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Research Article

Evaluation of Protective Action of Sesame Seed Oil on Chlorpromazine-associated Extrapyramidal Side Effects in Experimental Animals

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ABSTRACT

Sesame plant (*Sesamum indicum* Lin.) belonging to family *Pedaliaceae*. Sesame seed oil also known as queen of oil which contains number of phytochemicals like thiamine, riboflavin, niacin, pyridoxine, folic acid, vitamin B12, ascorbic acid, stearic acid, linoleic acid, palmitic acid and various lignance like sesamolin, pinoresinol, sesamin, sesamol, tocopherols which are potent antioxidants. They have neuroprotective action hence, sesame seed oil (SSO) was used in the present study to investigate its protective action on chlorpromazine (CPZ) associated extrapyramidal side effects (EPSE) in the animal model. The study was designed by giving CPZ (3 mg/kg ip) for 21 days to induce EPSE in animals. CPZ significantly induces EPSE (Catalepsy, muscular rigidity and hypolocomotion) in 21 days. The effect of SSO on CPZ induced EPSE in rat was evaluated using doses 200 and 400 mg/kg PO. Syndopa (10 mg/kg) was used as standard drug. During 21 days of study animals behavioral parameters were evaluated after each 7 days of interval by block test (catalepsy), rotarod test (muscular rigidity) and actophotometre (locomotors activity). Animals showed significantly reduced catalepsy score, increased fall of time and increased locomotor activity in dose dependant manner. Biochemical estimation of dopamine (DA) and antioxidant parameters were also evaluated. SSO-treated animal groups showed significant increase in level of DA, Catalase and reduced glutathione preferably at higher dose. Thus present study validates that SSO has an antioxidant and protective effect on CPZ-associated EPSE. Further research is required to elucidate its specific mechanism of action and isolation of responsible active principles.

Introduction

Schizophrenia is serious, complex mental health disorder that affects individuals thinking, and behavior. They lose touch with reality, disorganized speech, delusions and hallucinations also seen. Different molecular mechanisms were involved into the development of schizophrenia i.e., dopamine hypothesis, neurodevelopment hypothesis and glutamate hypothesis. Psychosis is a group of symptoms: hallucinations, delusions, and difficulties concentrating and completing tasks. A person may unable to meet the regular demands of life.[1]

Neuroleptic's are a group of medication typically used to deal with psychosis (counting misbelieve, hallucinations, and disordered thought), schizophrenia and split personality disorders. Firstly it is introduced in clinical practice with Delay *et al.* in 1952. After introduction of neuroleptics, clinicians had a numbers of compounds which could diminish psychotic excitement without producing sedation. The number of patients in chronic mental hospitals began to drop, for first time an individual's Became manageable as outpatients. But after availability of prominent management for schizophrenia and psychosis it was found that neuroleptic's produces a range of

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extrapyramidal side effects.[2] Drug-induced movement disorders (DIMDs) also referred as Extrapyramidal symptoms (EPS) Broadly EPS were differentiated into four categories. Drug induced Parkinsonism, Akathisia, Acute Dystonia and Tardive Dyskinesia among these, drug induced parkinsonism is common. Neuroleptic-induced movement disorders has reported prevalence 61.6%.Neuroleptic induced akathisia 31.3%, neuroleptic induced Parkinson 23.2%, neuroleptic induced tardive dyskinesia 32.3% was reported.[3] About 80% of schizophrenic's consuming neuroleptic's shows evidence of more than one type of EPSE .The survey conducted on extrapyramidal side effects produced by antipsychotics like Chlorpromazine (CPZ) represent's that about 40% patients exhibits Parkinson like symptoms.[4]

Need of Investigation

Recently, many antiparkinsonian agents have been useful in drug induced-EPSE. Some antihistaminic's in combination with other anticholinergics like ethopropazine, trihexyphenidyl; benzotrophins were used to treat neuroleptic induced extra-pyramidal side effects. This combination found less effective but it causes various usual side effects.[5] Decrease the dose or discontinuation of neuroleptic's is best approach to reduce its serious EPSE. But dyskinesia may settle for months or years after discontinuation of therapy or may be lifetime. It has no cure and treatment offered presently is symptomatic. There is no satisfactory solution for this problem.[6]

Now, researchers are looking for more specific drugs with higher safety and lower cost. The researchers working in this field have attention of medicinal plants because these plants have long been used to treat various diseases with minimum adverse effects than synthetic and chemical drugs. By screening natural sources like plants, the search for novel chemical entities had led to the finding of various clinically beneficial drugs that play a vital role in the treatment of diseases with various complications.

