administration of drotaverine and hesperidin showed significant improvement in exploratory activity. Reserpine treated animal showed elevated level of lipid peroxidation along with significant reduction in antioxidant enzyme such as CAT indicating generation of free radicals. However, administration of drotaverine and hesperidin significantly attenuated this reduction in enzymatic defence generated against free radical and reduced lipid peroxidation, suggesting their possible antioxidant action. The results obtained from this study supported the oxidative stress hypothesis of tardive dyskinesia and suggested a beneficial role of drotaverine and hesperidin in treatment of neurodegenerative disorders. In histopathological findings, reserpine treated brain revealed the presence of ghost cells, vacuolated cytoplasm compared to vehicle treated group. Whereas drotaverine and hesperidin treated rats showed a smaller number of ghost cells, vacuolated cytoplasm compared with the reserpine treated group. Thus, drotaverine and hesperidin showed the neuroprotective potential against orofacial dyskinesia. Drotaverine is phosphodiesterase-4

inhibitor and have the abilities to decrease oxidative stress, inflammation, and endothelial dysfunction resulting from a variety of pathophysiological events. Drotaverine also showed the calcium channel blocking property which is linked to cAMP/CREB pathway involved in controlling the activity of dopamine and choline. Hesperidin is a flavonoid, possesses a direct scavenging activity on oxygen radicals with antioxidant properties, which play a key role in neurodegenerative disorder. Hesperidin also increases the GABAergic transmission and also restores the NMDA function. Anxiety as well as psychosis are prevented by the enhanced GABAergic transmission, which showed an inhibitory regulation of dopaminergic hyperactivity. From all these evidences, it has been concluded that drotaverine and hesperidin showed neuroprotective effects against reserpine induced orofacial dyskinesia.

9. Conclusions

From the results of the study, it has been concluded that drotaverine and hesperidin could be drugs of choice for psychotic anxiety accompanying oxidative stress and related activation of several other pathways. It is evident from the study that administration of drotaverine and hesperidin slowed down the formation of free radicals and ameliorated the oxidative stress along with inhibition of inflammatory mediators with improvement in VCMs, TPs and exploratory behaviours. Drotaverine and hesperidin also improved the level of catalase (CAT) and decrease the lipid peroxidation (LPO). It can be concluded that drotaverine and hesperidin have significant antioxidant activity and neuroprotective activity against reserpine induced orofacial dyskinesia. Drotaverine could target Ca^{2+} dependant/cAMP/PKa/CREB pathway and hesperidin stimulates GABAergic transmission, thus the symptoms of psychotic anxiety ameliorated. Hence drotaverine and hesperidin may be considered as an alternative therapy for psychotic anxiety.

List of Abbreviations:

VCMs	Vacuous chewing moments
TPs	Tongue protrusions
CAT	Catalase
LPO	Lipid peroxidation
OCD	Obsessive-compulsive disorder
PTSD	Post-traumatic stress disorder
OD	Orofacial dyskinesia
TD	Tardive dyskinesia
cAMP	Cyclic adenosine monophosphate
CREB	cAMP response element binding protein
PK A	Protein kinase A
ERK	Extracellular signal-related protein kinase
Hesp	Hesperidin
Ca^{2+}	Calcium
CPCSEA	Committee for the Purpose of Control and Supervision of Experiments on Animals
IAEC	Institutional Animal Ethical Committee
MDA	Malondialdehyde
TBA	Thiobarbituric acid
H & E	Haematoxylin and eosin
Vc	Vacuolated cytoplasm
G	Ghost cell
ANOVA	Analysis of variance
VMAT	Vesicular monoamine transferase

DECLARATIONS:

Ethics approval and consent to participate:

The research was carried out in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), based in New Delhi's (India). The study protocols

(IAEC/2023/01) was approved by the Institutional Animal Ethical Committee of M.V.P.S College of Pharmacy in Nashik.

