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PharmTech

International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.4, No.2, pp 669-675, April-June 2012

Neuroprotective activity of S-Allylcysteine on Haloperidol induced Parkinson's disease in albino mice.

S.Manikandaselvi[1]*, R.Mahalakshmi[1] ,R.Thinagarbabu[2] ,A.R.Angumeenal[2]

[1]Department of Biochemistry, Sengamala Thaayar Educational Trust Women's College, Mannargudi- 614001, Tamil Nadu, India.
[2]School of Chemical&BioTechnology, SASTRA University, Thanjavur, Tamil Nadu, India.

Corres.author :selvithinagar@gmail.com

Abstract: Neuroprotective activity of S-allylcysteine was examined against Haloperidol induced Parkinson's disease in mice. The oral administration of S-allylcysteine (30mg/kg body weight) for 7 days against haloperidol affected mice significantly increased the level of antioxidants (glutathione peroxidase, super oxide dismutase, reduced glutathione and catalase) and decreased the level of thiobarbutaric acid reactive substances and lipid peroxidation. Histochemical analysis of the midbrain of the mice confirmed the brain injury with haloperidol administration. The results of the present study provide clear evidence of defense provided by S-allylcysteine against haloperidol induced Parkinson's disease in mice mid brain.
Keywords: S-Allylcysteine, Haloperidol, Neuroprotective activity, Parkinson's disease.

INTRODUCTION

In 40% of men and 23% of women diagnosed with schizophrenia, the condition arises before the age of 19[1-3]. The first line treatment for schizophrenia is antipsychotic medication [4]. Most antipsychotics take around 7–14 days to have their main effect. The typical neuroleptics used to treat schizophrenia are highly effective, but are associated with severe extra pyramidal side effects. The most predominant among these symptoms are dystonia, parkinsonian-like syndrome, and tardive dyskinesia. These extra pyramidal side effects have been and still are major concerns in the society, as the drug that treats the patients for schizophrenia leaves them with lifelong disabilities [5].

Haloperidol is a typical potent antipsychotic drug. Haloperidol blocks postsynaptic dopamine D_2 receptors in the mesolimbic system and increases dopamine turnover by blockage of the D_2 receptors somatodendritic auto receptor. After approximately 12 weeks of chronic therapy, depolarization blockage of dopamine tracts occur. Decrease in dopamine neurotransmission has been found to correlate with the antipsychotic effects [6]. Haloperidol possesses extremely weak anticholinergic and β-adrenergic receptor blocking effects.

Parkinson's disease is the second most common neurodegenerative disorder, affecting 1% of the population aged 65 years of age and increasing up to 3% of the population over 80 years of age [7]. Parkinson's disease is characterized by resting

tremor, rigidity, postural abnormalities, stooped posture, bradykinesia, akinesia and festinating gait [8].The major pathological change in patients with Parkinson's disease is the loss of melanin containing dopaminergic neurons in zona compacta of the substantia nigra [9]. These pigmented neurons have been identified as nigrostriatal dopamine neurons [10], loss of these neurons results in decrease of dopamine content in striatum [11]. Clinical symptoms appear only when dopaminergic neuronal death exceeds a critical threshold 70-80% of striatal nerve terminals.

Many health foods and plant-derived substances touted to improve health are sold around the world. Garlic (*Allium sativum*) is believed by many people to be useful for disease prevention. S-Allylcysteine(SAC) is one of the water-soluble organosulfur compounds in garlic. The concentration of SAC in the garlic increases during extraction/aging[13]**.** S-Allylcysteine protected vascular endothelial cell from H_2O_2 induced injury [14] and inhibits Cu^{2+} induced LDL oxidation [15, 16]. S-Allylcysteine also decreases the morphological alterations in heart and liver of mice exposed to the anticancer drug doxorubicin [17-20].

