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Protective effect of *Zincum metallicum* on rat model of Parkinson's disease

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ABSTRACT

Background: Parkinson's Disease (PD) is one of the major neurodegenerative disorders, and oxidative stress has been implicated in playing an important role in the pathogenesis of the disease. *Zincum metallicum*, produces symptoms mentioned in Homoeopathic Materia Medica which are akin to PD on which basis it might be considered as one at the intermediate to treat the disease.

Materials and Methods: Rats were divided into eight groups; surgery was done by stereotaxic apparatus. 6-hydroxydopamine was used to induce parkinsonism thereafter on 16th day of lesioning animals were assessed by the video path analyzer. Animals were sacrificed and biochemical assays (Lipid peroxidation [LPO], glutathione [GSH], glutathione peroxide [GPx], glutathione reductase [GR], glutathione-S-transferase [GST]) and level of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA), were estimated. Further dopaminergic D_2 receptor binding was also done to confirm the induced parkinsonism.

Results: The behavior activities (locomotor, distance travel, stereroevent) were decreased whereas the rest time was increased in lesion group animals as compared to the sham group. The locomotor activity and the distance traveled were protected significantly with 6C whereas rest time was protected significantly with 30C and 200C of Homoeopathic medicine *Zincum metallicum*. On the other hand, S + 30C and S + 200C groups have shown increased locomotor activities as compared to S group. The rest time was also increased significantly in S + 6C and S + 30C group animals as compared to S group. The elevated level of LPO and DA D_2 receptor binding density in PD group was protected significantly with *Zincum metallicum* (6C, 30C, and 200C). The depleted level of GSH and activity of antioxidant enzymes (GPx, GR, and GST) and DA and its metabolites DOPAC and HVA were protected significantly with *Zincum metallicum* (6C, 30C, and 200C).

Conclusion: The study indicates the *Zincum metallicum* may be helpful in slowing down injury in parkinsonism and could be a beneficial drug for the prevention of PD.

Keywords: Parkinsonism, Zincum metallicum, Homoeopathy, Anti-oxidant activity

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INTRODUCTION

Parkinson's disease (PD) is the second most common age-related neurodegenerative disease, which is characterized by the gradual loss of dopaminergic cells within the nigrostriatal pathway and the subsequent loss of dopamine (DA).[1] The earliest parkinsonian signs such as bradykinesia, gait disturbances, rigidity and tremor are observed clinically when 80–90% of striatal DA and 50% of the nigral dopaminergic neurons are lost.[2] Levodopa therapy, still considered one of the most effective treatments, only provides a transient relief of the motor impairments caused by the disease by replacing the lost DA and does not address the basis of the disease, neuronal degeneration.[3] Consequently, current therapeutic interventions of PD are aimed at controlling the symptoms of the disorder and fail to halt the underlying degenerative process.[4] Therefore, one promising therapeutic approach for the treatment of the disease is the use of homoeopathic drugs to promote the survival of dopaminergic neurons. The ultimate goal is to slow or halt neuronal degeneration at an early stage in order to preserve existing dopaminergic neurons.

Zinc is a mineral that is found naturally in sulfur compounds. As a trace element, it plays a significant role in boosting the metabolism. It is considered a power source for the nervous system and used to treat anxiety and the headaches that accompany it, as well as mental and physical weakness and exhaustion. *Zincum metallicum* is helpful for memory, depressive thoughts, lethargy, headache, difficult comprehension, incoherent ideas, thoughtlessness and confusion.[5] It is also helpful in treating nervous strain and exhaustion. *Zincum metallicum* also improves a person's ability to focus, renewing vitality, and stamina. A proven remedy for neuralgia, backaches, muscle twitches nervous weakness, exhaustion, constipation, and spasmodic coughing.

The present study appears to be the first report of its kind on the protective role of Homoeopathic medicine, *Zincum metallicum* in different potencies on 6-hydroxydopamine (6-OHDA) rat model of PD.

MATERIAL AND METHODS

Chemicals

6-hydroxydopamine, 5,5'-dithiobis-2-nitrobenzoicacid, DA, 3,4-dihydroxybenzylamine (DHBA), bovine serum albumin, ethylenediaminetetraacetic acid (EDTA), thiobarbituric acid (TBA), and haloperidol were purchased from Sigma-Aldrich Chemical Co., Pvt., Ltd. (Bengaluru, India). ^{3}H-Spiperone was purchased from Perkin Elmer, USA. *Zincum metallicum* was procured from Dr. Reckeweg and Co., Germany. Chloral hydrate was purchased from SD Fine-Chem Ltd. Other chemicals were of analytical grade.

