RESEARCH ARTICLE

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Amelioration of 6-Hydroxydopamine-Lesion Induced Neurotoxicity by Different Extracts of *Ocimum sanctum* Leaves in Rodent Model of Parkinson's Disease

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Abstracts: Neuroprotective effect of Petroleum-ether (PE), Ethyl acetate (EA) and Chloroform fractions ($CHCl_3$) from leaves of *Ocimum sanctum* (OS) were investigated on 6-hydroxy dopamine (6-OHDA) lesioned rodent model of Parkinson disorder (PD). PE, EA and $CHCl_3$ fractions from ethanolic extract of the leaves of OS were pretreated (300 mg/kg) three weeks before unilateral injection of 6-OHDA on 22nd day (10µg/2µl) into the right striatum. The behavioral observations, oxidative biochemical markers, Dopamine D_2 receptor binding assay, Quantification of Dopamine (DA) and its metabolites and tyrosine hydroxylase (TH) immunohistochemistry were evaluated after three weeks of leison. Increase in apomorphine-induced rotations and deficits in locomotor activity & muscular coordination due to lesion were significantly restored in EA treated group, PE and $CHCl_3$ fractions leaves of OS. Pretreated animals showed significant protection against neuronal degeneration compared to leison animals by normalizing the deranged levels of biomarkers with EA, PE, and $CHCl_3$ fractions of the leaves of OS. The results were further supported by D_2 receptors binding assay, Quantification of DA and its metabolites and TH immunohistochemistry study. Different extract of OS significantly prevented neurodegenration associated with PD in rats. Further, OS annetuated the neuropsychological symptoms associated with animal models of PD. These neuroprotective effects observed with OS can be attributed to its anti-oxidant activity.

INTRODUCTION

Parkinson's disease (PD) is one of the major neurodegenerative disorder characterized by the progressive loss of the dopaminergic neurons in the substantia nigra pars compacta and accompanied by the symptoms of muscular rigidity, rest tremor, bradykinesia and stooped posture[1]. This degeneration of the dopaminergic neurons involves a corresponding decrease in the levels of dopamine (DA) and its metabolites 3, 4-Dihydroxyphenylacetic acid (DOPAC) & Homovanillic acid (HVA) [2]. Despite repeated attempts, no researchers have been successful in developing a suitable cure to this deadly disease. The only successful therapy i.e. L-DOPA treatment, when given prolonged, is associated with irreversible adverse effects and development of motor complications such as fluctuations and dyskinesia[3]. Such adverse effect limits the ongoing symptomatic treatment for PD and has motivated search for new natural moieties for PD with minimum or no side effects.

6-OHDA, a specific dopaminergic neurotoxin, is a hydroxylated analog of neurotransmitter DA and has been commonly adapted by researchers to produce experimental animal models of PD[4]. A direct stereotaxic injection of 6-OHDA into the stratum causes injury to dopaminergic neurons due to formation of various oxidants and free radicals, lipid peroxidation, damaged proteins and mitochondrial complex I defect leading to depletion of reduced glutathione content, SOD and Catalase activities[5]. Adding up to this issue, it was earlier reported that L-DOPA administration has resulted in endogenous 6-OHDA formation in rat brain[6].

Plant *Ocimum sanctum,* Tulsi (OS) belongs to family "Labitae" and is considered a sacred medicinal plant by Hindus[7]. The essential oils extracted from fresh leaves of *Ocimum sanctum L.* has been found to show therapeutic potential mainly due to eugenol (l-hydroxy–2-methoxy–4–allylbenzene) which is the major constituent, a phenolic compound [8]. Review of literature reveals that Tulsi leaves exhibits to be antidiabetic, wound healing, antibacterial, anti-tussive, anti-ulcer and immunomodulatory[9, 10, 11, 12]. OS can reduce oxidative stress, which is one of the causative factors for PD and furthermore it contains linalool which can enhance DA concentration[13], therefore the present study was designed to evaluate effect of different extracts of OS in experimentally induced PD like condition.