MATERIALS AND METHODS

ANIMALS

Albino mice (25 to 35 g) in the Central animal house, Department of experimental medicine, Raja muthaiah medical college, Annamalai University were used in the present study. Animal handling and experimental procedures were approved by Institutional Animal Ethical committee, Annamalai University (Ethical no.479, Reg.no.1602010). The animals were kept under 12-h light / dark cycles,22^{0}C and 60% humidity with food and water ad libitum.

CHEMICALS

Haloperidol 4-[4-(4-chlorophenyl)-4-hydroxy piperidinol]-4'-Fluorobutyrophenone, thiobarbituric acid (TBA), reduced glutathione and 3, 5-dithio-bis-nitrobenzoic acid were purchased from Sigma Aldrich. All other chemicals were of analytical grade.

INDUCTION OF PARKINSON'S DISEASE:

Haloperidol powder was dissolved in saline (0.02 mg / 0.5 ml) and a dose of about 1mg / kg b.w / day was administered intraperitoneally for all the animals to induce Parkinson's disease. All animals were observed for 30 minutes post injection and then hourly intervals for next 3 hours.

EXPERIMEN TAL (Phase I and II):

The mice were randomized and divided into four groups of four animals each.

Group I - Normal mice with i.p injection of saline served as control mice.

Group II- Mice received intraperitoneal injection of haloperidol (1 mg/kg b.w) for seven days.

Group III - Mice are treated with S-Allylcysteine (30 mg /kg b. w) orally then received haloperidol for 7 consecutive days.

Group IV- Mice are treated with S-Allylcysteine (30mg/kg body weight) alone orally for seven days.

Phase I

At the end of the experiment (8th day), the animals were analysed for behavioural studies such as Open field (number of rearings, grooming and number of visits to the central and peripheral compartments were calculated during a single 5 minutes session), swim test [21] and catalepsy were performed.

Phase II

At the end of the experiment (8th day), measurement of Parkinson's disease were performed in all the groups. Following the behavioural studies, the mice were sacrificed by cervical dislocation and the brain was dissected for analysis.

Brain tissue preparation

The brains were post-fixed overnight at 4.C with cold 4% Para formaldehyde in 0.1M Phosphate buffer and soaked in 0.5M phosphate buffered saline containing 30% sucrose for cryoprotection. Serial 30 µm thick coronal sections were cut on a free freezing microtome and stored in cryoprotectant(25% ethylene glycol, 25% glycerol, 0.05M phosphate buffer) at 4.C until use for immune histochemical study.

Immunohistochemistry

Fixed mesencephallic cells on cover slips and sections were washed in phosphate buffered saline before immune staining, they were pre-treated with 1% hydrogen peroxide for 15 minutes then they were incubated with rabbit anti tyrosine hydroxylase antibody overnight at 25.C in the presence of 0.3% Triton X 100 and normal goat serum. They were incubated with antirabbits IgG for 90 minutes at room temperature. Samples were mounted on gelatine coated slices dehydrated and cover slipped in histomount medium.

RESULTS

Effect on behavioral patterns (phase1)
Haloperidol treatment showed increase in Vacuous Chewing Movements (VCM), tardive dyskinesia, facial jerking and catalepsy. Pretreatment with S-Allylcysteine inhibited the increase of haloperidol induced vacuous chewing movements, tardive dyskinesia, facial jerking and catalepsy (Table 1).

Open field Assessment:
Significant reduction in peripheral movements and central movements along with rearing and grooming in haloperidol injected mice (group III) compared to controls mice (group I). Pretreatment of S-Allylcysteine to haloperidol administered mice (group IV), made them to exhibit increased peripheral movements and central movements along with rearing and grooming significantly (Table1).

Swim Test Assessment:
Haloperidol treatment decreased swimming ability of mice. Pretreatment with S-Allylcysteine inhibited the haloperidol induced swimming impairment (Table2).