Animals

Male Wistar rats were obtained from Central Animal House of Jamia Hamdard (Hamdard University), weighing 200-230 g. They were housed in polypropylene cages, four animals per cage and had free access to food and water. They were maintained on 12-hours dark-light cycle (light on from 06:00 to 18:00 hours) and provided free access to rat chow and water. The experiments were in accordance with University guidelines and approved by the Animal Ethics Committee of the University. The animals were divided into eight groups and each group has six animals [Table 1]. The animals were pretreated orally with 1 drop (21 μl) of 6C, 30C, for 7 days and with 200C for 1st and 5th day, while the sham-operated control animals received one drop (21 μl) of 85% alcohol (homoeopathic medicine

Table 1: Grouping of the animals with different potencies *Zincum metallicum*

Group	Number of animals	Symbolic representation	Number of days per treatment	
			Pre-treatment	Post-treatment
Control (sham)	6	S	1-7	10-19
Control (sham)+6C	6	S+6C	1-7	10-19
Control (sham)+30C	6	S+30C	1-7	10-19
Control (sham)+200C	6	S+200C	1-5	7-19
Lesion (experimental)	6	L	1-7	10-19
Lesion+6C	6	L+6C	1-7	10-19
Lesion+30C	6	L+30C	1-7	10-19
Lesion+200C	6	L+200C	1-5	7-19

used in study was prepared in 85% alcohol). On day 7, 6-OHDA was injected intra-striatal into the right striatum to develop lesion. The sham-operated group received only the vehicle. After lesioning, the animals of each lesion group were further treated with their respective Homeopathic medicines for 19 days. The animals were kept watching for 2 weeks from the lesioning to develop complete behavioral impairment. After 16 days of lesioning, the animals were assessed in the video path analyzer to monitor the locomotory behavior to evaluate the efficacy of the Homoeopathic medicine used for the treatment of PD.

Experimental Procedure

Experiment 1

Rats were pre-treated 3 times a day orally with 1 drop of *Zincum metallicum* 6C or 30C for 7 days and with 200C for 1st and 5th day [Table 1]. On 8th day, 2 μl of 6-OHDA (12.5 μg 6-OHDA in 0.1% ascorbic acid saline) or vehicle was infused into the right striatum of the animals. After 2 days of lesioning, the rats were post treated 3 times a day for 19 days with *Zincum metallicum* of 6C or 30C. The potency of 200C was given on day 7th and 19th to evaluate the activities of antioxidant enzymes, contents of Glutathione (GSH), and Lipid Peroxidation (LPO).

Experiment 2

Rats were pre-treated 3 times a day orally with one drop of *Zincum Metallicum* of 6C or 30C for 7 days. The potency of 200C was given on day 1st and 5th. On 8th day, 2 μl of 6-OHDA (12.5 μg 6-OHDA in 0.1% ascorbic acid saline) or vehicle was infused into the right striatum of the animals while sham-operated groups were infused with 0.1% ascorbic acid saline. After 2 days of lesioning, the rats were post-treated 3 times a day for 19 days with *Zincum metallicum* of 6C or 30C. The potency of 200C was given on day 7th and 19th to evaluate the content of DA D_2 receptor, DA, and its metabolites.

Intra-striatal administration of 6-hydroxydopamine

The animals were anesthetized with 400 mg/kg i.p. Chloral hydrate. The rats were placed on a stereotaxic frame and a hole of 1.0 mm was done on right side in the skull with the help of drill machine fixed on the stereotaxic frame and having a bit of 1.0 mm. Through the skull hole, a 28-gauge Hamilton syringe of 5 μl was attached to micro injector unit and the piston of the syringe was lowered manually to the right striatum. Lesion co-ordinates were used as described by Lee *et al.*[6] Briefly, they were AP-0.5, L-2.5, V-5 mm relative to bragma and ventral from dura with the tooth bar set at 0 mm.[7] Rats were either infused 2 μl of 0.1% Ascorbic acid saline or 12.5 μg 6-OHDA in 2 μl 0.1% Ascorbic acid saline over 5 minutes and the needle was left in place for 5 minutes before retracting it slowly.

Postoperative Care

Recovery of anesthesia took approximately 4–5 hour. Each rat was kept in an individual cage till gained full consciousness and then housed together in a group of four animals per cage in air condition room at 25°C ± 2°C. Food was kept inside the cages for the 1st week so that animals could easily access it without any physical trauma due to overhead surgery. Then the animals were treated normally. Food, water, and bedding of the cages were changed every day as usual.

Behavioral Studies

The behavioral tests were started after 2 weeks of lesioning and performed for one-week. The experiment was performed between 09.00 hours and 16.00 hours in the laboratory at standard optimal conditions. All performed tests were analyzed by persons blind to the experiment.

Spontaneous locomotor activity

On day 16th, all the animals were tested for locomotor activity in a computerized animal activity monitor, Video Path Analyzer (Coulbourn Instruments, Allentown, PA, USA). It consists of a chamber (50 cm × 50 cm × 35 cm), a video camera fixed over the chamber by an adjacent rod, an activity monitor, a programmer/processor, and a printer. The animal was placed in the chamber, and its locomotor activity was monitored by activating the camera and viewed on the screen. The activity chamber was furnished with black paper to provide contrast on the screen. The data were fed to the printer to print out the intervals (minutes), wall hugging(s), locomotion(s), rest(s), rearing(s), stereo events (number), rotations (clockwise and anticlockwise), and distance traveled (cm). Rats were individually placed in the chamber, acclimatized for 5 minutes and their locomotor activity scores were recorded for 15 minutes.[1,3] The activity chamber was swabbed with 10% alcohol every time to avoid the interference due to animal odors. Results were expressed in terms of activity/15 minutes.

Tissue Preparation

After 3 weeks of lesioning, the animals were killed by cervical dislocation and brains were taken out

quickly and kept on ice. Striatum was dissected out by cutting coronal section of 1.5 mm thickness using rat brain matrix in the light of rat brain atlas.[8] For enzymes, GSH and LPO assays, right striatum was homogenized in phosphate buffer (10 mM, pH 7.4) to give 5% homogenate w/v. Homogenate was taken for the LPO and rest of the homogenate was centrifuged at 10,500 g for 20 min at 4°C to get post mitochondrial Supernatant (PMS).

