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OCIMUM SANCTUM Linn. ATTENUATES HALOPERIDOL INDUCED TARDIVE DYSKINESIA AND ASSOCIATED BEHAVIOURAL, BIOCHEMICAL AND NEUROCHEMICAL CHANGES IN RATS

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Abstract:

The present study investigated the effect of *Ocimum sanctum*, an antioxidant, in haloperidol-induced tardive dyskinesia, biochemical (lipid peroxidation, reduced glutathione levels, antioxidant enzyme level (catalase) and neurochemical (neurotransmitter levels) parameters. Tardive dyskinesia is a complex hyperkinetic syndrome consisting of choriform, athetoid or rhythmically abnormal involuntary movements. The irreversibility of this hyperkinetic disorder has been considered a major clinical issue in the treatment of schizophrenia. The pathophysiology of tardive dyskinesia is not completely known. Numerous studies showed that an enhanced oxidative stress and increased glutamatergic transmission as well as inhibition in the glutamate uptake after the chronic administration of haloperidol are the cause for tardive dyskinesia. Haloperidol is a widely used neuroleptic drug for the treatment of acute and chronic psychosis. The use of haloperidol is limited by extrapyramidal movement disorders. Chronic administration of haloperidol (1 mg/kg i.p. for 21 days) significantly increased vacuous chewing movements (VCM's in rats which was dose-dependently inhibited by *Ocimum sanctum*. Pretreatment with *Ocimum sanctum* reversed these behavioral changes. Besides, haloperidol also induced oxidative damage in brain which was attenuated by *Ocimum sanctum*. On chronic administration of haloperidol, there was a decrease in turnover of dopamine, GABA and norepinephrine in the brain which was again dose-dependently reversed by treatment with *Ocimum sanctum.* The results of the present study suggested there is involvement of free radicals in the development of neuroleptic-induced tardive dyskinesia and *Ocimum sanctum* as a possible therapeutic potential to treat this disorder.

Key Words: Tardive dyskinesia, Haloperidol, *Ocimum sanctum.*

Introduction

Tardive dyskinesia is a complex hyperkinetic syndrome consisting of choriform, athetoid or rhythmically abnormal involuntary movements. Tardive dyskinesia (TD) occurs in 20–40% of the patients who are on chronic neuroleptic medication (Egan *et al*., 1997; Casey, 2000; Kulkarni and Naidu, 2001). The classical to typical antipsychotics such as haloperidol and chlorpromazine often cause distressing extrapyramidal side effects. These include motor disturbances like tardive dyskinesia and Parkinsonism (Grohman *et al*., 1990). TD appears months or years after the initiation of antipsychotic treatment and it may persist even after drug withdrawal and may be irreversible in 20% of schizophrenic patients (Andreassen and Jorgensen, 1994). The pathophysiology of tardive dyskinesia is not completely known. Increase in the density of striatal

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dopaminergic D2 receptors observed in humans and in experimental rodent models of tardive dyskinesia coincide with the appearance of extrapyramidal side effects. Role of the dopaminergic hypothesis as the main molecular mechanism of TD has been questioned on several grounds (Klawans and Rubovits, 1972). The participation of free radicals derived from the metabolism of dopamine and/or from an enhancement of the glutamatergic transmission, secondary to presynaptic dopamine receptors blockade has gained ample experimental support (Casey, 2000; Naidu and Kulkarni, 2001a, b; Tsai *et al*., 1998). Other neurochemical hypotheses proposed for the development of tardive dyskinesia include dopaminergic hypersensitivity, disturbed balance between dopamine and cholinergic systems, dysfunctions of striatonigral GABAergic neurons and excitotoxicity. Various animal studies have demonstrated an enhancement to glutamatergic participation as well as inhibition in the glutamate uptake after the chronic administration of haloperidol (Grimm *et al*., 1998; Burger *et al*., 2005a,b).

Ocimum sanctum Linn (OS), commonly known as Holy Basil, is considered as a sacred plant in India and grown in every rural household. Traditionally, fresh juice or decoction of OS leaves are used to promote health and in the treatment of various disorders as advocated in Ayurveda, the Indian System of Medicine. Indian Materia Medica describes the use of aqueous, hydroalcoholic and methanolic extract of OS leaves in a variety of disorders, like bronchitis, rheumatism and pyrexia (Nadkarni, 1976; Kritikar and Basu, 1965). Several recent investigations using these extracts have indicated that OS possesses significant anti-inflammatory (Singh *et al*., 1996), antioxidant (Maulik *et al*., 1997), immunomodulatory and antistress (Sen *et al*., 1992) properties. In addition, it has been reported to have radioprotective and anticarcinogenic property (Devi, 2000).