MATERIALS AND METHODS

Plant Materials

The fresh leaves of OS (locally named 'Tulsi') were collected from medicinal plant garden, Delhi Institute of Pharmaceutical Sciences and Research (DIPSAR), Sec-3, Pusph Vihar, University of Delhi, New Delhi. The leaves were dried and grinded to a coarse powder which was used for cold extraction. The authenticity of the OS was identified by Department of Botany, DU.

Preparation and Fractionation of Crude Extracts

The coarse powder was submerged in ethyl alcohol (96%) and allowed to stand for several days (7-10) with occasional shaking and stirring. When the solvent become concentrated, the liquid alcohol content was filtered through cotton and then through filter paper (Whatman filter paper #1). Then the solvents were allowed to evaporate using rotary evaporator at temperature 40-45°C. Thus the highly concentrated crude extracts were obtained. They were then fractionated using PE, EA and $CHCl_3$. The

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Table 1: Scatchard analysis of ^{3}H-spiperone binding activity to striatal membranes of rats in Sham, Lesion, L + PE ext, L + EA ext, L + Chloro ext

Parameter	Sham	Lesion	L + PE Ext	L + EA Ext	L + Chloro Ext
Bmax	507.7 ± 9.22	958.1 ± 7.54	762.3 ± 11.01[a]	564.2 ± 08.24[a,b]	865 ± 11.27 [a+,b,c]
Kd	0.91 ± 0.02	0.49 ± 0.01	0.74 ± 0.03[a]	0.89 ± 0.04 [a,b++]	0.67 ± 0.01 [a,b+,c]

Bmax-maximum number of binding sites expressed in pmoles bound/g protein; Kd-dissociation constant expressed in nM. Values are in mean ± S.E.M. (n=6). Levels of significance: [a] P≤0.001; [a+]P≤0.01 compared with Lesion; [b]P≤0.001; [b+]P≤0.01; [b++]P≤0.05 compared with Lesion+PE Ext; [c]P≤0.001; [c++]P≤0.05 compared with Lesion+EA Ext

Table 2: Effect of Curcumin, DMC & BDMC on striatal dopamine, DOPAC and HVA levels (mg/g of brain tissue) in in rats lesioned by 6-OHDA

	Dopamine (DA)	3,4-Dihydroxy phenyl acetic acid (DOPAC)	Homovanillic acid (HVA)
S	13.17±0.79	1.867±0.05	6.333±0.12
L	2.5±0.42	0.4333±0.04	1.567±0.14
L+PE Ext	8.167±0.47[a]	1.003±0.06[a]	4.033±0.19[a]
L+EA Ext	10.0±0.57 [a,b+]	1.333±0.05 [a,b+]	5.133±0.20 [a,b+]
L+Chloro Ext	6.833±0.60 [a,b,c]	1.067±0.09 [a,b++]	3.8±0.18 [a,b,c+]

Values are in mean ± S.E.M. (n=6). Levels of significance: [a] P≤0.001; [a+]P≤0.01 compared with Lesion; [b]P≤0.001; [b+]P≤0.01; [b++]P≤0.05 compared with Lesion+PE Ext; [c]P≤0.001; [c++]P≤0.05 compared with Lesion+EA Ext

dried fractionated extracts were then preserved in the refrigerator for the experimental use.

Chemicals

6-OHDA hydrochloride was purchased from Sigma-aldrich chemicals Co Pvt. Ltd. Enzymatic assay kits were of Cayman chemical company, Michigan, USA. 1 phenyl-4-3H-spiperone (18.5 ci/mMoles, Perkin Elmer, USA), Tris-HCl (CDH, Mumbai, India). All chemicals and reagents used were of analytical grade except ethanol (which was of commercial grade obtained from Excise department, New Delhi, India).

Animals, Group Distribution and Treatment

Male albino wistar rats (200-250 g), procured from the animal house of DIPSAR, New Delhi, were used. Rats were housed in temperature controlled room; 25 ± 2 °C with a 12-hour light/dark cycle and 57 ± 7 % relative humidity under standard hygienic conditions and had free access to fresh tap water & pelleted diet (Amrut rat feed, India). Animals were acclimatized for seven days prior to experimental use.