Effect on the levels of TBARS and Antioxidants:
Table 3 depicts the levels of Thio Barbutiric Acid Reactive Substances (TBARS) and antioxidants in midbrain of the control and experimental groups. Lipid peroxidation levels in midbrain region were significantly elevated in haloperidol treated animals (group II) relative to the control mice (group I). Prior treatment of S-Allylcysteine to haloperidol administered mice (group III) significantly reduced the levels of lipid peroxidation.The levels of reduced glutathione, superoxide dismutase, catalase, glutathione peroxidase decreased in midbrain region in haloperidol treated animals (group II) compared to the control mice (group I). Prior treatment of S-Allylcysteine to haloperidol administered mice (group III) significantly increased the reduced glutathione, superoxide dismutase, catalase and glutathione peroxidase levels (group I).

Neurochemical Estimations
Haloperidol injection to the mice showed significant reduction in the levels of dopamine, 3, 4, dihydoxyphenyl aceticacid and homo vallinic acid (GroupII). The S-Allylcysteine pretreated and Haloperidol administered mice showed a significant increase in level of dopamine and its metabolites(3,4,dihydoxyphenyl acetic acid and homo vallinic acid) compared to haloperidol intoxicated mice (Table3).

Immuno histochemical Analysis
Injection of haloperidol to the mice (Group II) showed the decrease in the dopaminergic neuron number, which is indicated by immuno histochemistry of Tyrosine hydroxylase positive cells. Tyrosine hydroxylase were normal in control (Group I) and S-Allylcysteine pretreated lesioned mice, the damage was reduced (Group III) (Fig.1)

Expression of neurotensin
Injection of haloperidol (GroupII) showed the increase in the expression of neurotensin, which is indicated by immunohistochemical analysis. Expression of neurotensin was normal in control and S-Allylcysteine (Group IV) pretreated mice. (Fig.2).

Table: 1. Changes in the behavior patterns of control and experimental mice.

Parameter	Group I	Group II	Group III	Group IV
Vacuous Chewing Movement	3.1 ± 0.12	23.4 ±2.89	12.9 ±1.48	2.61 ± 0.25
Tarsive Dyskinesia	1.1 ± 0.01	6.4 ± 0.39	3.11 ± 0.29	1.06 ± 0.01
Facial Jerking	0.06 ± 0.001	7.41 ± 0.69	3.98 ± 0.19	1.06 ± 0.05
Catalepsy	4.12 ± 0.08	15.41 ± 1.19	7.98 ± 1.39	4.89 ± 0.09
Peripheral movements (PMs/5Min.)	70 ± 7.0	30 ± 2.9	50 ± 4.8	70 ± 6.8
Central movements (CMs/5Min.)	60 ± 5.7	20 ± 1.9	40 ± 3.7	60 ± 5.6
Rearing	15 ± 1.4	7 ±0.69	13 ± 1.2	17 ± 1.6
Grooming	6.5 ± 0.62	4.0 ± 0.39	5.0 ± 0.48	6.0 ± 0.56

Values not sharing a common superscript letter differ significantly at $p<0.05$(DMRT).
Values are given as mean ± S.D for 4 mice in each group

Table 2: Swim Test Assessment of Control and Experimental mice.

SWIM TEST (Scoring method)			
Group I	**Group II**	**Group III**	**Group IV**
3.9 ± 0.37	3.8 ± 0.36	3.2 ± 0.30	2.8 ± 0.26
3.0 ± 0.29	2.8 ± 0.25	2.0 ± 0.16	1.8 ± 0.16
3.7 ± 0.32	3.4 ± 0.31	2.5 ± 0.21	2.0 ± 0.19
4.0 ± 0.38	4.4 ± 0.39	3.0 ± 0.28	3.1 ± 0.29