Alteration in catecholamine metabolism is one of the reasons of increased oxidative damage after haloperidol administration. Decreased levels of norepinephrine after the chronic administration of haloperidol reflect increased metabolism which can be correlated with the induction of oxidative damage. *Ocimum sanctum* has been reported to exhibit several medicinal properties. In the present study we have investigated the possible protective effect of *Ocimum sanctum* on haloperidol-induced tardive dyskinesia and related behavioural, biochemical and neurochemical alterations.

Methods and materials

Plant collection

The leaf of *Ocmum sanctum* Linn. have been collected from Sri Venkateshwara University near Tirupathi, Andhra Pradesh during the month of December 2009 and dried under shade. The plant was authentified by Dr. K. Madhava chetty, Assistant Professor, Department of Botany of S. V. University, Tirupathi.

Preparation of extracts

Leaves of *Ocimum sanctum* were shade dried and the dried leaves were powdered to get coarse granules. About 350g of dried powder was extracted with 80% methanol by continuous hot percolation, using soxhlet apparatus. The resultant black extract was concentrated upto 80ml on rotavapour under reduced pressure. The concentrated crude extract were lyophilised into powder (10g) and used for the study.

Animals

Adult male wistar rats weighing about 180-200g were taken for the study. Male rats were chosen to avoid fluctuations due to estrous cycle (Sugioka *et al*., 1987). The rats were housed in under standard laboratory conditions with 12 h light/dark cycle. The rats were fed with standard laboratory chow (Hindustan Lever Ltd., Mumbai) and water ad libitum. Animals were acclimatized to the laboratory conditions prior to experimentation. All the experiments were carried out between 900 and 1200 h. Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA (Ref No. IAEC / XIII / 05 / SVCP /2009 - 2010).

Acute Toxicity Study

The acute toxicity of 80% methanolic extract of *Ocimum sanctum* was determined as per the OECD guideline no. 423 (Acute Toxic Class Method). It was observed that the test extract was not mortal even at 2000mg/kg dose. Hence, 1/40th (50mg/kg), 1/20th (100mg/kg) and 1/10th (200mg/kg) of this dose were selected for further study (OECD, 2002).

Drugs and study protocol

The following drugs were used in the present study. Haloperidol (Serenace, Searle, India) was diluted with normal saline. *Ocimum sanctum* was dissolved in tween 80. Haloperidol and/or *Ocimum sanctum* was/were administered intraperitoneally and per-orally respectively in a constant volume of 0.5 ml per 100 g of bodyweight of rat. Animals were divided into six groups of six animals each. First group received vehicle (tween 80), second group received haloperidol (1 mg/kg, i.p) plus vehicle, third, fourth and fifth groups received haloperidol (1 mg/kg) plus *Ocimum sanctum* L (50, 100, and 200 mg/kg, p. o respectively). Haloperidol and *Ocimum sanctum* were administered simultaneously once daily (0900) in the morning for a period of 21 days

Induction of vacuous chewing movements (VCMS)

Haloperidol (1 mg /kg i.p) was administered chronically to rats for a period of 21 days to induce vacuous chewing movements (Naidu and Kulkarni, 2001a, 2001b, Naidu *et al*., 2002). All the behavioral assessments were carried out after 24 h of last dose of haloperidol.

Behavioural assessment of (VCMS)

On the test day rats were placed individually in a small (30 × 20 × 30 cm) Plexiglas cage for the assessment of VCMS. The floor and the back wall of the cage consisted of mirrors so as to permit observation of VCMS when the animal was faced away from the observer. Animals were allowed 10 min to get used to the observation cage before behavioral assessments. In the present study VCM are referred to as single mouth openings in the vertical plane not directed toward physical material. If tongue protrusion or VCM occurred during a period of grooming, they were not taken into account. Counting was stopped whenever the rat began grooming, and restarted when grooming stopped. Mirrors were placed under the floor and behind the back wall of the cage to permit observation of VCMS when the animal was faced away from the observer. The behavioural parameters of VCMS were measured continuously for a period of 5 min. In all the experiments, the scorer was unaware of the treatment given to the animals (Naidu *et al*., 2003).