Sesamum indicum L. an ancient plant belonging to family Pedaliaceae, the chemical constituents which are present in sesame seed are vitamins, minerals and antioxidants, such as thiamine, riboflavin, niacin, pyridoxine, folic acid, vitamin B12, ascorbic acid, tocopherols, omega-3, omega-6 an essential fatty acids, valenolinic acid, linoleic acid, sterols, minerals, sodium potassium, calcium, iron, zinc and magnesium. Lignance i.e. sesamin, sesamol, sesaminol, sesamolin, pinoresinol are main constituents of SSO.[7,8]

The medicinal plants such as *Nigella sativa* seeds, Juniperus communis and polyherbal formulation it has been reported a promising ameliorating effect on catalepsy, muscular rigidity and hypolocomotion induced by Chlorpromazine. This effect is possibly due to its potent antioxidant and neuroprotective action because they have mentioned that, antipsychotics causes' free radical-mediated damage in rats and oxidative stress by varying the levels of antioxidant enzymes.[9-11]

Sesame seeds oil mainly contains monosaturated fatty acids (MUFA), stearic acid, linoleic acid, palmitic acid etc. SSO also contains tocopherols, sesamin, sesamolin, sesamol are potent antioxidants. Sesamol involves in triggering of endogenous antioxidant enzymes like catalase, GSH and prevents neurodegeneration.[12]

Neuroprotective effect of SSO in 6-Hydroxydopamine induced neurotoxicity was by restoring striatal dopamine and by enhancing and activating the antioxidant defense mechanism which indicates that it acts as ROS scavenger. SSO also increases expression of tyrosine hydroxylase in substantial nigra as well as dopamine.[12]

There is lots of work on SSO has done which proves that SSO has a potential neuroprotective action by enhancing antioxidant defense mechanism. Therefore, we examine the neuroprotective role of SSO in neuroleptic-induced EPSE in animal model.

If proved, the effectiveness can be used to reduce serious extra pyramidal side effects and future development of parkinsonism produced by CPZ in patients. Its protective action will be also useful in other neuroleptic-induced EPS. If it proved effective, it can be given as adjuvant therapy to increase the effectiveness and compliance of existing neuroleptics.

Materials And Methods

Drugs and Chemicals

Chlorpromazine (Research lab fine chem. Industry Mumbai), dopamine (Sisco Research Laboratories Pvt. Ltd.), 5,5-dithio bis nitro-benzoic acid (DTNB), Pet–ether, glacial acetic acid, tricholoacetic acid (TCA), tris buffer, sodium hydroxide, EDTA (all from Research lab fine chem. industries, Mumbai). Pyrogallol (Sigma Aldrich, Pvt. Ltd), thiobarbituric acid (TBA) (Loba chemicals, Mumbai), hydrogen peroxide (Ranbaxy, Mumbai), ethanol (Datta sugar factory, Shirol). All chemicals were of analytical grade.

Equipments

Rota rod machine (Sigma Company, Ambala), actophotometre (HILAB), rotary evaporator, analytical weighing balance (Shimadzu Instru, Mumbai), homogenizer (Remi motors LTD), centrifuge (Remi motors LTD), UV spectrophotometer (Jasco, V-730 India).

Experimental Animals

Experiment was carried out using wistar Albino rats of either sex weighing around 150–200 g. Animals were procured from animal house of Appasaheb Birnale College of pharmacy Sangli, were used for the study. Animals were housed in well- ventilated room at 23 ± 20°C, with humidity of 65–70%, and maintained 12 hours light and 12 hours dark cycle, they were fed on a conventional diet and

Table 1: Experimental design

S. no	Groups	Treatment
1.	Normal	Normal saline solution
2.	Control	Chlorpromazine 3 mg/kg, i.p
3.	Standard	Chlorpromazine 3 mg/kg, i.p + Syndopa (10 mg/kg .P.O.)
4.	Test-I	Chlorpromazine 3 mg/kg, i.p + sesame seed oil.200 mg/kg P.O.
5.	Test-II	Chlorpromazine 3 mg/kg, i.p + sesame seed oil.400 mg/kg P.O.

had free access to water. Form B protocol were prepared and submitted to Institutional Animal Ethics Committee (AIEC). To the laboratory condition the animals were acclimatized for as a minimum 5 days before conduct of experimentation. The experimental protocol (IAEC/ABCP/03/2019-20) was approved by the IAEC. The Procedures involving laboratory animals were performed in agreement with the strategies of the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Procurement, Authentication, Drying and Extraction of Seeds

Plant (*S. indicum* Lin.) was collected in month of August 2019 from Kolhapur region (Dist-Kolhapur) Maharashtra (India). The Plant (*S. indicum* L.) material was authenticated by Prof. M.D.Wadmare Sir Department of Botany, Smt. Kasturbai Walchand College, Sangli.

Preparation of Extraction of Oil from Seeds

The powdered seed of plant (*S. indicum* L.) was exhaustively extracted with pet Ether in a Soxhlet's apparatus for 24 hours. The oil obtained was separated by rotary evaporator then it collected and stored at room temperature. Average %yield of the oil from sesame seeds was found to be 36%. Physicochemical investigation of Extracted oil was also done.[13]

Determination of LD_{50}

LD_{50} of extracted SSO was determined following the OECD guideline Acute Toxicity class method (OECD guideline no. 423). The animals were observed for 24 hours for toxic symptoms such as behavioral changes and mortality.