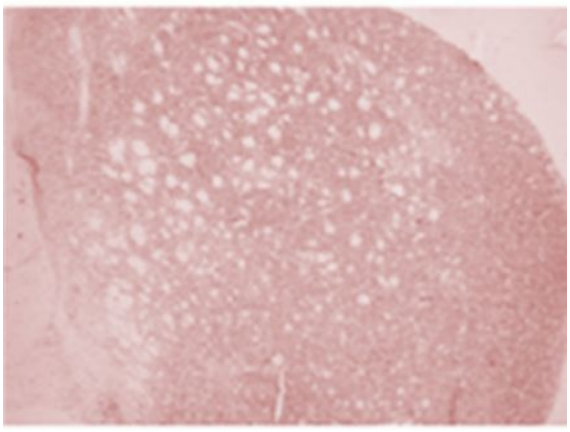
Values not sharing a common superscript letter differ significantly at $p<0.05$ (DMRT).
Values are given as mean ± S.D for 4 mice in each group

Table 3: Effect on the levels of TBARS, Reduced glutathione,Super oxide dismutase, Catalase , Glutathione peroxidase, Neurochemical substances in midbrain

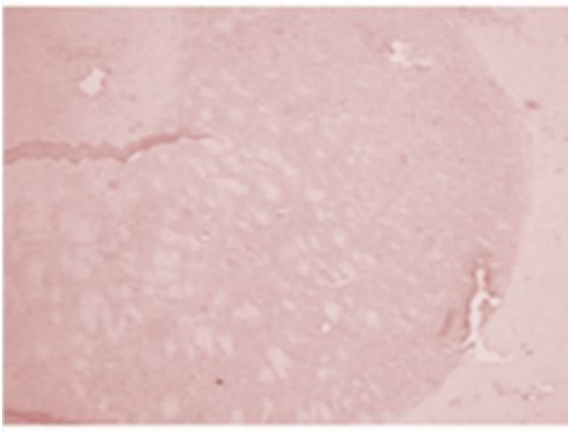
Group	TBARS	Reduced glutathione	Super oxide dismutase	Catalase	Glutathione peroxidase	Dopamine	Dihydroxy phenyl acetic acid	Homo vallinic acid
Group I	1.64±0.12	13.4±1.32	1.56±0.15	1.94±0.1	13.2±1.30	19.11±1.9	2.17±0.14	1.79±0.15
Group II	3.49±0.31	6.8±0.65	0.89±0.08	0.9±0.09	8.69±0.82	8.34±0.8	1.12±0.11	1.05±0.07
Group III	2.07±0.21	9.6±0.92	1.04±0.10	1.31±0.1	10.74±1.7	12.17±1.2	1.78±0.11	1.41±0.04
Group IV	1.61±0.11	13.03±1.30	1.63±0.6	1.97±0.2	13.86±1.5	20.14±2.1	2.19±0.15	1.83±0.11

Values not sharing a common superscript letter differ significantly at $p<0.05$ (DMRT).
Values are given as mean ± S.D for 4 mice in each group

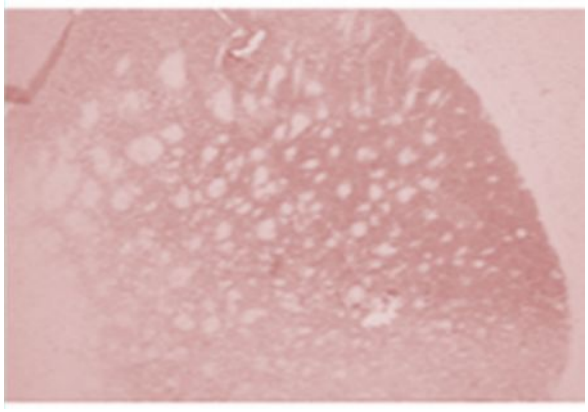
Fig.1.Tyrosine hydroxylase expression in striatum