Biochemical Analysis

Lipid peroxidation

Homogenate was used for the estimation of TBA reactive substance (TBARS). The method of Utley *et al*.[9] as modified by Islam *et al*.[10] was used for the estimation of LPO. Briefly, 0.25 ml homogenate was pipetted out in a test tube and incubated at 37°C ± 1°C in a metabolic water bath shaker for 60 minutes. Another 0.25 ml of the same homogenate was pipetted in an eppendorf tube and placed at 0°C. After 1 hour of incubation, 0.25 ml of 5% trichloroacetic acid and 0.5 ml of 0.67% TBA were added in both samples (i.e., 0°C and 37°C). The reaction mixture from the test tube was transferred to the centrifuge tube and centrifuged at 1200 g for 15 minutes. The supernatant was transferred to another tube and placed in a boiling water bath for 10 min. Thereafter, the test tubes were cooled, and the absorbance of the color was read at 535 nm. The rate of LPO was expressed as nmol of TBARS formed/h/mg protein using molar extinction coefficient 1.56×10^5 M^{-1} cm^{-1}.

Estimation of reduced glutathione

Reduced GSH was determined by the method of Jollow *et al*.[11] In brief, equal volume of PMS was precipitated with an equal volume of sulfosalicylic acid (4%). The sample was kept at 4°C for at least 1 hour and then subjected to centrifugation at 1200 g for 15 minutes at 4°C. The assay mixture contained 0.1 ml of supernatant, 1.7 ml Phosphate buffer (0.1 M, pH 7.4) and 0.2 ml 5,5'-dithio-bis-2-nitrobenzoic acid (4 mg/ml of phosphate buffer, 0.1 M, pH 7.4) in a total volume of 2.0 ml. The yellow color developed was read immediately at 412 nm. GSH was calculated in terms of μM GSH/mg protein using a molar extinction coefficient 13.6×10^3 M^{-1} cm^{-1}.

Glutathione peroxidase

Glutathione peroxide (GPx) (EC 1.11.1.9) activity was measured at 37°C by a coupled assay system in which oxidation of GSH was coupled to Nicotinamide Adenine Dinucleotide Phosphate (NADPH) catalyzed by Glutathione Reductase (GR).[12] The reaction mixture consisted of 0.1 ml H_2O_2 (0.2 mM), 0.1 ml GSH (1 mM), 1.4 units of 0.1 ml GR, 0.1 ml NADPH (1.43 mM), 0.1 ml Sodium Azide (1 mM), 1.4 ml phosphate buffer (0.1 M, pH 7.4) and 0.1 ml PMS. The enzyme activity was quantitated by measuring the disappearance of NADPH at 340 nm. GPx activity was defined as nmol NADPH oxidized min/mg protein using the molar extinction coefficient of 6.22×10^3 M^{-1} cm^{-1}.

Glutathione Reductase

Glutathione Reductase (GR) (EC 1.6.4.2) activity was measured by the method of Carlberg and Mannervik.[13] The assay system consisted of 1.65 ml phosphate buffer (0.1 M, pH 7.6), 0.1 ml EDTA (0.5 mM), 0.05 ml oxidized GSH (1 mM), 0.1 ml NADPH (0.1 mM), and 0.1 ml PMS in a total volume of 2.0 ml. The enzyme activity was quantified at 25°C by measuring the disappearance of NADPH at 340 nm and was calculated as nmol NADPH oxidized/min/mg protein using the molar extinction coefficient of 6.22×10^3 M^{-1} cm^{-1}.

Glutathione-S-Transferase

Glutathione-S-Transferase (GST) (EC 2.5.118) activity was measured by the method of Habig *et al*.[14] The reaction mixture consisted of 2.5 ml phosphate buffer (0.1 M, pH 6.5), 0.2 ml reduced GSH (1 mM), 0.2 ml 1-Chloro-2,4-Dinitrobenzene (CDNB) (1 mM), and 0.1 ml of PMS in a total volume of 3 ml. The changes were recorded at 340 nm by using a Spectrophotometer (λ-20, PerkinElmer) and the enzymatic activity was calculated as nmol CDNB conjugate formed/min/mg protein using a molar extinction coefficient of 9.6×10^3 M^{-1} cm^{-1}.

Quantification of dopamine, 3,4-dihydroxyphenylacetic acid, and homovanillic acid

The striatal tissue levels of DA and its metabolite 3,4-Dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were measured by High-performance liquid chromatography (Waters, Milford, MA, USA), using electrochemical detector (Waters 464 detector).[15] The right striatum (20% w/v) was sonicated in 0.4 N perchloric acid containing 100 ng/ml of the internal standard DHBA, followed by centrifugation at 15,000 × g for 10 minutes at 4°C and the filtered through a 0.25-μm membrane. The filtrate was injected manually through a 20-μ1 loop over the ODS-C 18 column for separation and quantification. The mobile phase consisted of 0.1 M Potassium

phosphate (pH 4.0), 10% methanol, and 1.0 mM Heptane sulfonic acid. Samples were separated on an ODS-C 18 column using a flow rate of 1.0 ml/min. The concentrations of DA and its metabolite DOPAC and HVA were calculated using a standard curve generated by determining ratio between three known amounts of the amine or its metabolites and a constant amount of internal standard DHBA and were represented as ng/mg of tissue.