The study was priory approved by the Institutional Animal Ethics Committee (IAEC/DIPSAR/2010-I/02) of DIPSAR, New Delhi and all followed experiments were carried out in accordance with the guidelines laid down by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India.

The animals were randomly divided into 5 groups of 8 animals in each group.

- Group I served as sham (S) control and received 2µl of 0.1% ascorbic acid in 0.9 % NaCl (normal saline) on 22nd day.
- Group II animals, lesioned (L) were treated once with 6-OHDA (10 µg/2 µl; 0.1 % ascorbic acid-saline) in the striatum.
- Group III animals (L+PE Ext) received PE fraction from petroleum extract of the leaves of OS (300 mg/kg body weight p.o. suspended in 0.5% CMC) for three weeks followed by single injection of 6-OHDA in the striatum on the 22nd day.
- Group IV animals (L+EA Ext) received ethyl acetate fraction (300 mg/kg/p.o. suspended in 0.5% CMC) for three weeks as a pretreatment followed by 6-OHDA administration as in group III.
- Group V animals (L+$CHCl_3$) received chloroform fraction (300 mg/kg/p.o. suspended in 0.5% CMC) as pretreatment for three weeks followed by 6-OHDA administration as in group III.

EXPERIMENTAL DESIGN

Surgery

Injection of 6-OHDA

The food was withdrawn 10-12 h before the surgical procedure. The rats were anesthetized with chloral hydrate 350 mg/kg i.p. After anesthesia, the head of the rat was mounted in a stereotaxic apparatus (Inco, Ambala, India) frame. The steriotaxic cooridinates were used as described by Paxinos and Watson, (1982), and were measured accurately as anterioposterior 0.5 mm, lateral 2 .5 mm and ventral 5.0 mm relative to bregma and ventral from dura with the tooth bar set at 0 mm. All the animals in the experimental group were lesioned in right unilateral region by injecting 10 µg/2 µl 6-OHDA (in 0.1% in ascorbic acid–saline) into the right striatum through the drilled hole, with the help of a very fine Hamilton syringe (28 Gauge, Outer diameter 0.362mm and Internal diameter of 0.184 mm, Hamilton bonaduz AG, Switzerland). The sham group was followed with same surgical procedure but injection of 6-OHDA was replaced with 2.0µl of 0.1% ascorbic acid–saline.

BEHAVIORAL STUDIES

Apomorphine Induced Rotational Behavior Study[14]

On 22nd day after the lesion, apomorphine was injected subcutaneously at a dose of 0.5mg/K.G. to monitor neurodegenerative effect of 6-OHDA and protective effect of different fractions of OS on contralateral rotations. The rotational scores were collected over a period of 60 minutes.