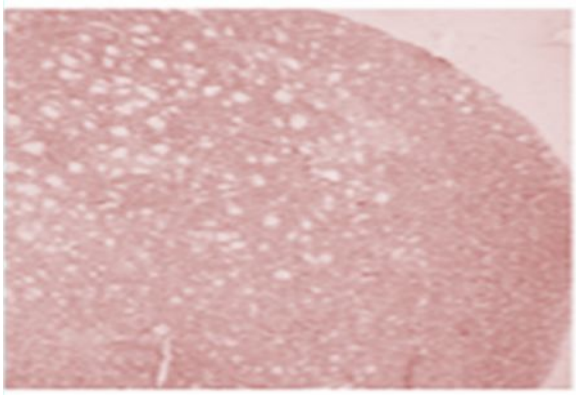
Group I



Group II



Group III



Group IV

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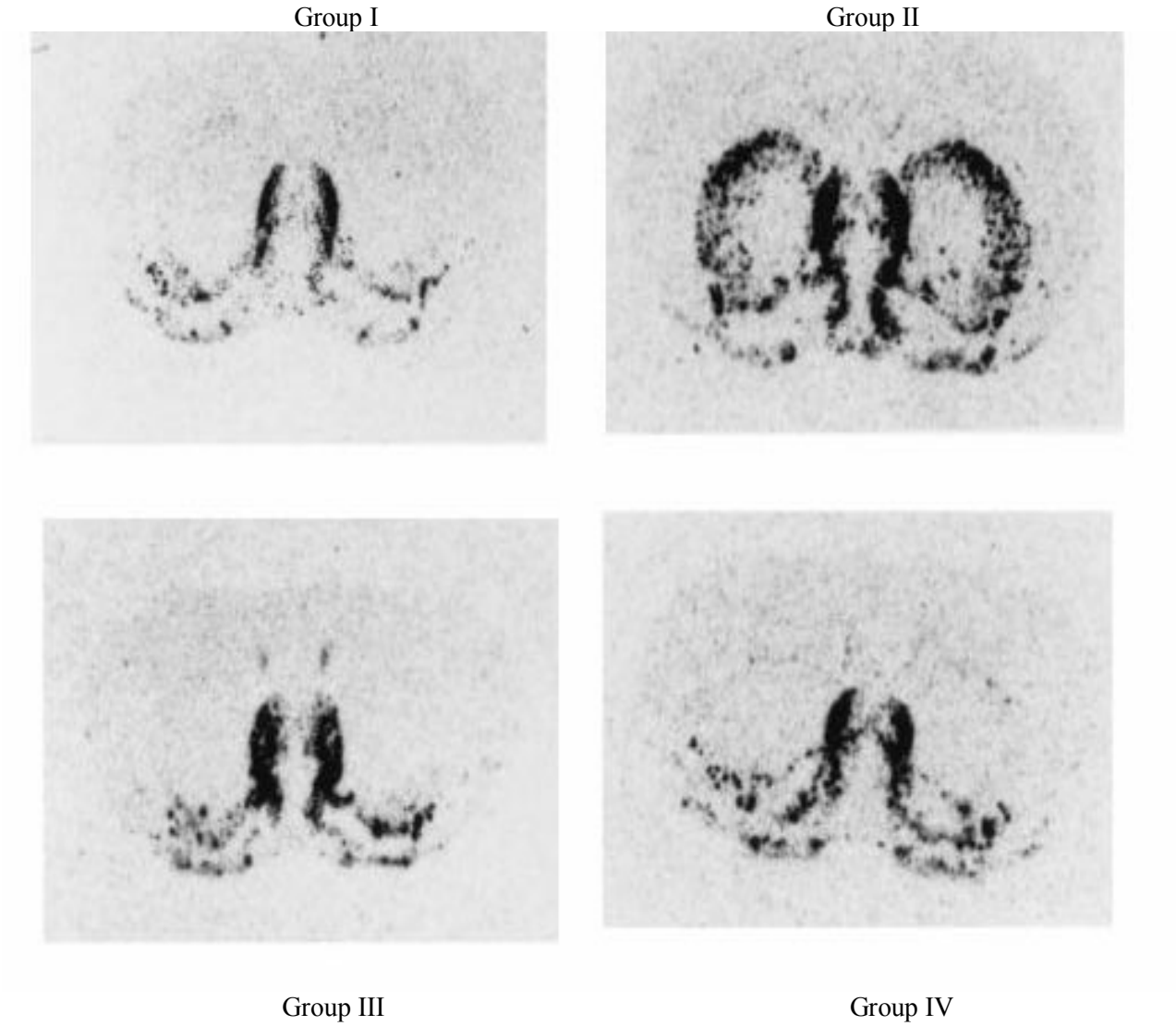
Fig. 2. Expression of striatal neurotensin