Dose Selection

According to OECD guidelines as the LD_{50} of Extracted SSO was found to be 1000 mg/kg then two doses are selected for further evaluation as $1/5^{th}$ and its doubled dose *i.e.* 200 and 400 mg/kg, respectively.

Experimental Design [11,14,15]

The animals were divided into 5 groups (6 mice in respectively group). All animals were administered the drugs/SSO (according to treatment) orally by using tuberculin syringe (1-mL) and gavage needle.

Table 2: Scoring for catalepsy

No.	*Stages*	*Scoring*
I	Rat moves commonly when positioned on table	0
II	Rat moves just when touched or pushed	0.5
III	Rat positioned on the table with front paws set alternatively on three cm high block, rat fails to acceptable position in ten sec. score 0.5 for each paw.	1
IV	Rats fails to remove its paw when positioned on the other hand on nine cm block score -	2
V	The maximum score of single rat that revealed total catatonia	3.5

Fig. 1: a) Catalepsy test b) Rotarod test c) Actophotometre

Treatment was continued for 21 days. Various parameters like catalepsy (Block test), locomotor activity (Actophotometer Test) and muscular rigidity (Rota rod test) for all groups were accessed on 7^{th}, 14^{th} and 21^{th} day. After 21days, animals were sacrificed; their brain were removed and weighed. A 10% tissue homogenate was prepared in 0.1 M phosphate buffer (PH-8) for Dopamine, GSH and CAT estimation.

Behavioral Study Test

Measurement of Catalepsy

Catalepsy is extreme tonus; muscular rigidity which is characterizes by a tendency to remain in a fixed position for long period, hence unable to accurate an outwardly imposed, unusual position over a lengthened times span. At the time of testing rat was grasped lightly in the region of the shoulder and below the forepaws and positioned on 3 cm high block with extended forelimbs. Same was done for 9 cm high block. Time taken by animal to remove the paws from block or to correct the imposed posture was recorded. Scoring was done according to stages observed.

Actophotometer Test

Most of central nervous system acting drugs influence the locomotors activity in man and animals. The locomotors activity can be easily measured using an actophotometer which operates on photoelectric cells which are connected in circuit with a counter. At the time of testing individual rat was placed (for 5 minutes) in automated locomotors activity cage, *i.e.*, Actophotometer consists of a clear Perspex lid. During test time locomotors activity was recorded by means of breaking of beam of light falling on photocell was counted. Locomotor activity score was recorded for all groups.

Rotarod Test

This test is used to evaluate the activity of drugs interfering with motor coordination. In 1956, Dunham and Miya suggested that the test compound could be evaluated by testing the ability of rat to remain on a revolving rod. The rod is on a height of about 50 cm above the tabletop in order to discourage the animal from jumping off the rod. Before the test every animal was given one min. exposure to rotating rod. The rat was positioned on rotating rod. (Speed 20–25 rpm) for 3 minutes. Latency to drop from the revolving rod for all groups was noted. Movement impairment was posses by the incapability of animal to stay on the revolving rod for a 3 minutes test periods.

Biochemical Estimation [11,16]

Dissection and Preparation of Brain Tissue Sample

Rats were sacrificed on last day of experiment by using CO_2 euthanasia chamber. Tissue was weight and homogenized in 5 mL HCL–butanol solution for about 1-minute. Then sample was centrifuged for time 10 minutes at 2000 RPM. After removing an (01 mL) aliquot supernatant phase it was taken in centrifuge tube holding 2.5 mL heptane and 0.31 mL 0.1M HCL. After 10 min. the content in centrifuge tube was centrifuged for time 10 minutes at 2000 RPM. to separate the 2 phases, and overlaying organic phase was removed. The aq. phase (0.2 mL) was used for estimation of DA.

Estimation of Dopamine

By Schlumpf M. Method:

Procedure

To the 0.2 mL of aqueous phase, 0.05 mL 0.4M.HCl and 0.1 mL of EDTA/Sodium acetate buffer (pH 6.9) were added, followed-by 0.1 mL iodine solution (0.1 M in ethanol) for oxidation. The reaction was stopped after 2 minutes by addition of 0.1 mL Na_2SO_3 solution. 0.1 mL Acetic acid was added after 1.5 minutes The solution was then heated to 100°C for 6 minutes when the sample again reached room temperature, excitation and emission spectra were read from the spectrofluorimeter. The readings were taken at 330–375 nm.