Consent for publication: Not Applicable

Availability of data and material:

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Author's contributions:

Author VN and SN conceived and designed the work. BP, LK, SB and TH analysed the data. The original manuscript was drafted by SN while VN

revised the manuscript. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

Funding Support

The authors declare that they have no funding for this study.

REFERENCES

[1] Tanaka M, Szabó Á, Vécsei L. Integrating armchair, bench, and bedside research for behavioral neurology and neuropsychiatry. Biomedicines. 2022 Nov 22;10(12):2999.

[2] Ioakeimidis V, Khachatoorian N, Haenschel C, Papathomas TA, Farkas A, Kyriakopoulos M, Dima D. State anxiety influences P300 and P600 event-related potentials over parietal regions in the hollow-mask illusion experiment. Personality Neuroscience. 2021 Jan;4: e2.

[3] Mohammadi MR, Pourdehghan P, Mostafavi SA, Hooshyari Z, Ahmadi N, Khaleghi A. Generalized anxiety disorder: Prevalence, predictors, and comorbidity in children and adolescents. Journal of anxiety disorders. 2020 Jun 1; 73:102234.

[4] Kalin NH. Novel insights into pathological anxiety and anxiety-related disorders. American Journal of Psychiatry. 2020 Mar 1;177(3):187-9.

[5] Volkan K. Schizophrenia: epidemiology, causes, neurobiology, pathophysiology, and treatment. J Health Med Sci. 2020 Nov;3(4):487-521.

[6] Rahme C, El Kadri N, Haddad C, Fekih-Romdhane F, Obeid S, Hallit S. Exploring the association between lifetime traumatic experiences and positive psychotic symptoms in a group of long-stay patients with schizophrenia: the mediating effect of depression, anxiety, and distress. BMC psychiatry. 2023 Jan 12;23(1):29.

[7] Orzelska-Górka J, Mikulska J, Wiszniewska A, Biała G. New atypical antipsychotics in the treatment of schizophrenia and depression. International journal of molecular sciences. 2022 Sep 13;23(18):10624.

[8] Zhu J, Hou W, Xu Y, Ji F, Wang G, Chen C, Lin C, Lin X, Li J, Zhuo C, Shao M. Antipsychotic drugs and sudden cardiac death: A literature review of the challenges in the prediction, management, and future steps. Psychiatry Research. 2019 Nov 1; 281:112598.

[9] Kumsa A, Girma S, Alemu B, Agenagnew L. Psychotropic medications-induced tardive dyskinesia and associated factors among patients with mental illness in Ethiopia. Clinical Pharmacology: Advances and Applications. 2020 Dec 1:179-87.

[10] Dhingra D, Goswami S, Gahalain N. Protective effect of hesperetin against haloperidol-induced orofacial dyskinesia and catalepsy in rats. Nutritional Neuroscience. 2018 Oct 21;21(9):667-75.

[11] Ward KM, Citrome L. Antipsychotic-related movement disorders: drug-induced parkinsonism vs. tardive dyskinesia—key differences in pathophysiology and clinical management. Neurology and therapy. 2018 Dec; 7:233-48.

[12] D'Cruz M, Andrade C. Potential clinical applications of Ashwagandha (Withania somnifera) in medicine and neuropsychiatry. Expert Review of Clinical Pharmacology. 2022 Sep 2;15(9):1067-80.

[13] Reinheimer JB, Bressan GN, de Freitas CM, Ceretta AP, Krum BN, Nogara PA, Rodrigues T, Schwerz JP, da Rocha JB, Fachinetto R. Effects of CATECHIN on reserpine-induced vacuous chewing movements: behavioural and biochemical analysis. Naunyn-Schmiedeberg's Archives of Pharmacology. 2020 Dec;393: 2439-52.

[14] Samad N, Khaliq S, Alam M, Yasmin F, Ahmad S, Mustafa S, Raza U. Tryptophan lessens reserpine induced anxiety, depression and memory impairment by modulating oxidative stress and serotonergic activity. Pakistan Journal of Pharmaceutical Sciences. 2021 Jul 2;34.