DISCUSSION

In the present study, chronic haloperidol treatment significantly increased vacuous chewing movement and tardive dyskinesia as compared to control animals. Neuroleptics act by blocking dopamine receptors [22]. Such blockage results in increased dopamine turnover, which in turn leads to increased production of hydrogen peroxide, resulting in oxidative stress [23,24]. Existing evidence indicates that excessive production of free radicals is associated with chronic neuroleptic use and might contribute to the onset of tardive dyskinesia and other movement disorders, such as dystonias and Parkinsonism[25].Swim test is an efficient method to examine motor damage for direct measurement of motor impairment with bilateral lesions in animal models [26].

Rats with vacuous chewing movements had significantly higher thiobarbituric acid reactive substances (TBARS) in the striatum, suggesting the possible involvement of lipid peroxidation and free radical production in the pathophysiology of Parkinson's disease. Dopamine is primarily metabolized through oxidation by monoamine oxidase to 3, 4-dihydroxyphenyl acetic acid. This reaction produces hydrogen peroxide. Dopamine is also metabolized by auto oxidation yielding superoxide radical. Hydrogen peroxide can further react with iron or copper ions to produce the hydroxyl radical, which is the most toxic free radicals. Increased dopamine turnover by neuroleptics could lead to excessive production of these potentially damaging free radicals [27].Oxygen free radicals are also reported to diminish the dopamine transporter function further increasing the extracellular dopamine levels.

Galili[28] *et al*., reported that the direct neurotoxic effects of haloperidol and its metabolites on mouse neuronal cultures and PC-12 cells were reversed by antioxidants. Burkhardt[29] *et al*., reported that, haloperidol inhibited complex I of the electron transport chain, this might be one of the possible mechanisms for the development of Parkinson's disease . Administration of single dose of haloperidol to mice led to increase oxidized glutathione (GSSG) levels in the striatum indicating generation of oxidative stress by the drug [30-32]. The histochemical analysis of Tyrosine hydroxylase, rate limiting enzyme in dopamine synthesis, was evaluated in mouse brain treated and untreated with S-allylcysteine. Haloperidol induce dopaminergic neuronal death, formation of TBARS and are selectively toxic to dopaminergic neurons that express the dopamine transporter. In the present study S-allylcysteine significantly protected dopaminergic neurons from haloperidol, resulting in an increase in the number of tyrosine hydroxylase positive cells.

CONCLUSION

In the present study, the activity of antioxidant enzymes, Tyrosine hydroxylase and the levels of dopamine, 3, 4, dihydroxy phenyl acetic acid and homo vallinic acid gets reduced after the administration of haloperidol. Oral administration of S-Allylcysteine protect haloperidol induced neurotoxicity through an inhibition of reactive oxygen species and increases the activities of antioxidant enzymes, Tyrosine hydroxylase and the levels of dopamine, 3, 4, dihydroxy phenyl acetic acid and homo vallinic acid. The findings of the present study suggested for the involvement of free radicals in the development of neuroleptic induced Parkinson's disease and point to S-Allylcysteine as a possible therapeutic option to treat this hyperkinetic movement disorder.