Dopaminergic D_2 receptor binding

Preparation of crude synaptic membrane suspension

Animals of all groups were sacrificed by cervical dislocation and brains were dissected out to remove the right striatum. Each right striatum was weighed and homogenized (5%w/v) in 40 mM Tris-HCl buffer pH 7.4 and centrifuged at 20,000 g for 20 minutes at 4°C. Supernatant was discarded, and pallet was washed with the same amount of the discarded supernatant and again centrifuged at 20,000 g for 20 minutes at 4°C. The pellet was resuspended by mixing gently and slowly with a glass rod and homogenized by hand in 0.5 ml homogenizer with glass pestle and used for the receptor binding.

Dopaminergic D2 receptor binding

The binding assay was performed by the method of Agrawal *et al*.[1] In brief, the incubation mixture of 1.0 ml consisted of the synaptic membrane (100 μl) along with 1.0 nM of 1-phenyl-4-^{3}H-spiperone in 40 mM Tris-HCl (pH 7.4). A parallel incubation was carried in the presence of 1.0 μM Haloperidol to ascertain nonspecific 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 (GF/C, Whatmann) through Millipore filtration Assembly. The filter discs were washed rapidly with 2 × 5 ml cold Tris-HCl buffer (40 mM, pH 7.4), and transferred to scintillation vials and dried properly. After adding 10.0 ml scintillation cocktail to vials, the radioactivity was counted in a β-scintillation counter (W ALLAC-1410) with an efficiency of 50% for tritium. Specific binding was calculated by subtracting nonspecific binding from total binding obtained in the absence of haloperidol. Results were expressed as pmol ^{3}H-spiperone bound/mg protein.

Protein

Protein was determined by the method of Lowry *et al*.[16]

Statistics

Results are expressed as mean ± standard error of mean ANOVA with Tukey–Kramer *post-hoc* analysis was used to analyze differences between the groups with the help of OriginPro 6.1. The value $P < 0.05$ was considered as significant.

RESULTS

Survival Rate of Operated Rats

The survival rate after the surgery was 100%.

Behavioral Observations

Effect of parkinsonism on behavior activity and its restoration by *Zincum metallicum*

Figure 1 shows the effect of *Zincum metallicum* on locomotor activity. A significantly decreased locomotor activity was observed in L group as compared to S group ($P < 0.05$). Pre- and post-treatment with 6C of *Zincum metallicum* restored the locomotion in L + 6C group as compared to L group ($P < 0.05$). On the other hand, 30C and 200C of *Zincum metallicum* have elevated the locomotor activity significantly in S + 30C and S + 200C as compared with S group ($P < 0.05$). The depletion of locomotor activity was not stored by 30C, and 200C of *Zincum metallicum* in L + 30C and L + 200C group as compared to S group.

Figure 2 shows the effect of *Zincum metallicum* on rest time. The rest time was significantly elevated in group L as compared to group S ($P < 0.05$). The pre- and post-treatment with *Zincum metallicum* has restored the rest time significantly in the groups L + 6C, L + 30C, and L + 200C as compared to L group ($P < 0.05$). On the other hand, the

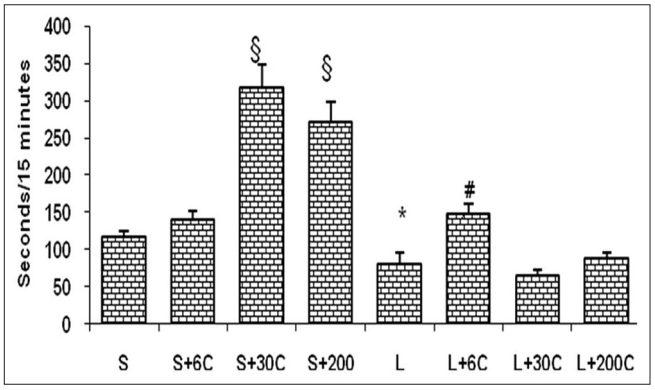

Figure 1: Effect of *Zincum metallicum* on locomotor activity(s). Values are expressed as mean ± standard error of six animals. *$P < 0.05$ significant lesion versus sham group, #$P < 0.05$ significant L + 6C versus L, §$P < 0.05$ significant S versus S + different potencies of the drugs, that is, S + 30C and S + 200C groups

rest time was decreased in pre- and post-treated S groups (S + 6C, S + 30C and S + 200C) animals as compared to S group animals ($P < 0.05$).

Figure 3 shows the effect of *Zincum metallicum* on distance traveled. The distance traveled was decreased significantly in group L as compared to group S ($P < 0.05$). The *Zincum metallicum*, 30C and 200C in groups L + 30C and L + 200C have decreased distance traveled as compared to L group. The pre- and post-treatment with *Zincum metallicum* has restored the distance traveled significantly in L + 6C group animals as compared to L group ($P < 0.05$). On the other hand, the distance traveled was increased in pre- and post-treated S group animals with different potencies of *Zincum metallicum*, S + 30C and S + 200C as compared to S group ($P < 0.05$).

Figure 4 shows the effect of *Zincum metallicum* on average speed, which was decreased significantly in group L as compared to group S ($P < 0.05$). The *Zincum metallicum* 6C has protected the average speed in pre- and post-treated animals as compared to L group ($P < 0.05$) but the potency of 30C and 200C in groups L + 30C and L + 200C has not protected the distance traveled as compared to L group. The average speed was increased in pre- and post-treated S group animals with different potencies of *Zincum metallicum*, S + 6C, S + 30C, and S + 200C as compared to S group ($P < 0.05$).