Antioxidant Parameters

- Estimation of reduced glutathione: 1-mL 10% tissue homogenate was mixed with 1-mL trichloroacetic acid (TCA), (10%) containing 1 mm EDTA, mixture was centrifuged at speed 1000 rpm for 10 minutes. In aliquot of supernatant 4 mL of phosphate buffer wad added then 0.5 mL of DTNB was added to final solution. Mixed well and absorbance was measured at 412 nm on UV visible double beam spectrophotometer. The amount of glutathione was calculated by molar extinction coefficient 13600 $M^{-1}cm^{1}$.
- Estimation of catalase activity: Tissue homogenate (0.1 mL) mixed with 1-mL of freshly prepared hydrogen peroxide (H_2O_2) and 1.9 mL phosphate buffer into cuvette. Without tissue homogenate blank was prepared. Absorbance of test solutions was measured at 240 nm against blank on UV-visible spectrophotometer, for 3 minutes. At 240 nm, CAT activity was showed in unit/mg of protein was calculated using molar extinction coefficient 43.6 $M^{-1}cm^{-1}$ (Aebi Method).

Statistical Analysis and documentation of Results

The values are expressed as Mean ± SEM for six rats in each group. The Statistical Analysis was done using one way ANOVA followed by Dunnett's test. (Graph pad prism version 8.4.3).

Results

Physical Properties of Extracted Sesame Seed oil

Table 3: Physical Properties of oil

1	colure	Pale yellow
2	Odour	characteristic
3	Density	0.94
4	Yield	36% v/w

Qualitative Physicochemical investigation of Extracted SSO

Table 4: Physicochemical investigation of SSO

S. no.	*Physicochemical parameter's*	*Standard oil*	*Extracted oil*
1.	Boiling point	160°C	157°C
2.	Rf value	0.724	0.689
3.	Density	0.95	0.94
4.	Refractive index	1.476	1.472
5.	Acid value (mg KOH/gm)	0.68	0.78
6.	Saphonification value (mg KOH/gm)	191	189

Anticataleptic Activity

Effect of SSO on CPZ-induced Catalepsy in Rats Screened by Block Test

In this test, animals were treated with two doses of SSO 200 mg/kg and SSO 400mg/kg showed significant $*p < 0.05$ decrease in catalepsy score on 7th day (0.75 ± 0.1708), on 14th day $**p < 0.01$ (1.250 ± 0.1110) and on 21th day $***p < 0.001$(1.583 ± 0.2007) resp. when compared with control group on 7th day (1.500 ± 0.2236), on 14th day (2.500 ± 0.2582) and on 21th day (3.00 ± 0.2582), respectively. Similarly animal treated with Syndopa (10 mg/kg) showed significant $****p < 0.0001$ decrease in catalepsy score (0.25 ± 0.1118, 0.750 ± 0.1116, 1.000 ± 0.2236) on 7th, 14th and 21th day when compared with control group.

Table 5: Effect of SSO on CPZ induced catalepsy

Sr. no	*Drug treatment*	*Catalepsy score*		
		7 th Day	14 th Day	21th Day
1	Control CPZ (3 mg/kg, IP)	1.500 ± 0.2236	2.500 ± 0.2582	3.00 ± 0.2582
2	Test-I CPZ+SSO (200 mg/kg, P.O.)	1.250 ± 0.2141	1.750 ± 0.1118*	2.000 ± 0.1826** (33%)
3	Test-II CPZ+SSO (400 mg/kg, P.O.)	0.75 ± 0.1708*	1.250 ± 0.1110**	1.583 ± 0.2007*** (47%)
4	Standard CPZ+Syndopa (10 mg/kg,P.O.)	0.25 ± 0.1118***	0.750 ± 0.1116****	1.000 ± 0.2236**** (66%)

Table 6: Effect of SSO on CPZ associated hypo-locomotion

Sr. no	*Drug treatment*	*Locomotor activity (Sec)*		
		7 th Day	14 th Day	21th Day
1	Normal	183.3 ± 4.201	183.3 ± 4.201	183.3 ± 4.201
2	Control CPZ (3 mg/kg, IP)	142.2 ± 9.196####	73.67 ± 4.944####	46.50 ± 2.754####
3	Test-I CPZ+SSO (200 mg/kg, P.O.)	150.5 ± 7.693	95.17 ± 8.581*	70.83 ± 2.442** (34%)
4	Test-II CPZ+SSO (400 mg/kg, P.O.)	170.2 ± 4.408*	155.5 ± 2.825***	134.0 ± 7.497**** (65%)
5	Standard CPZ+Syndopa (10 mg/kg, P.O.)	180.5 ± 4.311***	171.7 ± 3.818****	159.2 ± 2.182**** (70%)

Effect of SSO on CPZ-induced Hypolocomotion in Rats (Actophotometer)

SSO produces significant increase in locomotors activity. Group which receive CPZ (control group) showed significant decrease (####$p < 0.0001$) in locomotors activity when compared with normal group. Test animals treated with 200 and 400 mg/kg showed significant *$p < 0.05$ increase in locomotors activity on 7th day (170.2 ± 4.408), on 14th day ***$p < 0.001$ (155.5 ± 2.825) and on 21th day ****$p < 0.0001$ (134.0 ± 7.497), respectively when compared with control group on 7th day (142.2 ± 9.196) on 14th day (73.67 ± 4.944), and on 21th day (46.50 ± 2.754), respectively. Similarly animals treated with Syndopa (10mg/kg) showed significant ****$p < 0.0001$ increase in locomotors activity (180.5 ± 4.311, 171.7 ± 3.818, 159.2 ± 2.182) on 7th, 14th and 21th day, respectively. when compared with control group.