REFERENCES

1. Sim, K., Chua, T.H., Chan, Y.H., Mahendran, R. and Chong, S.A., Psychiatric comorbidity in first episode schizophrenia: a 2 year, longitudinal outcome study, *Journal of Psychiatric Research, 2006,* 40 (7),656–63.
2. Brown, S., Barraclough, B. and Inskip, H., Causes of the excess mortality of schizophrenia, *British Journal of Psychiatry*, 2000, 177, 212–217.
3. Cullen, K.R., Kumra, S. and Regan, J., Atypical Antipsychotics for Treatment of Schizophrenia Spectrum Disorders, *Psychiatric Times*,2008, 25,101-104.
4. Van, Os. J., Krabbendam, L., Myin-Germeys, I. and Delespaul, P., The schizophrenia envirome, *Current Opinion in Psychiatry* ,2005, 18 (2), 141–5.(*Retrieved* 2008-07-06).
5. Gorrod, J.W. and Fang, J.,On the metabolism of haloperidol.Xenobiotica, 1993,23, 495 - 508.
6. Naidu, P.S., Singh, A. and Kulkarni, S.K., Quercetin, a bioflavonoid attenuated haloperidol induced orofacial dyskinesia, *Neuropharmacology* ,2003,44, 1100–1106.
7. Lau, Y.S., Petroske, E., Meredith, G.E. and Wang, J.Q., Elevated neuronal nitric oxide synthase expression in chronic haloperidol treated rats,*Neuropharmacology , 2003,* 45, 986–994.
8. Bishnoi, M., Chopra, K. and Kulkarni ,S.K.,Involvement of adenosinergic receptor system in an animal model of tardive dyskinesia and associated behavioural, biochemical and neurochemical changes, *Eur J Pharmacol.* ,2006,552,55-66.
9. Hennah, W., Thomson, P., McQuillin, A., Bass, N., Loukola, A., Anjorin, A.Blackwood, D. and Curtis, D.,DISC1 association, heterogeneity and interplay in schizophrenia and bipolar disorder, *Mol. Psychiatry,2009,*14 (9), 865–73.
10. Lewis, R., Should cognitive deficit be a diagnostic criterion for schizophrenia, *Journal of Psychiatry and Neuroscience*, 2004, 29, (2), 102–113.
11. Meyer-Lindenberg, A., Miletich, R.S., Melsen, P.D., Esposito, G., Carson, R.E., Quarantelli, M., Weinberger, D.R. and Berman, K.F.,Reduced prefrontal activity predicts exaggerated striatal dopaminergic function in schizophrenia, *Nature Neuroscience*,2002,5, (3), 267–71.
12. Besret, L., Caldwell, M.A., Torres, E.M. and Dunnett, S.B., Antioxidant strategy to counteract the side effects of antipsychotic therapy: an in vivo study in rats, *Eur. J. Pharmacol.,* 2000,408(1),35-9.
13. Affany, A., Salvayre, R. and Douste-Blazy, L., Comparison of the predictive effect of various flavonoids against lipid peroxidation of erythrocyte membranes (induced by cumen hydroperoxide), *Fund. Clin. Pharm.* , 1987,1,451-7.
14. Widerlov, E., Lindstrom, L. H., Besev, G., Manberg, P. J., Nemeroff ,C.B., Breese ,G .R.,Kizer, J. S . and Prange, A., J. Jr. Am. J. sychiatry, 1982, 139, 1122–1126.