Biochemical measurements

On 22nd day blood samples were collected from retro-orbital plexus under light ether anesthesia.

Estimation of catalase

2.5ml of phosphate buffer was added to 0.1 ml of serum and incubated at 25^{0}C for 30 min. After transferring into a cuvette the absorbance was measured at 240 nm, 650 µl of hydrogen peroxide solution was added to initiate the reaction. The change in absorbance was measured for 3 min (Beers and Sizer, 1952).

Estimation of reduced glutathione

To 0.5ml of citrated blood, 0.5 ml of 5% trichloroacetic acid (TCA) solution was added to precipitate the proteins and centrifuged at 3000 rpm for 20 min. To 0.1ml of supernatant, 1 ml of sodium phosphate buffer and 0.5ml of DTNB reagent were added. The absorbance of the yellow color developed was measured at 412nm (Ellman, 1959).

Estimation of lipid peroxidation

0.1ml of plasma was treated with 2 ml of TBA 0.37%, 0.25N HCl, and 15% TCA (1:1:1 ratio) and placed in water bath for 15 min, cooled and centrifuged and then clear supernatant was measured at 535nm against reference blank (Nichans and Samuelson, 1959).

A fluorimetric micromethod for the simultaneous determination of noradrenaline and dopamine in brain

On the 22nd day after behavioural assessments all group rats were sacrificed, whole brain was dissected out. Weighed quantity of tissue was homogenized in 0.1 ml hydrochloric acid - butanol, (0.85 ml of 37% hydrochloric acid in one liter *n*- butanol for spectroscopy) for 1 min in a cool environment. The sample was then centrifuged for 10 min at 2,000 rpm. 0.08 ml of supernatant phase was removed and added to an Eppendorf reagent tube containing 0.2 ml of heptane (for spectroscopy) and 0.025 ml of 0.1 M hydrochloric acid. After 10 min of vigorous shaking, the tube was centrifuged under same conditions to separate two phases. Upper organic phase was discarded and the aqueous phase (0.02 ml) was used for estimation of Nor Adrenaline and Dopamine assay (Schlumpf *et al*., 1974).

Nor-adrenaline and dopamine assay

The assay represents a miniaturization of the trihydroxide method. To 0.02ml of HCl phase, 0.05ml 0.4M EDTA and 0.01ml Sodium acetate buffer (pH 6.9) were added, followed by 0.01ml iodine solution (0.1M in ethanol) for oxidation. The reaction was stored after two minutes by addition of 0.01ml Na_2SO_3 in 5ml NaOH. Acetic acid was added 1.5 minutes later. The solution was then heated to 100^o c for 6 minutes. When the sample again reached room temperature, excitation and emission spectra were read in the microcuvette. In some cases, the readings were limited to the excitation maxima. 395-485nm for NA and 330-375nm for DA uncorrected instrument values (Schlumpf *et al*., 1974).

Estimation of brain GABA content

The brain amino butyric acid (GABA content was estimated according to the method of Lowe *et al*., (1958). Animals were sacrificed by decapitation and brains were rapidly removed, weighed and placed in 5ml of ice-cold trichloroaceticacid (10% w/v), then homogenized and centrifuged at 10,000 rpm for 10 min at 0°C. A sample (0.1ml) of tissue extract was placed in 0.2ml of 0.14 M ninhydrin solution in 0.5M carbonate bicarbonate buffer (pH 9.95), kept in a water bath at 60°C for 30min, then cooled and treated with 5ml of copper tartrate reagent (0.16% disodium carbonate, 0.03% copper sulphate and 0.0329% tartaric acid). After 10 min fluorescence at 377/455nm in a spectofluorimeter was recorded (Lowe *et al*., 1958).

Statistical analysis

All the values are expressed as mean ± S.E.M. The data were analyzed by using analysis of variance (ANOVA) followed by Dunnett's test. In all tests, the criterion for statistical significance was $P< 0.05$.