Effect of SSO on CPZ- induces of Muscular Rigidity in Rats (Rotarod Test)

The twenty one days treatment of SSO produces significant increase in fall of time. Group which receive CPZ (control group) showed significant increase in (####$p < 0.0001$) fall of time when compared with normal group. Test animals treated with 200 and 400 mg/kg showed significant *$p < 0.05$ increase in fall off time on 7th day (69.83 ± 3.790), on 14th day **$p < 0.01$ (56.83 ± 1.515) and on 21th day ****$p < 0.0001$ (52.50 ± 1.727)resp. when compared with control group on 7th day (54.67 ± 2.741), 14th day (33.83 ± 1.922) and on 21th day (7.833 ± 0.009), respectively. Similarly animals treated with Syndopa (10 mg/kg) showed significant ****$p < 0.0001$ increase in fall of time (115.3 ± 3.201, 109.0 ± 2.595, 100.3 ± 1.333) on 7th, 14th , and 21th day resp. when compared with control group.

Effect of SSO on Level of DA on Rat's Brain Homogenate

Level of dopamine from brain tissue homogenate was also estimated. In this estimation animal treated with SSO 200 mg/kg showed significant *$p < 0.05$ (0.1755 ± 0.063) raised DA level also Test-II group treated with SSO 400 mg/kg shows significant ***$p < 0.001$ (0.2152 ± 0.026) increased level of DA when compared with control group. Likewise animals treated with Syndopa (10 mg/kg) showed significant ****$p < 0.0001$ raised level of DA in brain tissue homogenate when compared with control group.

Estimation of Antioxidant Parameters

Effect of SSO on Glutathione Level in CPZ-induced EPSE Model

The statistical Analysis of brain GSH level showed significant difference between normal (####$p < 0.0001$) and CPZ treated group. However groups treated with SSO (200 mg/kg) (*$p < 0.05$) and (400 mg/kg) showed significant ***$p < 0.001$ (2.455 ± 0.0803) increase in level of GSH compared with CPZ treated group. The standard group also shows significant (****$p < 0.0001$) in GSH level compared with CPZ treated group.

Effect of SSO on Catalase Activity in CPZ-induced EPSE Model.

The statistical Analysis of brain catalase enzyme showed significant difference (####$p < 0.0001$) between normal and CPZ treated group. In SSO (200 mg/kg) and (400 mg/kg) treated groups enzymatic activity were observed to improve significantly (***$p < 0.001$) than CPZ treated group.

The values were expressed in a Mean ± SEM. The results were analyzed statistically by the one - way ANOVA followed by Dunnett's test (n=6), were *$p < 0.05$,

Table 7: Effect of SSO on chlorpromazine associated muscular rigidity

Sr. no	*Drug treatment*	*Mean of fall of time(Sec)*		
		7 th Day	14 th Day	21th Day
1	Normal	134.3 ± 4.271	136.8 ± 4.06	136.3 ± 4.842
2	Control CPZ (3 mg/kg, IP)	54.67 ± 2.741####	33.83 ± 1.922####	7.833 ± 0.009####
3	Test-I CPZ+SSO (200 mg/kg, P.O.)	58.83 ± 3.516	43.67 ± 1.820*	25.00 ± 1.065**
4	Test-II CPZ+SSO (400 mg/kg, P.O.)	69.83 ± 3.790*	56.83 ± 1.515**	52.50 ± 1.727****
5	Standard CPZ+Syndopa(10 mg/kg,P.O.)	115.3 ± 3.201****	109.0 ± 2.595****	100.3 ± 1.333****

Table 8: Effect of SSO on concentration of dopamine

Sr. no.	*Groups*	*Treatment*	*Concentration of dopamine in µg/gm of brain tissue (Mean ± SEM)*
1.	Normal	Normal saline solution	0.3381 ± 0.084
2.	Control	CPZ (3 mg/kg)	0.1417 ± 0.0167####
3.	Test-I	CPZ +SSO(200 mg/kg)	0.1755 ± 0.063*
4.	Test-II	CPZ+ SSO (400 mg/kg)	0.2152 ± 0.026***
5.	Standard	CPZ+ Syndopa(10 mg/kg)	0.3022 ± 0.0149****

Table 9: Effect of SSO on reduced glutathione level in rat's brain

Sr. no.	*Groups*	*Treatment*	*GSH level in nM/mg of protein (Mean ± SEM)*
1.	Normal	Normal Saline solution	5.387 ± 0.1460
2.	Control	CPZ (3 mg/kg)	1.800 ± 0.0789####
3.	Test-I	CPZ +SSO (200 mg/kg)	2.190 ± 0.0789*
4.	Test-II	CPZ+ SSO (400 mg/kg)	2.455 ± 0.0803***
5.	Standard	CPZ+ Syndopa (10 mg/kg)	4.925 ± 0.0614****