Figure 5 shows the effect of *Zincum metallicum* on stereoevent, which was decreased significantly in group L as compared to group S ($P < 0.05$), which was also decreased with 6C, 30C and 200C in groups L + 6C, L + 30C and L + 200C as compared to L groups animals ($P < 0.05$, $P < 0.01$). The stereoevent was increased significantly in pre and post-treated S group animals with 30C of *Zincum metallicum* in group S + 30C as compared to S group.

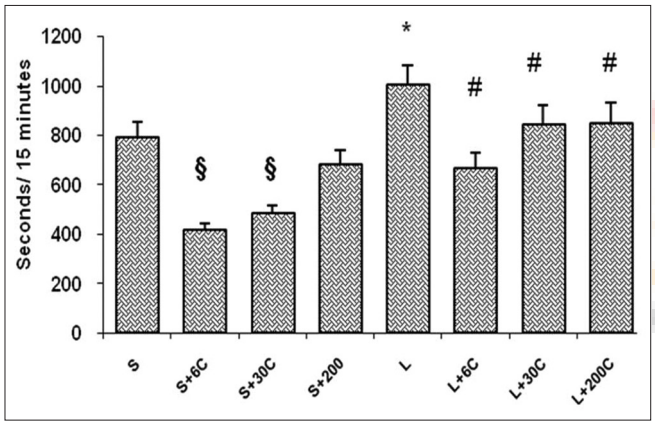

Figure 2: Effect of *Zincum metallicum* on rest time(s). Values are expressed as mean ± standard error of six animals. *$P < 0.05$ L versus S, #$P < 0.05$ L + different potencies of the drugs versus L, §$P < 0.05$ S + different potencies of the drugs (S + 6C and S + 30C) versus S group

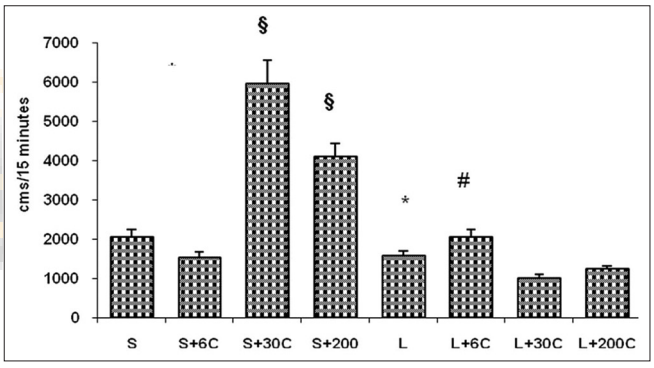

Figure 3: Effect of *Zincum metallicum* on distance traveled. Values are expressed as mean ± standard error of six animals. *$P < 0.05$ significant L versus S group, #$P < 0.05$ L + different potencies of the drugs versus L, §$P < 0.05$ significant S + different potencies of the drugs (S + 6C and S + 30C) versus S group

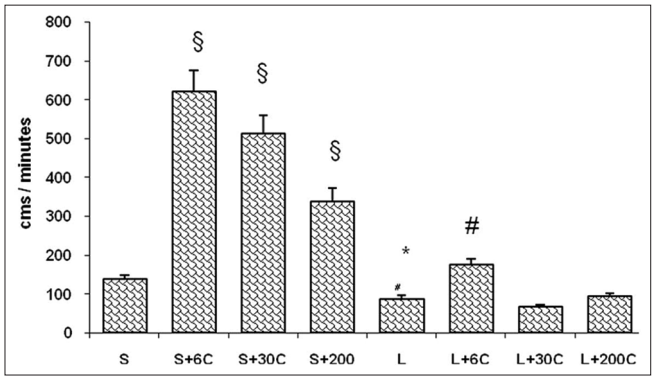

Figure 4: Effect of *Zincum metallicum* on average speed.Values are expressed as mean ± standard error of six animals. *$P < 0.05$ significant L versus S group, #$P < 0.05$ significant L + 6C versus L, §$P < 0.05$ significant S + different potencies of the drugs versus S

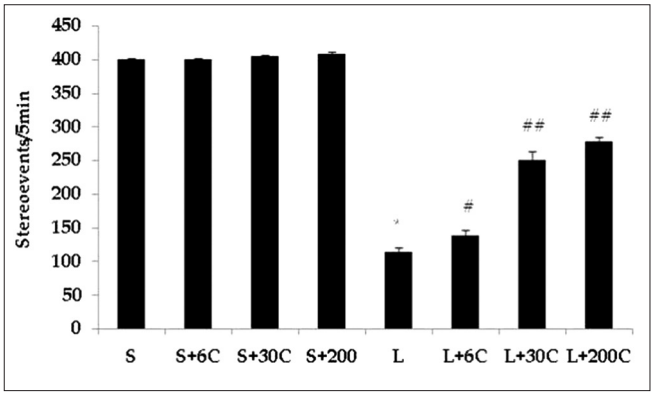

Figure 5: Effect of *Zincum metallicum* on stereo events.Values are expressed as mean ± standard error of six animals. *$P < 0.05$ significant L versus S group, #$P < 0.05$ L + 6C, ##$P < 0.01$ L + 30C and L + 200C versus L group

Biochemical Observations

Effect of *Zincum metallicum* on thiobarbituric acid reactive substance level

Figure 6 shows the effect of *Zincum metallicum* on TBARS content in the striatum. The TBARS content was increased significantly in L group as compared to S group ($P < 0.05$), which was restored significantly in L + 6C, L + 30C and L + 200C groups as compared to L group ($P < 0.05$). No significant change was observed in S + 6C, S + 30C and S + 200C groups as compared to sham group.