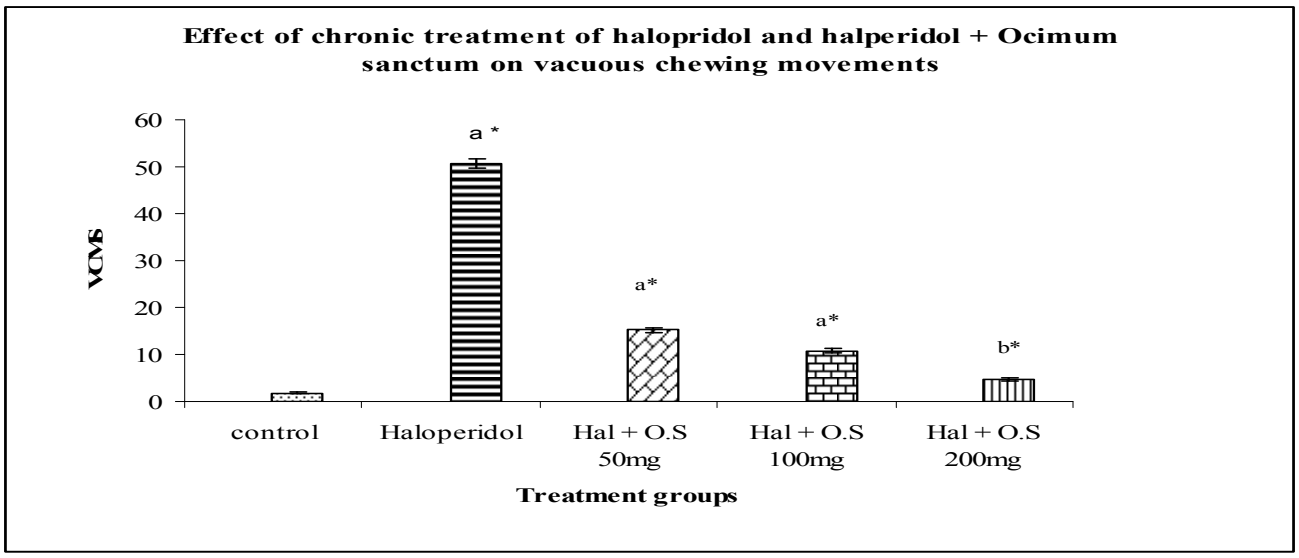
RESULTS

Assesment of vacuous chewing movement

Chronic haloperidol (1mg/kg) treatment resulted in significant increase in VCM's. Pretreatment with *ocimum sanctum* (50, 100, and 200 mg/kg) dose dependently decreased the haloperidol induced vacuous chewing movements. *ocimum sanctum* (50mg/kg) did not cause any significant change in VCM's.

Catalase activity:

Chronic haloperidol treatment (1mg/kg) significantly decreased the catalase levels when compared to control animals**.** Pretreatment with *ocimum sanctum* (100 and 200 mg/kg) significantly reversed the haloperidol induced decrease in catalase levels. *ocimum sanctum* (50mg/kg)did not significantly reversed the haloperidol induced decrease in catalase levels compared to control.



[a] $p<0.001$, [b] $p<0.01$ statistically significant compared to control

* $p<0.001$ statistically significant compared to haloperidol

Biochemical Estimations:

Biochemical assessment:

Groups	Lipid peroxidation (nmol/ml/hr)	Reduced glutathione (mmol/ml/hr)	Catalase (micromole H_2O_2Degraded/mg protein/ min)
Control	10.33 ± 0.30	1876.7 ± 18.9	48.132 ± 0.23
Haloperidol	15.83 ± 0.49 [a,*]	493.33 ± 14.29[a,*]	28.69 ± 0.15 [a,*]
Haloperidol +*Ocimum sanctum* 50mg	9.92 ± 0.27*	1650 ± 18.439[a,*]	55.8 ± 0.19 [a,*]
Haloperidol +*Ocimum sanctum* 100mg	8.58 ± 0.24 [a,*]	5343.3 ± 6.146 [a,*]	60.97 ± 0.16 [a,*]
Haloperidol +*Ocimum sanctum* 200mg	5.50 ± 0.35 [a,*]	6403.3 ± 14.981[a,*]	62.77 ± 0.47 [a,*]

[a] $p<0.01$ statistically significant compared to control

* $p<0.01$ statistically significant compared to haloperidol

Neurochemical assessment:

Groups	GABA (pg/mg)	Dopamine (pg/mg)	Noradrenaline (pg/mg)
Control	245 ± 0.96	345.52 ± 0.89	336.67 ± 0.96
Haloperidol	123.33 ± 0.61[a]	170.78 ± 0.86[a]	178.33 ± 0.87[a]
Haloperidol +*Ocimum Sanctum* 50mg	233.33 ± 1.12	290.42 ± 1.13[a]	248.33 ± 0.65[a]
Haloperidol +*Ocimum Sanctum* 100mg	281.67 ± 0.98[a]	375 ± 0.62	355 ± 1.36
Haloperidol +*Ocimum Sanctum* 200mg	365 ± 0.89[a]	445.67 ± 0.89[a]	433.33 ± 0.99[a]

[a] $p<0.01$ statistically significant compared to control

* $p<0.01$ statistically significant compared to haloperidol

Estimation of reduced glutathione

Chronic haloperidol treatment (1mg/kg) significantly decreased reduced glutathione levels when compared to control animals**.** Pretreatment with *ocimum sanctum* (100 and 200 mg/kg) significantly reversed the haloperidol induced decrease in reduced glutathione in levels. *ocimum sanctum* (50mg/kg) did not significantly reversed the haloperidol induced decrease in reduced glutathione levels compared to control.

Lipid peroxidation assay:

Chronic haloperidol treatment (1mg/kg) significantly increased lipid peroxidation levels when compared to control animals**.** Pretreatment with *ocimum sanctum* (100 and 200 mg/kg) significantly decreased the haloperidol induced increase in lipid peroxidation levels. *ocimum sanctum* (50mg/kg) did not significantly reversed the haloperidol induced increase in lipid peroxidation levels compared to control.

Neurochemical assessment:

Chronic haloperidol treatment (1mg/kg) resulted in decreased levels of dopamine, norepinephrine and GABA in brain. It was prevented by Pretreatment with *ocimum sanctum* (100 and 200 mg/kg). *Ocimum sanctum* (50mg/kg) did not significantly reverse the haloperidol induced decrease in dopamine, norepinephrine and GABA levels compared to control.

Discussion and conclusion:

In the present study, chronic treatment with haloperidol in rats showed increased frequencies of VCMs as compared to control animals. The administration of *ocimum sanctum* dose dependently showed protective effect against haloperidol induced vacuous chewing movements. Numerous studies showed that chronic treatment with typical neuroleptics results in excessive production of free radicals (Cadet *et al*., 1986; Peet *et al*., 1993; Adler *et al*., 1993a, b, 1998).

Haloperidol is a typical antipsychotic drug widely used for treatment of mental disorders; it is capable of causing an extrapyramidal syndrome in humans. Tardive dyskinesia is a major clinical issue in psychiatry and occurs in approximately 20% of antipsychotic-treated patients (Lohr *et al,* 2003a, b). The neuropathophysiology of TD has been related to the generation of free radicals and oxidative stress (Cadet *et al,* 1986; 1987; Lohr, 1991; Casey, 1995; Tsai *et al,* 1998; Naidu and Kulkarni, 2001; Burger *et al.,* 2003, 2005a, b). Typical neuroleptics block the D2 receptors in brain which is associated with increase in catecholamine turn over in brain resulting in over production of free radicals (Lohr *et al*, 2003; Ravindranath and Reed, 1990;). The increased free radical production is associated with decrease in antioxidant defense mechanism. The results of the present study indicated haloperidol treatment decreased the levels of CAT, GSH and increased the lipid peroxidation. Haloperidol decreases the genetic expression of MnSOD, CuZnSOD and CAT and thus decreases the enzymatic activities and protein content of SOD, GPx, CAT (Parikh *et al*., 2003).The antioxidant enzymes play a important role in maintaining physiological levels of free radicals by decreasing the production of organic peroxides and hydrogen peroxides. In our study *Ocimum sanctum* dose dependently decreased the vacuous chewing movement induced by haloperidol and increased the levels of antioxidant enzymes and decreased the lipid peroxidation.

The neurochemical hypothesis proposed for the development of TD during the last decades includes disturbed balance between dopaminergic and cholinergic systems, dysfunction of striatonigral GABAergic neurons, excitotoxicity promoted by glutamate and overproduction of free radicals (Andreassen and Jorgensen, 2000; Cadet *et al*., 1986, 1987; Lohr, 1991). Reduction in GABA is thought to be important in the etiology of TD. In fact, it has been described a decrease in the GAD activity and in the levels of GABA in brain regions of monkeys with dyskinetic symptoms induced by neuroleptics (Gunne *et al*., 1984). In the present study haloperidol decreased the dopamine noradrenaline and GABA levels in the brain.