Table 10: Effect of SSO on catalase activity

S. no.	*Groups*	*Treatment*	*CAT activity U/mg of protein (Mean ± SEM)*
1.	Normal	Normal saline solution	5.683 ± 0.09804
2.	Control	CPZ (3 mg/kg)	3.300 ± 0.1932####
3.	Test-I	CPZ +SSO (200 mg/kg)	3.900 ± 0.1211*
4.	Test-II	CPZ+ SSO (400 mg/kg)	4.467 ± 0.1085***
5.	Standard	CPZ+ Syndopa (10 mg/kg)	5.317 ± 0.1014****

** $p < 0.01$ *** $p < 0.001$ and **** $p < 0.0001$ as compared to control group. The values in bracket indicates that the % reduction in catalepsy.

Evaluation of Hypolocomotion (Actophotometer)

The values were expressed in a Mean ± SEM. The results were analyzed statistically by the one - way ANOVA followed by Dunnett's test (n=6), were * $p < 0.05$,** $p < 0.01$ *** $p < 0.001$ and **** $p < 0.0001$ as compared to control group. #### $p < 0.0001$ as compared to normal group (control group is compared with normal group). The values in bracket indicates that the % increase in activity when compared with control.

The values were expressed in a Mean ± SEM. The results were analyzed statistically by the one - way ANOVA follow by Dunnett's test (n=6) were * $p < 0.05$, ** $p < 0.01$ *** $p < 0.001$ and **** $p < 0.0001$ as compared to control group. #### $p < 0.0001$ matched to normal group (control group is compared with normal group.

Effect of SSO on concentration of Dopamine in CPZ induced EPSE Model

The values were expressed in a Mean ± SEM. The results were analyzed statistically by the one - way ANOVA followed by Dunnett's test (n=6) were * $p < 0.05$,** $p < 0.01$ *** $p < 0.001$ and **** $p < 0.0001$ as compared to control group. #### $p < 0.0001$ as compared to normal group, (control group is matched with normal group).

Estimation of Antioxidant Parameters

Evaluation of Effect of SSO on Glutathione Level in CPZ-induced EPSE Model.

The values were expressed in a Mean ± SEM. The results were analyzed statistically by the one-way ANOVA

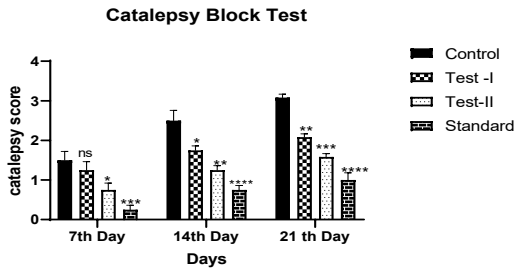

Fig. 2: Effect of SSO on chlorpromazine Associated Catalepsy

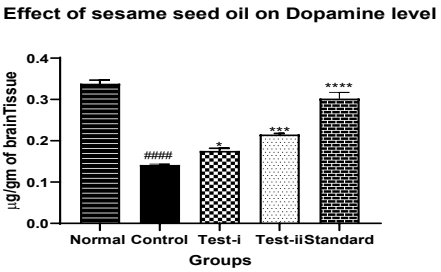

Fig. 5: Effect of SSO on concentration of Dopamine

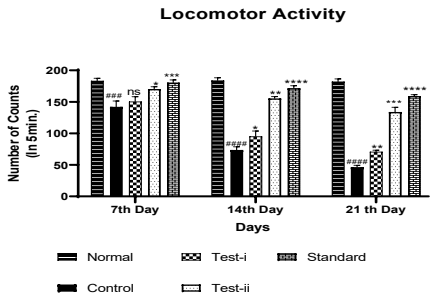

Fig. 3: Effect of SSO on CPZ associated hypo-locomotors action

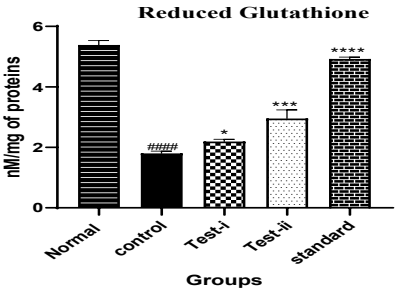

Fig. 6: Effect of SSO on Glutathione level in rat's Brain.

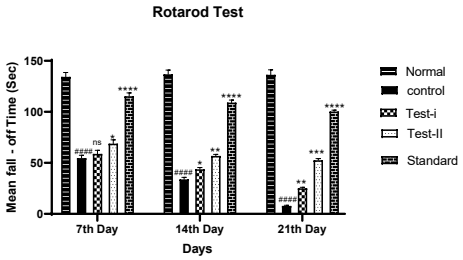

Fig. 4: Effect of SSO on chlorpromazine associated Muscular Rigidity.