[15] Soare AC, Meltzer V, Colbea C, Stanculescu I, Pincu E. Compatibility of drotaverine hydrochloride with ibuprofen and ketoprofen nonsteroidal anti-inflammatory drugs mixtures. Materials. 2022 Feb 8;15(3):1244.

[16] Akinsola Ao, Adeneye Aa, Olorundare Oe, Salahdeen Hm, Murtala Ba, Mukhtar H, Albrecht Rm. Vasorelaxant Mechanism (S) Of Clerodendrum Volubile Ethanol Leaf Extract In Normal And Doxorubicin-Treated Endothelium Intact Aortic Rings. Asian J Pharm Clin Res. 2022;15(7):135-43.

[17] Nazir S, Anwar F, Saleem U, Ahmad B, Raza Z, Sanawar M, Rehman AU, Ismail T. Drotaverine inhibitor of PDE4: Reverses the streptozotocin induced alzheimer's disease in mice. Neurochemical research. 2021 Jul;46:1814-29.

[18] Nazir S, Anwar F, Saleem U, Ahmad B, Raza Z, Sanawar M, Rehman AU, Ismail T. Drotaverine inhibitor of PDE4: Reverses the streptozotocin induced alzheimer's disease in mice. Neurochemical research. 2021 Jul;46:1814-29.

[19] Yang X, Long F, Jia W, Zhang M, Su G, Liao M, Zeng Z, Chen W, Chen J. Artesunate inhibits PDE4 leading to intracellular cAMP accumulation, reduced ERK/MAPK signaling, and blockade of influenza A virus vRNP nuclear export. Antiviral Research. 2023 Jul 1; 215:105635.

[20] Essali A, Soares-Weiser K, Bergman H, Adams CE. Calcium channel blockers for antipsychotic-induced tardive dyskinesia. Cochrane Database of Systematic Reviews. 2018(3).

[21] Sharma P, Kumari S, Sharma J, Purohit R, Singh D. Hesperidin interacts with CREB-BDNF signalling pathway to suppress pentylenetetrazol-induced convulsions in zebrafish. Frontiers in pharmacology. 2021 Jan 11; 11:607797.

[22] Kumar N, Yadav M, Kumar A, Kadian M, Kumar S. Neuroprotective effect of hesperidin and its combination with coenzyme Q10 on an animal model of ketamine-induced psychosis: behavioral changes, mitochondrial dysfunctions, and oxidative stress. Future Journal of Pharmaceutical Sciences. 2022 Jan 15;8(1):13.

[23] Kuzay D, Dileköz E, Özer Ç. Effects of thymoquinone in a rat model of reserpine-induced depression. Brazilian Journal of Pharmaceutical Sciences. 2022 Jul 13;58: e19847.

[24] Wang MH, Yang CC, Tseng HC, Fang CH, Lin YW, Soung HS. Naringin ameliorates haloperidol-induced neurotoxicity and orofacial dyskinesia in a rat model of human tardive dyskinesia. Neurotoxicity Research. 2021 Jun; 39:774-86.

[25] Dara S, Dhamercherla S, Jadav SS, Babu CM, Ahsan MJ. Machine learning in drug discovery: a review. Artificial Intelligence Review. 2022 Mar;55(3):1947-99.

[26] Nandi A, Yan LJ, Jana CK, Das N. Role of catalase in oxidative stress-and age-associated degenerative diseases. Oxidative medicine and cellular longevity. 2019 Nov 11;2019.

[27] Angelova PR, Esteras N, Abramov AY. Mitochondria and lipid peroxidation in the mechanism of neurodegeneration: Finding ways for prevention. Medicinal Research Reviews. 2021 Mar;41(2):770-84.

[28] Kajero JA, Seedat S, Ohaeri J, Akindele A, Aina O. Effects of cannabidiol on vacuous chewing movements, plasma glucose and oxidative stress indices in rats administered high dose risperidone. Scientific Reports. 2022 Nov 16;12(1):19718.