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Research Article

STUDY OF HALOPERIDOL INDUCED BEHAVIOURAL & BIO-CHEMICAL ABNORMALITIES IN RATS: A NOVEL TOOL IN EVALUATION OF ANTI-PARKINSONIAN AGENTS

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ABSTRACT

Parkinson's disease (PD) is a complex hyperkinetic syndrome of abnormal involuntary dyskinesia movements.The present research work was aimed to study behavioural and biochemical abnormalities in haloperidol induced dyskinesia in experimental animals i.e rats. Experimental model i.e Male wistar rats, weighing 180-250 g (4-6 months old) were used in the study. Animals were acclimatized to laboratory conditions at room temperature prior to experimentation. Haloperidol at a dose of 1mg/kg s.c was administered chronically to the rats for a period of 21 days to induce PD.Various parameters assessed like behaviour response, rota rod test, narrow beam walking test, biochemical estimation, lipid peroxidation and reduced Glutathione. The animals treated with chronic haloperidol showed decrease in muscle co-ordination in narrow beam walk and in rota rod activity, glutathione concentration and increased in nitrite concentration as compared to control group. Haloperidol treatment produces behavioural abnormalities like increase in frequency of foot slips, loss of grip strength of hind paws, increase in tongue protrusions and facial jerking. Haloperidol treatment increased oxidative stress in rat brain as evidenced by increase in lipid peroxidation and nitrite production. It can be concluded that haloperidol administration to rats produces behavioural and biochemical abnormalities similar to as observed in PD patients.

Key words: Parkinson's Disease, In-vivo study, Tarditive Dyskinesia.

INTRODUCTION

Parkinson's disease (PD) is a complex hyperkinetic syndrome of abnormal involuntary hyperkinetic movements. In late 1950 a complex syndrome of involuntary hyperkinetic movements was first described and associated with the prolonged use of neuroleptics to treat the symptoms of schizophrenia in psychiatric patients [1]. The syndrome was given the name "Dyskinesia" to signify a movement disorder that could manifest itself, and persist for years, even after the discontinuation of treatment with the precipitating agent [2]. PD most often affects the mouth, lips and tongue, and tic-like motions of the lips, eyes, and eyebrows [3]. PD is a combination of two terms i.e. tardive means late onset and dyskinesia means disorder resulting in involuntary muscle movement [4]. PD is not a disease. It is a major side effect of antipsychotic drugs. The long term use of these agent shows extra pyramidal symptoms (EPS) due to dopamine supersenstivity which decrease in schizophrenia. Schizophrenia is a mental disorder characterized by disintegration of thought processes and of emotional responsiveness [5]. Abnormalities in various neurotransmitter systems are involved in the pathophysiology of PD such as dopamine supersenstivity, GABA insufficiency, serotonin receptor dysfunction,

glutamate receptor dysfunction, oxidative stress, neuroinflammation [6,7]. Dopamine is a major neurotransmitter that plays an important role both in the central and peripheral nervous system and dopamine affects a variety of processes such as motor control, cognition, emotion, neuroendocrine regulation etc. [8]. It proposes that the nigrostratial dopamine system develops increased sensitivity to dopamine as a consequence of chronic dopamine receptor blockade induced by neuroleptic drugs. Behavioural evidence of dopamine hypersensitivity following neuroleptics treatment is seen in various animal models. Biochemical changes due to increased number of dopamine D_2 receptor are usually found after repeated neuroleptic treatment but ths is not the case in all studies. A modification in the D_2 receptor hypersensitivity hypothesis incorporates a role for dopamine D_1 receptors. This possibility is supported by the wealth of data documenting the functional interactions between D_1 and D_2 receptors. Basically PD develops from an imbalance between D_1 and D_2 mediated effects in the basal ganglia. Second hypothesis for the pathophysiology of PD involves gamma aminobutyric acid insufficiency, due to the acute and chronic treatment with neuroleptics which cause the decreased glutamic acid decarboxylase, the GABA synthesizing enzyme, was noted in substantia nigra, medial globus pallidus and subthalamic nuclei in dyskinetic monkeys as compared to neuroleptic treated non PD monkeys [9]. Although biochemical data suggests for the involvement of GABA but there has not been enough clinical evidence to support the fact. The postmortem studies have shown a trend towards decreased GAD activity in the medial globus pallidus, but normal levels in the substantia nigra in PD patients. Chronic use of neuroleptics alters both dopaminergic and serotonergic neurotransmission as indicated by changes in DA, D_1, D_2 and 5-HT_1, 5-HT_2 receptor binding [10]. Serotonin may modulate dopamine activity and thus be involved with PD. The involvement of excitotoxicity in acute neuronal damage is well described, but in chronic degeneration and PD, the exact mechanism of the excitotoxicity is not clear. The long term use of neuroleptics treatment has been shown to decrease the expression of the GLT-1 transporter in the rat striatum and also include extrapyramidal side effects which occur in glutaminergic synapses [11]. Modest but sustained elevation of extracellular glutamate levels could be involved. An intriguing possibility is that impairment of cellular energy metabolism by classical neuroleptics could be involved. Disruption of ATP synthesis in the neuron may lead to a decreased membrane potential and to a decreased ion pump activity. This will facilitate the activation of NMDA receptors due to the opening of the voltage dependent calcium channels by magnesium, leading to an excess of calcium influx triggering the cascade of excitoxic reactions. Oxidative stress has also been involves in the pathophysiology of PD. Oxidative stress increases the release of inflammatory mediators like TNF- alpha and interleukin-1-beta, IL-6 which initiate the apoptotic pathway, a central mechanism of neuronal cell death. Anti-psychotics significantly inhibited the production of NO and pro inflammatory mediators results in neuroinflammation [12].