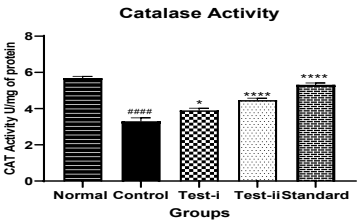

Fig. 7: Effect of SSO on Catalase Activity in rat's Brain

followed by Dunnett's test (n=6) were *$p < 0.05$,**$p < 0.01$ ***$p < 0.001$ and ****$p < 0.0001$ as compared to control group. $^{\#\#\#\#}p < 0.0001$ as compared to normal group (control group is matched with normal group).

Evaluation of Effect of SSO on Catalase Activity in CPZ-induced EPSE Model.

The values were expressed in a Mean ± SEM. The results were analyzed statistically by the one - way ANOVA followed by Dunnett's test (n=6) were *$p < 0.05$,**$p < 0.01$ ***$p < 0.001$ and ****$p < 0.0001$ as compared to control group. $^{\#\#\#\#}p < 0.0001$ as compared to normal group (control group is matched with normal group).

Discussion

Neuroleptic induced movement disorders comprise a global crisis in the management of schizophrenia. In single investigation of chronic institutionalized patient's suffer with schizophrenia; regular use of neuroleptics was linked with movement disorder in 61.6% of patients.[17]

The term extrapyramidal side effects usually refer to the disorders such as acute akathisia, parkinsonism and dystonia that develop as early onset side effect. Symptoms of parkinsonism may develops during starting days of therapy.[18]

Approximately 75% of neuroleptic-medicated schizophrenic patients exhibited parkinsonism-like symptoms rigidity, tremor, akinesia, Postural instability, hypomania and dysphasia.[19]

DIP occurs prior in antipsychotic treatment, with approximately 50–75% of cases appearing between a first month, and 90% of cases in first three months.[20] CPZ is one of from antipsychotic drugs which was scheduled as an essential drug by WHO in2003 for treatment of acute and chronic psychosis.[6] It has been linked with side effects such as (antidopaminergic) extrapyramidal syndromes, urinary retention, blurred vision, (anticholinergic), temperature, BP disturbances, and muscle control, dry mouth narcoleptic erectile impotence, diminished libido, and ejaculation inhibition in male patients.

Chronic treatment with CPZ increases the DA receptor binding site in the striatum and mesolimbic region, which could account for DA hypersensitivity that induced tardive dyskinesia. Chlorpromazine develops parkinsonism by interfering with storage of catecholamine's in intracellular granules which can leads to monoamine diminution in nerve terminals and in the initiation of hypolocomotion and muscular rigidity; thus, CPZ produces the PD-like symptoms followed by chronic treatment of rats for 21 days.[10]

The management of these neuroleptic induced PD like symptoms firstly involve discontinuation of therapy if possible or reduction of dose. One of the anticholinergics antiparkinsonian drugs may be given concurrently. But regular combination of the anticholinergics from the beginning of treatment in all cases is not allowed, because they tend to deteriorate memory and weaken intellect, in-addition to dry mouth and urinary retention. Ethopropazine, trihexyphenidyl, benzotrophins strongly anticholinergic drugs combined with other antihistaminic were used to treat neuroleptic induced extra-pyramidal side effects. This treatment combinedly found less effective but it develops various usual side effects.[5]

The surgical procedure for treatment of PD is presently considered in complex patients when the optimized medicinal management was unsuccessful in treating motor fluctuations and dyskinesia. These surgical procedures also have a various disadvantages, risk of hemorrhage, device malfunction, infection, worsening mental status etc. Most medicinal plants attenuate extra-pyramidal side effects by increasing dopamine receptor binding, increasing antioxidant enzyme activity like glutathione, increasing tyrosine hydroxylase (TH) expression and diminishing lipid peroxidation in brain.[21]

Since herbal medicines, plants and their phytochemicals can attenuate extra-pyramidal side effects like catalepsy, muscular rigidity, locomotors behaviors by different mechanisms are considered the best hope for treatment.[21]

The medicinal plants such as *N. sativa* seeds, Juniperus communis and polyherbal formulation it has been reported a promising ameliorating effect on catalepsy, muscular rigidity and hypolocomotion induced by chlorpromazine, this effect in due to its potent antioxidant and neuroprotective properties.[9-11]

Sesame seeds oil mainly contains monosaturated fatty acids (MUFA), stearic acid, linoleic acid, palmitic acid etc. SSO also contains tocopherols, sesamin, sesamolin, sesamol are potent antioxidants. Sesamol involves in triggering of endogenous antioxidant enzymes like Catalase, GSH and prevents neurodegeneration. [12]

There is lots of work on SSO has done which proves that SSO has a potential neuroprotective action by enhancing its antioxidant defense mechanism. Therefore, we examine the neuroprotective role of SSO in neuroleptic induced EPSE in the animal model.