Rota Rod Study

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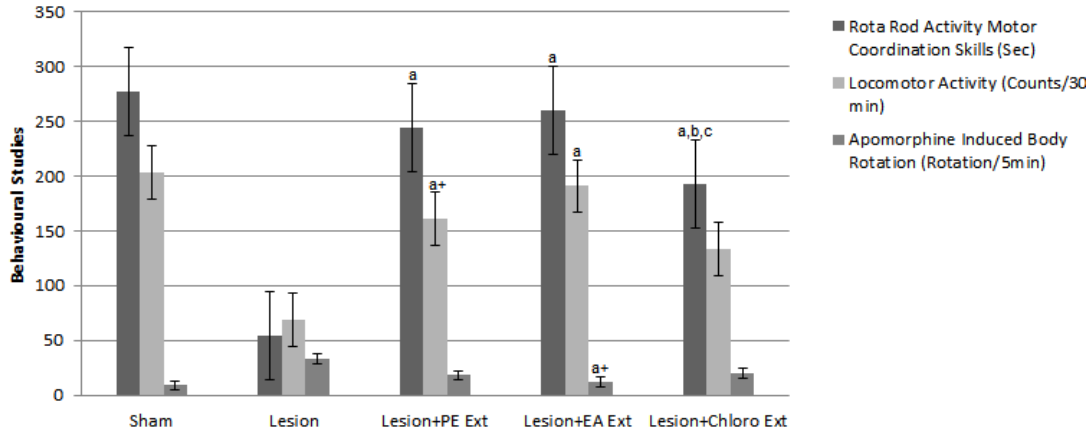
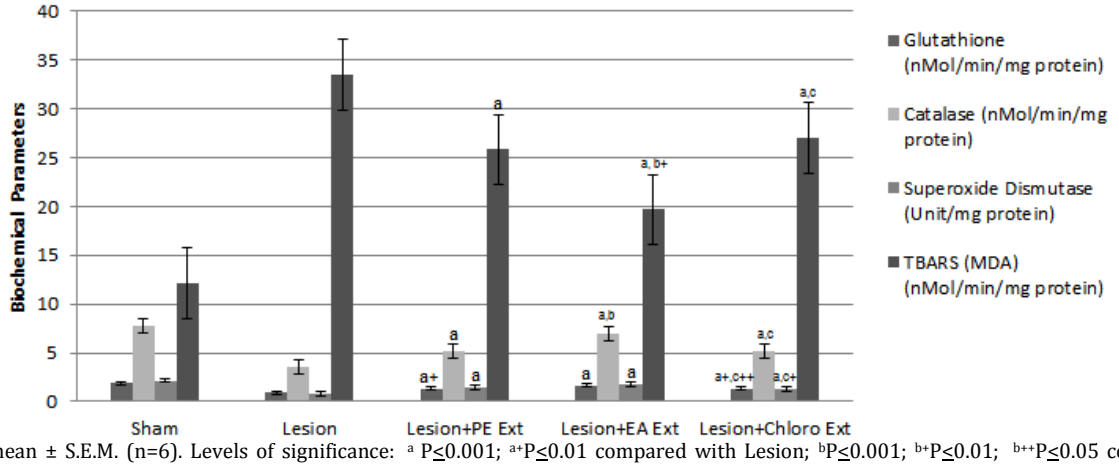


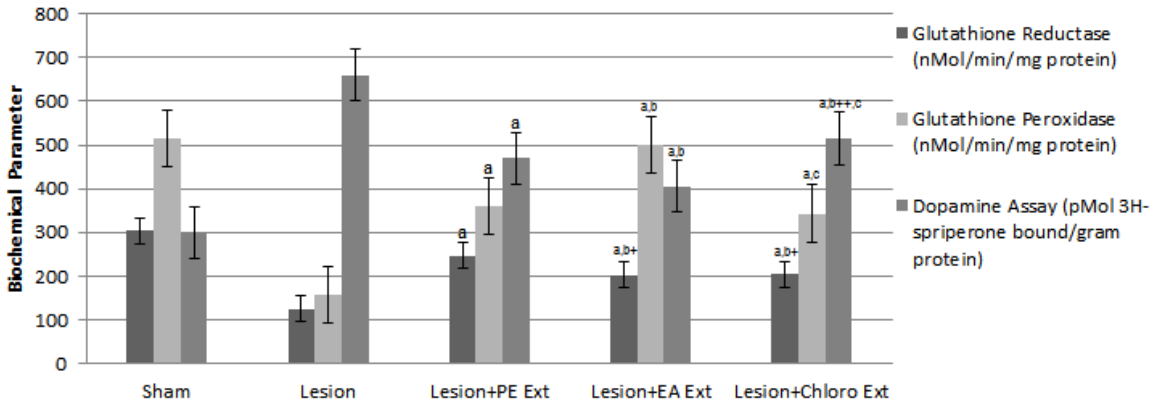
Values are in mean ± S.E.M. (n=6). Levels of significance: [a] P≤0.001; [a+]P≤0.01 compared with Lesion; [b]P≤0.001; [b+]P≤0.01; [b++]P≤0.05 compared with Lesion+PE Ext; [c]P≤0.001; [c++]P≤0.05 compared with Lesion+EA Ext