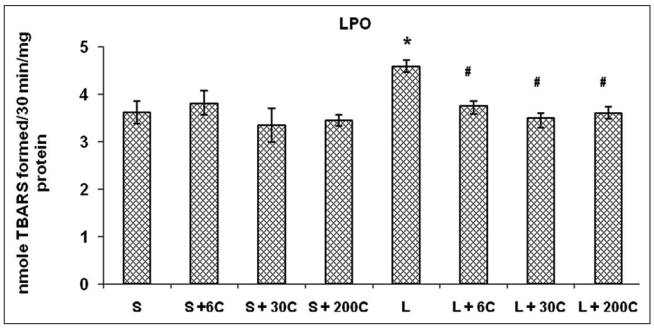

Figure 6: Effect of *Zincum metallicum* on thiobarbituric acid reactive substance content. Values are expressed as mean ± standard error of six animals. *$P < 0.05$ significant L versus S group, #$P < 0.05$ L + different potencies of the drugs versus L

Effect of *Zincum metallicum* on glutathione content

The content of GSH was decreased significantly in L group as compared to S group ($P < 0.05$) and treating the animals with *Zincum metallicum* has restored the content significantly in L + 6C, L + 30C, and L + 200C groups as compared to L group $P < 0.05$) [Figure 7]. No significant change was observed in S + 6C, S + 30C and S + 200C groups as compared to S group.

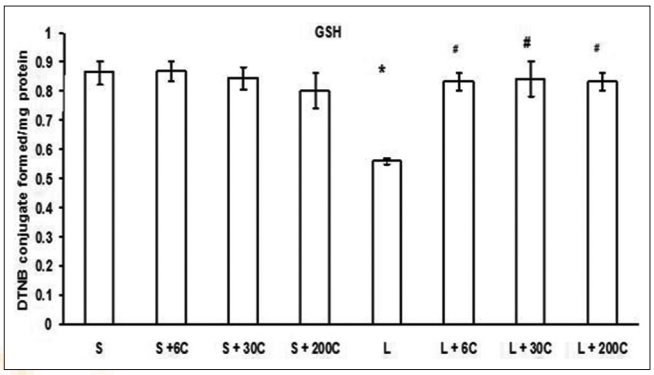

Figure 7: *Zincum metallicum* on reduced glutathione content. Values are expressed as mean ± standard error of six animals. *$P < 0.05$ significant L versus S group, #$P < 0.05$ significant L + different potencies of the drugs versus L

Effect of *Zincum metallicum* on antioxidant enzymes activity in parkinsonian rats

The activity of antioxidant enzymes (GPx, GR, and GST) in S + 6C, S + 30C, and S + 200C groups was not attenuated significantly as compared to S group. But the activity of these enzymes was decreased significantly in L group as compared to S group [Table 2] ($P < 0.05$). On the other hand, *Zincum metallicum* administration in L + 6C, L + 30C and L + 200C groups decreased the activities of these enzymes significantly as compared to L group ($P < 0.05$).

Dopamine, 3,4-Dihydroxyphenylacetic acid and Homovanillic acid content

A significantly decreased ($P < 0.01$) level of DA, DOPAC and HVA was observed in the striatal region of 6-OHDA-lesioned rats as compared to S group, indicating a significant loss of dopaminergic neurons in L group animals. DA, DOPAC and HVA level in L + 30C group exhibited more pronounced and significant increase in comparison to L group rats ($P < 0.05$), indicating the functional viability of dopaminergic neurons [Table 3]. No significant change was observed in the pretreated sham group (S + 30C) as compared to S group.

Effect of *Zincum metallicum* on dopamine D_2 receptor binding

The DA D_2 receptor binding [Figure 8] was increased significantly ($P < 0.001$) in L group as compared to S group. The increase in D_2 binding was restored significantly in pre and post-treatment of L group with the potency of 30C (L + 30C) as compared to L group ($P < 0.001$).

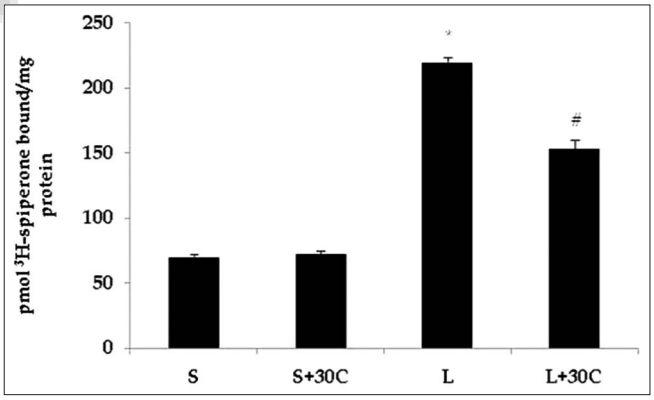

Figure 8: 6-hydroxydopamine infusion led to a significant increased in D_2 receptor binding in the L group as compared with the S group. Pretreatment with *Zincum metallicum* significantly decreases the receptor binding in the L + 30C group as compared with the L group. Values are expressed as mean ± standard error of the mean of eight animals. *$P < 0.001$, L versus S, #$P < 0.001$, L + 30C versus L group