15. Zielinska, M., Kostrzewa, A., Ignatowicz, E. and Budzianowski, J., The flavonoids, quercetin and isorhamnetin 3-O-acylglucosides diminish neutrophil oxidative metabolism and lipid *peroxidation*, *Acta. Biochim. Pol*., 2001, 48, 183-189.
16. Nair, V., Arjuman, A., Gopalakrishna, H.N. and Nandinim, M., Effect of Withania somnifera root extract on haloperidol-induced catalepsy in albino mice, *Phytother. Res*., 2008, 22, 243-246.
17. Rice-Evans, C.A., Miller, N.J. and Paganga, G. , Structure-antioxidant activity relationships of flavonoids and phenolic acids, *Free Radic. Biol. Med.,1998,*20, 933-56.
18. Shivakumar, B.R.and Ravindranath, V.,Oxidative stress and thiol modification induced by chronic administration of haloperidol, *J. Pharmacol. Exp. Ther*., 1993, 265, 1137 - 1141.
19. Robak, J. and Gryglewski, R.J., Bioactivity of flavonoids, Pol. *J. Pharmacol., 1996,* 48, 555–564.
20. Manji, H.K., Gottesman, I. and Gould, T.d., Signal transduction and genes-to-behaviors pathways in psychiatric diseases, Science's STKE: *signal transduction knowledge environment, 2003,*207,49.
21. Donnan, G.A., Willis, G.L., Kaczmarczyk, S.J. and Rowe, P.,Motor function in the 1-methyl-4-phenyl-1, 2, 5, 6-tetrahydropyridine treated mouse, *J. Neurol. Sci.,1987,* 77,185–91.
22. Creese, G., Burt, D. and Synder, S.I.L., Dopamine receptor binding and pharmacological potencies and antischizophrenic drugs, *Science*,1976, 92, 481 - 483.
23. Chuahan, A., Chauhan, V. and Brown, W.T., Oxidative stress in autism: increased lipid peroxidation and reduced serum levels of ceruloplasmin and transferrin–the antioxidant proteins, *Life Sci.,2004,* 75,2539-2549.
24. Elkashef, A.M. and Wyatt, R.J., Tardive dyskinesia: possible involvement of free radical and treatment with vitamin E, *Schizophr. Bull*.,1994, 25, 731 - 740.
25. Burger, M.E.R., Zeni, G. and Rocha, J,B., Ebselen attenuates haloperidol-induced orofacial dyskinesia and oxidative stress in rat brain ,Pharmacol. Biochem. Behav.,2005, 81,608-15.
26. Costall, B. and Naylor, J.R., Mesolimbic involvement with behavioural effects indicating antipsychotic activity, Eur. J. Pharmacol.,1994, 27,46-58.
27. Fleckenstein, A.E., Metzger, R.R., Beyeler, M.L., Gibb, J.W. and Hanson, G.R., Oxygen radicals diminish dopamine transporter function in rat striatum, *Eur. J. Pharmacol*.,1994, 334, 111 - 114.
28. Galili, R., Mosberg, W., Weizman, A., Melamed, E. and Offen, D., Haloperidol-induced neurotoxicity – possible implications for tardive dyskinesia, 2000, *J. Neural. Transm*. , 107,479 - 490.
29. Burkhardt, C., Kelly, J.P., Lim, Y.H., Filley, C.M. and Parker, .D.J., Neuroleptic medications inhibit complex I of the electron transport chain, *Ann. Neurol*.,1993, 33, 512 - 517.
30. Cohen, G. and Spina,M.B., Hydrogen production in neurones:Implications for understanding Parkinson's disease, In: Progress in Parkinson's Research., ed. Hefti, F. & Weiner, W.J., 1997, 119-126.
31. Cadet, J.L., Lohr, J.B. and Jeste, D.V., Tardive dyskinesia and schizophrenic burnout: the possible involvement of cytotoxic free radicals. In Handbook of Schizophrenia, The Neurochemistry and Pharmacology of Schizophrenia. ed. Henn, F.A. and DeLisi, L.E.,1987, 425 – 438, Amsterdam: Elsevier.
32. Michael, F., Mazurek, Shiela, M., Savedia, Raja,S. and Bobba, Particia I Rosebush Persistent loss of tyrosine hydroxylase immunoreactivity in the substantia nigra after neuroleptic withdrawal, J.*Neurol. Neurosurg. Psychiatry.,1998,* 64,799-801.

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