Figure 1: Effect of PE ext, EA ext & Chloro ext of *Osimum santacum* pretreatment on muscular coordination, locomotor activity and neuroprotective effect on apomorphine induced body rotations in rats lesioned by 6-OHDA



Values are in mean ± S.E.M. (n=6). Levels of significance: [a] P≤0.001; [a+]P≤0.01 compared with Lesion; [b]P≤0.001; [b+]P≤0.01; [b++]P≤0.05 compared with Lesion+PE Ext; [c]P≤0.001; [c++]P≤0.05 compared with Lesion+EA Ext

Figure 2: Effect of PE ext, EA ext & Chloro ext of *Osimum santacum* on glutathione, catalase, superoxide dismutase activity and malondialdehyde (MDA) content in lesioned rats striatum by 6-OHDA



Values are in mean ± S.E.M. (n=6). Levels of significance: [a] P≤0.001; [a+]P≤0.01 compared with Lesion; [b]P≤0.001; [b+]P≤0.01; [b++]P≤0.05 compared with Lesion+PE Ext; [c]P≤0.001; [c++]P≤0.05 compared with Lesion+EA Ext

Figure 3. Effect of of PE ext, EA ext & Chloro ext of *Osimum santacum* on glutathione reductase, glutathione peroxidase content and dopamine receptor (DA-D_2) binding activity in lesioned rats striatum by 6-OHDA

Protective effect of PE, EA and $CHCl_3$ partitionates of OS leaf extracts on motor coordination was studied in rats by Rota Rod apparatus (Inco, Ambala, India) following a standard procedure[15]. The apparatus consists of a metal rod of 4 cm in diameter, 75 cm in length with 6 equally divided sections and speed adjusted to 8 rotations / minute.

Spontaneous Locomotor Study

The spontaneous locomotor activity of each animal was recorded in square arena of actophotometer (Inco, Ambala, India) individually for ten minutes[16].

Neurochemicals Study

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Figure 4: Photo micrographs of TH immunoreactivity on substantia nigra tissue sections in representative rats of various groups. Rats receiving 6-OHDA intrastriatal injection (b) exhibited significant loss of dopaminergic neurons when compared to sham group rats (a). Pretreatment with 300 mg/kg PE Ext (c), EA Ext (d), Chloro Ext (e) of *Osimum santacum* for 21 days in 6-OHDA lesioned rats showed less loss of dopaminergic neurons with EA Ext group showing maximum protection followed by PE Ext and Chloro Ext. Scale bar = 100 µm**.**

Rats, after three weeks of the surgery, were sacrificed by cervical decapitation. Brains were removed and washed in ice cold saline. The right striatum was dissected and Homogenate were processed immediately for Parameters related to oxidative stress and dopaminergic receptor binding assay, following the standard protocol to understand the preventive efficacy of PE, EA and $CHCl_3$ partitionates of OS leaf extracts.

Tissue Preparation

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Animals were sacrificed and striatal tissue from lesioned sites was carefully dissected and homogenized in phosphate buffer (10 mM phosphate buffer, pH 7.0, having 10 µl/ml) protease arrests (5 mM leupeptin, 1.5 mM aprotinin, 2 mM phenyl ethyl-sulfonylfluoride, 3 mM peptastatin A, 10 mM EDTA, 0.1 mM EGTA, 1mM benzamidine and 0.04% butylated hydroxyl toluene) and centrifuged at 800 g for 5 min at 4 °C to separate the nuclear debris.