Table 2: The effect of *Zincum metallicum* on the activity of GPx, GR, and GST in striatum

Groups	nmol NADPH oxidized/min/mg protein		
	GPx	GR	GST
S	328.32±0.04	215.09±5.28	273.37±12.06
S+6C	409.24±32.19§	211.85±9.86	286.46±26.85
S+30C	389.09±42.10	225.70±14.56	210.51±14.56§
S+200C	383.67±36.86	183.65±19.25	265.23±26.32
L	196.42±39.54*	147.34±11.05*	158.07±38.58*
L+6C	244.97±79.35#	162.49±21.05#	209.01±15.25#
L+30C	279.88±41.00#	181.82±15.21#	241.87±46.85#
L+200C	261.03±77.00#	153.38±39.58	235.12±19.02#

The activity of these enzymes was decreased significantly (P<0.05) in lesion group as compared to sham group and their activities were restored dose dependently in L+6C, L+30C and L+200C groups as compared to L group. Values are expressed as mean±SEM of six animals. *P<0.05 L versus S group, #P<0.05 L+different potencies of *Zincum metallicum* versus L, §P<0.05 S+30C and S+6C versus S. SEM: Standard error of mean; GPx: Glutathione peroxidase; GR: Glutathione reductase; NADPH: Nicotinamide adenine dinucleotide phosphate reduced form; GST: Glutathione-S-transferase

Table 3: The effect of *Zincum metallicum* on the content of DA and its metabolites (DOPAC and HVA)

Group	ng/mg tissue		
	DA	DOPAC	HVA
S	5.97±0.68	1.01±0.87	0.90±0.71
L (%)	1.64±0.128[a] (−72.52)	0.34±0.028[a] (−66.33)	0.21±0.028[a] (−76.66)
L+30C (%)	2.61±0.06[b] (+59.14)	0.60±0.09[b] (+76.47)	0.35±0.04[b] (+66.66)

The 6-OHDA injection in striatum significantly (P<0.05) decreased in the DA, DOPAC and HVA levels in lesion group as compared to the Sham group. The depleted levels of DA and its metabolite were significantly increased by the pre- and post-treatment of *Zincum metallicum* (30C) as compared to the L group. Values are expressed as mean±SEM of six animals. [a]P<0.05 L versus S, [b]P<0.05, L+30C versus L. Values in percentage increase (+) or decrease (−) as compared to S or L group. DA: Dopamine; DOPAC: 3,4-Dihydroxyphenylacetic acid; HVA: Homovanillic acid; 6-OHDA: 6-hydroxydopamine; SEM: Standard error of the mean

DISCUSSION

The present study demonstrates the protective effects of *Zincum metallicum* in parkinsonian rats. *Zincum metallicum* is used in homoeopathic parlance to treat some deep-seated brain troubles in patients suffering from central nervous system diseases. Some of the symptoms in which *Zincum metallicum* is used are slow and gradual unconsciousness; rolling of the head for days; eyes lusterless; body emaciated; involuntary discharges of feces and urine on the bed; tongue dry and parched, face shrunken, every day looks older; paralysis of one hand or one foot, or it seems that whole muscular system is paralyzed. Symptoms of neurological origin were successfully restored by *Zincum metallicum*.

The altered neurobehavior activity was protected significantly by the pre- and post-treatment of *Zincum metallicum*. The enhancement of DA content by *Zincum metallicum* might have restored the alterations in neurobehavior activity and muscle coordination. On the other hand, some potency has shown the elevated level of locomotor activity, distance traveled, average speed, stereo events, and increase in test time as compared to the sham group. The pre and post-treatment effect of *Zincum metallicum* on these behavior activities has shown an excitement in the control (sham) group animals treated with the *Zincum metallicum* as compared to sham group animals which were given vehicle only. In other way, we can say that the pre and post-treatment with *Zincum metallicum* has decreased the aging due to which animals of these groups were quite active. Further study is required to confirm the hypothesis.

In view of our findings, it is suggested that it is reasonable to infer that the depletion of GSH triggers lipid peroxidation, leading to the degeneration of nigrostriatal neurons, which in turn would deplete DA and subsequently its metabolites. Conversely, GSH is converted to GSSG, which is reconverted to GSH by GR, thus maintaining the pool of GSH, which, in conjunction with the NADPH, can reduce LPO, free radicals, and H_2O_2. The increase in the content of GSH and decrease in the extent of LPO with the treatment of *Zincum metallicum* in our study, is in concordance with earlier reports[15,17-19] where antioxidants had been studied for the similar model of PD.