Chronic haloperidol administration resulted in a decrease in dopamine, norepinephrine and serotonin turnover in cortex as well as subcortical regions of the brain (Cahir *et al*., 2004; Bishnoi *et al*., 2007a,b). Chronic administration of haloperidol may increase the number of dormant receptors, hence resulting in decrease in dopamine turnover in extracellular spaces in the brain (Bishnoi *et al*., 2007a, b; Cara *et al*., 2001). Ocimum sanctum dose-dependently prevented the decrease in dopamine, nor adrenaline and GABA turnover in brain.

Increased expression of cyclooxygenase and lipooxygenase gene and PGE2 and LTB4 production has been implicated in neurodegeneration in several neurological diseases (Wang *et al*; 2005). Cyclooxygenase has been implicated in the signaling mechanisms of receptors like dopamine including glutamate and GABA which plays an important role in motor behaviour. The glutamate receptor agonist NMDA stimulates eicosanoid synthesis (Lazarewicz *et al*., 1990; Mollace *et al*., 1995), whereas NMDA-induced allodynia and brain c-fos expression are both prostaglandin-dependent processes (Lerea *et al*., 1997; Dolan and Nolan, 1999). The NMDA receptor antagonist, MK-801, completely blocks glutamate and NMDA toxicity. MK-801 also attenuates COX2 mRNA induction in glutamate-treated neural cultures (Hewett *et al*., 2000; Strauss and Marini, 2002). From these findings it indicates that excitotoxic levels of glutamate receptor agonists (kainate >> NMDA >> AMPA) increase neuronal COX2 expression, implicating COX2 as an effector of neurological deficits. The COX2 activity is intimately linked to excitotoxic neuronal death. Glutamate produces its toxicity by increasing the intracellular calcium levels. The intracellular calcium levels represent the final common pathway of several injurious stimuli and it is responsible for triggering the pro-oxidants and protease system that ultimately cause neuronal death (Mahendra Bishnoi *et al*; 2008). Recently it was reported that cyclooxygenase enzyme inhibitors attenuates the development of tardive dyskinesia in rats (Naidu and Kulkarni, 2001a,b).The fixed oil in *ocimum sanctum* is a potent inhibitor of both the pathways, i.e; cyclooxygenase and lipoxygenase and its protective action is due its COX and LOX inhibitory activity. (Surender Singh *et al*, 1996). In the present study the neuroprotective action of *Ocimum sanctum* may be due to its COX and LOX inhibitory activity.

Experimental studies have shown that phenolic compounds, particularly flavanoids and catechins, are important antioxidants and superoxide scavengers. Their scavenging efficiency depends on the concentration of phenol and the number and location of the hydroxyl groups. Some researchers reported the presence of phenols and flavanoids in *O. sanctum* (Benavente-Garcia *et al*., 2002 and Kelm *et al*., 2000). The antioxidant activity of *Ocimum sanctum* is the main cause for its pharmacological actions. *Ocimum sanctum* extract contains phenolic compounds like eugenol,crisilineol, isothymusin, isothymonin, rosmarinic acid (Kelm etal;200), orientin, and vicenin (Vrinda and Uma Devi, 2001) have been proved as good antioxidant compounds and zinc , an antioxidant mineral, has been found to be high in *Ocimum sanctum* (Samudralwar and Garg,1996). The quantification of phytochemicals in the *Ocimum sanctum* sample used for this study has also revealed the presence of substantial amount of phenolics, flavonoids and carotenoids.

In conclusion, the results of the present study suggests that *Ocimum sanctum* reverse the behavioural, biochemical and neurochemical changes induced by chronic haloperidol administration by virtue of its potent antioxidant activity and inhibition of cyclooxygenase and lipoxygenase enzymes. Other than its antioxidant action *Ocimum sanctum* also may have a direct action on the central monoaminergic system. On chronic use *Ocimum sanctum* may prevent the development of neuroleptic induced tardive dyskinesia. However further studies must be carried out to elucidate its exact mechanism in neuroprotection.

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