BIOCHEMICAL ANALYSIS

Assessment of Oxidant-antioxidant Status

The measurement of thiobarbituric acid reactive substances (TBARS) is a well-established method for screening and monitoring lipid peroxidation[17]. The MDA-thiobarbituric acids (TBA) adduct formed by the reaction of MDA and TBA under high temperature (90-100°C) and acidic conditions is measured colorimetrically at 530nm. Glutathione (GSH) estimation utilizes enzymatic recycling method, using glutathione reductase (GR), for the quantification of GSH [18]. Glutathione peroxidase (GPx) activity was measured, indirectly by a coupled reaction with GR. Oxidized glutathione, produced on reduction of H_2O_2 by GPx, is recycled to its reduced state by GR and NADPH. GR activity was estimated by measuring the rate of NADPH oxidation. The oxidation of NADPH to $NADP^+$ is accompanied by a decrease in absorbance at 340 nm and was directly proportional to the GR activity. SOD Assay Kit utilizes a tetrazolium salt for detection of Superoxide radicals generated by xanthine oxidase and hypoxanthine. Catalase (CAT) estimation utilizes the peroxidatic function of CAT for determination of enzyme activity.

PARKINSONISM MARKERS

Quantification of Dopamine and Its Metabolites

Resuspended pellet fraction were taken in the equal amount of 40 mM Tris-HCl buffer pH 7.4 and was homogenized by hand in a glass and Teflon homogenizer, followed by centrifugation (10,500 g; 20 min; 4 °C). The supernatants were purified using the earlier described method [19]; in brief, 1.0 ml sample of supernatant was pipetted onto Sephadex G-10 columns and washed with 1.8 ml of 0.01 M HCl. DA, DOPAC & HVA were collected with 1.7 ml of 0.01 M HCl and 1.0 ml of 0.02 M NH_3. The acidic metabolites, HVA and DOPAC, were collected by washing the columns with 1.0 ml of 0.02 M NH_3 & 0.01 M KOH respectively. Twenty µl of 2.6 mM sodium Pyrosulfite and 5.7 mM ascorbic acid was added to the tubes containing dopamine, DOPAC and HVA, respectively. The samples were assayed for the concentration determination of Dopamine, DOPAC and HVA using HPLC. Protein was determined by standard method[20].

Dopamine Receptor Binding

Radioligand receptor binding technique was employed to assay DA-D_2 receptors in crude synaptic membranes of corpus striatum following the standard procedure[21]. Briefly, the incubation mixture of 1 ml consisted of synaptic membrane equivalent to 250-300 µg protein along with 1.0 nM of 1 phenyl-4-^{3}H-spiperone (18.5 ci/mmoles) in 40 mM Tris-HCl, pH 7.4. A parallel incubation was carried out in presence of 1 µM haloperidol to ascertain non-specific binding. The assay was run in triplicate. Reaction mixture was incubated for 15 min at 37°C, terminated by cooling at 4°C, and filtered through glass fiber filters (approximately 0.3 µm pore size) through Millipore Filtration Assembly. The filter discs were washed rapidly with 2 × 5 ml cold Tris buffer (40 mM), dried and counted in a β-scintillation counter with an efficiency of 50% for tritium. Specific binding was calculated by subtracting non-specific binding from total binding obtained in absence of 1µM haloperidol. Final results are expressed as pmols bound per g protein) Scatchard analysis was run using varying concentrations (0.1-10 nM) of ^{3}H-spiperone, affinity and the maximum number of binding sites was calculated using linear regression analysis.

Statistical Analysis

Analysis of each data set was performed by one-way analysis of variance (ANOVA). Statistically significant effects were further evaluated with Bonferroni's multiple comparison tests. Differences were considered significant at $P < 0.05$. Results are expressed as means ± SEM.

RESULTS AND OBSERVATIONS

Effect of OS on Behavior Studies

The results of Morris' water maze are summarized in Fig. 1. The contralateral body rotations (362%) induced by apomorphine in the lesioned were significant as compared to the sham group. The different extracts had restored body rotations (L+EA, 131.86%, L+PE, 197.8%; L+$CHCl_3$, 219.78%,) in pretreated groups as compared to lesioned group.

Impairment in motor coordination was observed in lesioned (21.35%) as compared to sham group, which were significantly controlled in OS Ext pretreated groups. A highly significant improvement in recovery of muscular coordination, as analyzed by rota rod activity, was observed in L+EA group (93.96%) as compare to L+PE group (88.11%). L+$CHCl_3$ group rats (69.86%) showed less significant improvement, when compared with leisoned group (Figure 1).