Our findings are consistent with previous reports that free radicals inactivate GR and GPx. GPx plays a predominant role in removing excess free radicals and hydroperoxides and is a major defense system against oxidative stress in the brain.[20] Meanwhile, GST catalyzes the detoxification of oxidized metabolites of catecholamine (O-quinone) and may serve as an antioxidant system preventing degenerative cellular processes.[21] The enzymes that remove both superoxide and H_2O_2 protect the cells against intermediates of oxygen generated during normal aerobic metabolism, but when the production of O_2 and H_2O_2 crosses the normal threshold, the system is compromised. The role of antioxidants in providing protection against 6-OHDA induced deleterious effects have been reported.[15,17,18]

The restoration of these main antioxidant defenses was further emphasized by the normalization of denervation-related supersensitivity of dopaminergic D_2 receptors in the striatum and the restoration of striatal DA content by the *Zincum metallicum*. The denervation-related upregulation of these receptors is a compensatory mechanism for DA deficiency, in parkinsonism. Depletion of striatal DA content is considered the hallmark of PD, and restoring its level has always proven beneficial and mostly advocated in patients with the disease. Because the major constituents of the *Zincum metallicum* is Zinc, which is an important enzymatic cofactor, is involved in many metabolic processes, we are optimistic that this homoeopathic medicine may provide anti-parkinsonian effects as observed in our studies. This homoeopathic remedy is safe and without side effects and is not known to interfere with conventional drugs. However, homoeopathic preparations are regulated by Drugs and Cosmetic Act of India. It is premature to propose the mechanism(s) involved in the observed anti-parkinsonian effects of *Zincum metallicum*, and we do not consider that these effects were elicited through a single mechanism.

CONCLUSION

On the basis of the present findings, it is proposed that these anti-parkinsonian effects could be due to anti-oxidant, free radical scavenging or DA enhancing properties of the *Zincum metallicum*. However, we consider the findings very encouraging for good anti-parkinsonian potential and worthy of further investigation using various molecular biological and genetic approaches to elucidate proper mechanisms.

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Nil.

Conflicts of Interest

There are no conflicts of interest.

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पार्किंसन रोग के मूषक प्रतिदर्श पर ज़िंकम मेटेलिकम का रक्षात्मक प्रभाव

सार

पृष्ठभूमि: पार्किंसन रोग (पी.डी.) प्रमुख तंत्रिकाअपह्रासी विकारों में से एक है, और रोग के रोगजनन में ऑक्सीकारी तनाव की महत्वपूर्ण भूमिका की संलिप्तता ज्ञात है।

सामग्रियां एवं विधियां: चूहों को आठ समूहों में विभाजित किया गया था; त्रिविम अनुचलन (स्टीरियोटैक्सिक) उपकरण द्वारा शल्यक्रिया की गई थी। पार्किंसन रोग प्रेरित करने के लिए 6–हायड्रॉक्सीडोपामाइन का उपयोग किया गया था, उसके बाद, असामान्य संरचना बन जाने के 16वें दिन दृश्य पथ विश्लेषक द्वारा जंतुओं का आकलन किया गया। जंतुओं का बलिदान करके जैवरासायनिक आमापनों (लिपिड परॉक्सिडेशन एल.पी.ओ. ग्लूटाथायोन जी.एस.एच. ग्लूटाथायोन परॉक्साइड जी.पीएक्स. ग्लूटाथायोन रिडक्टेज़ जी.आर. ग्लूटाथायोन–एस–ट्रांसफ़रेज़ जी.एस.टी.,) डोपमाइन (डी.ए.), 3,4–डाईहायड्रॉक्सीफेनिलएसिटिक अम्ल (डी.ओ.पी.ए.सी.), एवं होमोवेनिलिक अम्ल (एच.वी.ए.) के स्तरों का आकलन किया गया। इसके अतिरिक्त, प्रेरित पार्किंसन रोग की पुष्टि करने के लिए डोपामिनर्जिक डी2 ग्राही आबंधन भी किया गया।

परिणाम: असमान्य संरचना समूह जंतुओं में स्वाँग समूह की तुलना में व्यवहार संबंधी गतिविधियां (चलन, तय दूरी, त्रिविम घटना) घटीं जबकि विश्राम समय बढ़ गया। ज़िंकम मेटेलिकम की 6सी से चलन गतिविधि एवं तय दूरी का उल्लेखनीय रूप से संरक्षण हुआ जबकि 30सी और 200सी से विश्राम समय का उल्लेखनीय रूप से संरक्षण हुआ। दूसरी ओर, एस + 30सी एवं एस + 200सी समूहों ने एस समूह की तुलना में चलन गतिविधियों में वृद्धि दर्शाई है। एस समूह की तुलना में एस + 6सी एवं एस + 30सी समूह के जंतुओं में विश्राम समय में भी उल्लेखनीय वृद्धि हुई। ज़िंकम मेटेलिकम (6सी, 30सी एवं 200सी) के साथ पी.डी. समूह में एल.पी.ओ. के बढ़े हुए स्तर एवं डी.ए. डी2 ग्राही आबंधन को उल्लेखनीय संरक्षण मिला। ज़िंकम मेटेलिकम (6सी, 30सी एवं 200सी) के साथ जी.एस.एच. के निःशेष स्तर एवं प्रतिऑक्सीकारी एंजाइमों (जी.पीएक्स., जी.आर. एवं जी.एस.टी.) और डी.ए. एवं उसके उपापचयजों डी.ओ.पी.ए.सी. तथा एच.वी.ए. की गतिविधि को उल्लेखनीय संरक्षण मिला।

निष्कर्ष: अध्ययन इंगित करता है कि ज़िंकम मेटेलिकम पार्किंसन रोग में क्षति को धीमा करने में सहायक हो सकती है एवं यह पी.डी. की रोकथाम में एक लाभकारी औषधि सिद्ध हो सकती है।