In present study EPSE were produced in animals by administering Chlorpromazine (3 mg/kg) for 21 days. Behavioral parameters like Catalepsy, muscular rigidity and locomotors activity were mainly used for screening.

Catalepsy represents intense rigidity in the body. Catalepsy is the maintenance of disorted posture. The time it takes to return to normal posture gives an indication of extent of catalepsy. The drugs that block dopaminergic system in the nigrostriatal pathway in the brain produce catalepsy and bradykinesia (hypolocomotion), dyskinesia etc. EPSE are commonly reported with antipsychotics drugs like CPZ, haloperidol etc.[22]

Dunham and Miya suggested that test compound could be evaluated by testing the ability of rat to remain on a revolving rod. This forced motor activity has subsequently been used by many investigators. The dose which impairs the ability of 50% of the rat to remain on revolving rod is considered as end point. Therefore we employed block test, actophotometer and rotarod test for investigation.[15]

Effect on catalepsy on 7^{th}, 41^{th} and 21^{th} day we observed that SSO significantly (***$p < 0.001$) reduced catalepsy score as compared to control group and reveals dose dependant Anticataleptic activity as shown in previous reported work.[10] The percentage decrease in catalepsy score of test-I (33%), test-II (47%) and standard group (66%) which Was comparable with catalepsy score of control group on 21^{th} day. In this test catalepsy reflects intense rigidity in the body and inability to correct imposed posture. Any decrease in catalepsy scoring reflects anticataleptic activity. This was found to be similar with previous scientific reports.[9]

In locomotors behavior test (actophotometre), results showed that administration of SSO significant (**$p < 0.01$) and (****$p < 0.0001$) increase in locomotors activity on 14^{th} and 21^{th} day, respectively. A decrease in locomotors activity of control group (74%) was observed when it compared with normal group on 21^{th} day. The percentage increase in locomotors activity of test-i (34%), test-ii (65%) and standard group (70%) was comparable with control group. These results were in accordance with previous reported study.[11]

Muscle activity (Rotarod test) increase in time spend by animal on rotating rod was observed in SSO treated groups. Results showed that SSO 200 (**$p < 0.01$) and 400 mg/kg (****$p < 0.0001$) significant increase in fall of time when compared with CPZ treated group (control group) and it was also comparable with standard group. These findings were similar to the previous study.[10]

The oxidative stress was measured by GSH, CAT level in brain tissue. In present study dame results were observed in the brain homogenate of CPZ treated control group.[10]

Brain protects against oxidative stress by catalase and glutathione peroxidase. Control group showed a significant $^{\#\#\#\#}p < 0.0001$ reduction in GSH and CAT as compared

to the normal group. All observations showed that CPZ increases oxidative stress in brain of animal. The two doses of SSO were used 200 and 400 mg/kg were showed a significant ***$p < 0.001$ increase in levels of GSH and CAT. Increased level of these GSH and CAT reduces duration of catalepsy. [10]

Brain homogenate of control group showed a decreased level of dopamine and there is direct relationship between motor dysfunctioning and loss of dopamine.[11,23,24] The groups receiving SSO 200 and 400 mg/kg showed significant protective effect i.e., raised levels of DA after 21 days of treatment. Observations showed that SSO restores of dopamine level by enhancing antioxidant defense mechanisms which indicates that SSO may act as an intracellular ROS scavenger. This was found to be similar with previous scientific reports.[11]

Study finding suggests that SSO may be a good neuroprotective agent. The increase in locomotor activity, muscle activity and anticataleptic activity of SSO is due to presence of potent antioxidant compounds, sesamine, sesamolin, tocopherols, Sesamin oleic, linoleic, palmitic and stearic acid, Phenylalanine, tyrosine which reduces oxidative stress and prevents neurodegeneration.

The exact mechanism through which SSO shows protective action on CPZ associated EPSE was not clear by performing this study. The effects here reported may be a result of one chemical substance, or different secondary metabolites of the oil.

Conclusion

Based upon results obtained from present investigation, it suggests that sesame seed oil can prevent extrapyramidal side effects associated to long term therapy of chlorpromazine in animal model. Group received Sesame seed oil shows significant decrease in catalepsy score in block test also significantly increase in activity score in locomotors test and significant increase in fall off time in Rotarod test. Further it is found that group treated with 400mg/kg dose shows more significant decrease catalepsy score, increased locomotors activity as well as increases fall of time.

Sesame seed oil also helps to restore Glutathione level, CAT activity and Dopamine level by enhancing antioxidant defense mechanisms. In the conclusion, we can say that sesame seed oil has a potential to avert CPZ associated extrapyramidal side effects. However, next to its antioxidant action precise mechanism by which it protects future development of CPZ associated extrapyramidal side effects is not completely understood.

The chemical constituents present in SSO which contributes for present action it is still not clear. Further extensive studies on the isolation of phytochemicals are needed to explore the detailed mechanism for the prevention and treatment of EPSE.

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