The time spend in locomotion was significantly decreased (33.91%) in lesioned as compare to sham group. Pretreatment with OS extracts significantly restored the locomotion activity with L+EA group rats showing maximal recovery (93.91%) which was highly significant when compare with lesioned, while L+PE and L+$CHCl_3$ group rats showed 79.12% & 64.45% recovery respectively (Figure 1).

CHANGE IN BIOCHEMICAL PARAMETERS

Effect of OS on Oxidative Parameters in PD Rats

MDA content in right striatum was significantly elevated in the lesioned (275.26%) as compare to sham group which was significantly restored in pretreated groups as compared to lesioned group (Figure 2 & 3).

The content of GPx, SOD, Catalase, GSH & GR enzyme in right striatum were depleted significantly in lesioned

(30.74%, 35.12%, 46.43%, 49.56% & 41.25%) as compared to the Sham group. These depleted levels of enzymes were significantly restored in OS pretreated groups as compare to lesioned group (Figure 2 & 3).

Effect on Dopamine, its Metabolites and DA-D2 Receptor Binding Activity

In lesion group, The DA and its metabolite (DOPAC and HVA) content were significantly ($p<0.001$) decreased as compared to sham group. L+EA, L+PE & L+$CHCl_3$ when compared with lesion group showed significant restoration in DA, DOPAC & HVA content (Table 2).

DA-D_2 receptor binding assay showed significant ($p<0.001$) increased binding activity in lesioned as compare to sham group. The elevated DA-D_2 receptor binding was significantly restored in PE, EA and $CHCl_3$ pretreated groups as compared to lesioned group (Table 1 & Figure 3).

Immunohistochemical Studies

The immunohistochemical analysis of contralateral striatal part showed depletion in percentage staining of TH immunopositive neurons with marked damage to specific subnuclei of the substantia nigra, pars compacta, with severe obliteration of their neuromelaninladen projection neurons in lesioned as compared to sham group. OS Ext. pretreated groups did not show any marked reduction in percentage area of TH-immuno positive neuron in all OS Ext pretreated groups (Figure 4a-e).

DISCUSSIONS

The present study, for the first time, demonstrates the neuroprotective effects of different extract of OS in 6 OHDA induced PD in rats. EA Ext of OS significantly ameliorated the neurodegeneration induced by the above neurotoxin in rats.

In the present study, we have demonstrated that experimental hemiparkinson rats when systematically pretreated with PE, EA & $CHCl_3$ ext. reduces 6-OHDA induced degeneration of dopaminergic terminals.

Oxidative stress, loss of ability to process oxidative modified substrate, leads to disturbance in neuronal homeostasis due to imbalance between production of free radicals and ability of cell to shield against them [22], is the leading hypothesis for dopaminergic neuron degeneration.

Single-site intrastriatal 6-OHDA injection protocol was followed as it produces behavioral, biochemical and histopathological changes similar to PD [23], due to a partial dopaminergic lesion, which was evidenced by behavioral impairment, altered neurochemical biomarkers and histological changes in the experimental animals. Antioxidants play an important role in prevention and control of neurological disorders including PD[24]. In our study, OS, which is reported to pertain antioxidant potential[25], might have shown protection in the same way.

The Lesion function was confirmed by subcutaneous administration of apomorphine, a dopaminergic agonist, which produces a contralateral rotation behavior, reveling nigrostraiatal dopamine depletion. Previous studies have shown that this rotation might occur when the lesion is nearly complete or fully complete[26]. This apomorphine induced rotational behavior of rats were significantly reduced by OS extracts, with EA showing the maximum effect followed by PE and $CHCl_3$ ext indicating its major contribution in preventing neuronal damage in nigrostriatal dopaminergic pathway.

Since deteriorating effect of oxidative stress on the neuronal cell functions are of major concern, the biomarkers detection of oxidative damage is the most viable way to unearth a possible role of unbalanced oxidation/reduction of cellular components (viz. protein, lipid, DNA & RNA) in pathogenesis of neurodegenerative disorders[27]. Exposure of 6-OHDA to mitochondria, key source of reactive species, leads to mitochondrial dysfunction and elucidates mode of action of this toxin as a mitochondrial complex I inhibitor (NADH dehydrogenase) [28]. Any changes in the electron transport chain oxidation and reduction of molecular oxygen leads to formation of super oxide, H_2O_2 and ATP generation. Therefore, functional compromise of this organelle has a larger impact on oxidative homeostasis. In the same context, estimation of lipid peroxidation is a reliable experimental approach to study oxdative damage in different animal models. The fact of having higher concentration of polysaturated fatty acid in the brain [29], lipid peroxidation, is major outcome of oxidative stress induced brain tissue injury[30]. Thus measuring of MDA, which is formed due to interaction between various lipid peroxidation products and TBARS is effectively used to detect oxidative stress [31]. Our results showed marked reduction in lipid peroxidation levels in 6-OHDA induced neurodegeneration compare to lesion group; with maximal protection in OS pretreated group.

6-OHDA induced neuro-toxicity though H_2O_2 intracellular production, leading to generation of reactive hydroxyl radicals, induces cell damage. The decrease of SOD and Catalase enzyme activity in the striatum could be attributed to this catecholaminergic toxin induced free radical generation and cyto-toxicity. Moreover, due to overproduction of free radicals in the striatum, the oxidative damage to membranes lipid and protein levels is enhanced and ultimately leading to depletion of GSH, GPx & GR. The reduction of GPx activity could be the cause of reduction in GSH level. Our findings are in total agreement with prior studies showing oxidant radicals deactivating GR & GPx[32]. Data from the enzymatic assay of GSH, GPx, GR, SOD and Catalase reveals that all enzyme levels, which were diminished during PD, were significantly restored by ocimum sanctum pretreated group as compared to lesioned group. In contrast, the activity of PE & $CHCl_3$ ext group was found equipotent and less significant when compared to the EA Ext group.

OS has been reported to increase the brain mono amine levels by inhibiting MAO-A and MOA-B in mice brain. Restoration of behavioral effect due to lesion, as shown in our result, may be attributed to the increased pool of dopamine made available by this pathway as MAO-B inhibitors are reported to decrease dopamine reuptake

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(Kuhr *et. al.*, 1986). These findings are fully supporting the normalization of D_2 receptor bindings by ocimum sanctum with efficacy in the order of EA > PE > $CHCl_3$.

Differences in ocimum sanctum potency were observed in immunohistochemistry on tyrosine hydroxylase (TH) staining in the striatum of the rats. EA, PE and $CHCl_3$ significantly enhanced the depleted TH expression, where PE and $CHCl_3$ were found to be comparable while EA was much more effective.

OS leaf extract Phytochemical investigations shows isolation of many active principles, like phenols (isothymucin, isothymonin, eugenol, cirsilineol, apigenin and vosamarinic acid) and flavonoids (orientin and vicenin). These entities show potent antioxidant and anti-inflammatory activity. Eugenol, although biologically very active, has low toxicity and is a lead for search of new natural neuroprotective agents. Following this it seems that eugenol and other chemical entities of OS are responsible for the presently observed attenuation of oxidative damage during 6-OHDA lesion.

CONCLUSIONS

Reduced activity of oxidative enzymes in right striatum suggested free radical mechanism in contribution to neuronal death in PD. PE, EA and $CHCl_3$ Ext of OS leaves appears to shield progressive neuronal degeneration from increased reactive oxygen species attack in 6-OHDA lesioned rats through its free radical scavenging and DA enhancing capabilities in the sequence of efficacy; EA > PE > $CHCl_3$ Ext. It suggests that, an antioxidant therapy with proper scavenging capabilities, if designed effectively & targeted with systemic & timely administration to the effective site, prior to maximal tissue damage, can be of immense help to individuals in combating from this deadly neurodegenerative disorder. Further, OS may have potential utility in treatment of many more oxidative stress induced neurodegenerative disorders. For further studies, the physiological & molecular mechanisms, using the isolated components of OS needs to be explored.

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