Haloperidol and other antipsychotic drug used for the treatment of schizophrenia and is well established animal model to study the pathogenesis of PD. The long term use of Haloperidol results in blockade of dopamine receptors and produce neurotoxicity due to generation of reactive oxygen species, increased oxidative stress and alters neurochemical balance. Haloperidol has been widely recognized as valuable tool to study the neuropathology of PD. It is a well studied concept and has been validated and confirmed with the time again by several group of workers. Chronic administration of haloperidol to rodents results in dopamine supersenstivity, oxidative stress, and dopamine supersenstivity along with glutamate excitotoxicoty, GABAergic hypofunction are among well established concepts. There is no effective treatment available that can slow down the neurodegeneration. Primary prevention of

PD is achieved by using the lowest effective dose of a neuroleptic for the shortest time. If PD is diagnosed, the causative drug should be discontinued. Dyskinesia may persist after withdrawl of drug for months, years or even permanently. Only approved drug for the management of dyskinesias should be used.

Schizophrenia is a serious brain disorder that affects a person's ability to think clearly, manage emotions, distinguish reality from unreality, make decisions, and relate to others. It is a chronic, disabling brain disorder that has affected people throughout history. Available treatment for schizophrenia is with neuroleptics which has left a great impact in psychiatry by treating schizophrenic patients on one side and giving a way to another deadly disease. A variety of neurological syndromes involving extrapyramidal symptoms occur after acute and chronic administration of neuroleptics i.e akathisisa, dystonia, parkinsonism, dyskinesia.

PD, initially called "persistent dyskinesia" or reversible and "irreversible drug related dyskinesia" is a complex hyperkinetic syndrome consisting of choriform, athetoid and rhythmic abnormal involuntary movements. It involves the facial, buccal and masticatory muscles, extending often to the upper and lower extremities including the neck, trunk, fingers. PD is among the most serious extrapyramidal symptoms in terms of frequency, persistence, treatment resistance, overall imact on the well being of the patients and the caregivers. The syndrome is very annoying and usually has a delayed onset and the intensity of the disturbance may fluctuate over time. PD is a neurological condition caused by prolonged use of dopamine receptor blocking agents, such as antipsychotic drugs used for the treatment of schizophrenia. The involuntary movements can be embarrassing and affect basic life functions, such as eating, breathing, walking. The occurance and irreversibility of this hyperkinetic disorder has been considered a major clinical issue in the treatment of schizophrenia. Some other classes of drugs producing symptoms of PD such as anti-cholinergics, anti-emetics, anti-epileptics, anti-histamines, anti-depressants etc. PD can create other problems that make it even more difficult to manage. Denture problems, tongue ulcerations, dysarthria, respiratory disturbances gastrointestinal disturbances, motor function difficulty, fixed postures and difficulty in swallowing can often occur alongside PD. Cognitive deficits are considered as core features of the pathophysiology of psychotic disorders and schizophrenia.

REVIEW OF LITERATURE:

PD is a neurological condition caused by prolonged use of dopamine receptor blocking agents, such as antipsychotic drugs used for the treatment of schizophrenia. The most common symptoms are orofacial also may involve dancing or writing movements of the arms, legs, the trunk or involuntary finger movements, in which patient may appear to be playing a piano or guitar. PD is a combination of two terms i.e. tardive means late onset and dyskinesia means disorder resulting in involuntary muscle movement. The involuntary movements can be embarrassing and affect basic life functions, such as eating, breathing and walking. The incidence of cases of PD has been estimated at 5% per year on antipsychotic therapy with conventional antipsychotic medications. Patients are less likely to develop PD with atypical antipsychotics. The first of these agents became available in the late 1980_s for the treatment of patients with schizophrenia. Schizophrenia is a severe mental disorder which is characterized by some deterioration in personal functioning [14].

The most common clinical presentation of PD is orofacial dyskinesia which includes opening, protrusion and retrieval of the tongue and closing of mouth, chewing, licking, sucking, smacking (press together and open the lips quickly and noisily, as in eating or tasting), panting (breathe

rapidly in short gasps, as after exertion) and grimacing (making a sharp contortion of the face). Physically, PD can create other problems that make it more difficult to manage. Denture problems, tongue ulcerations, dysarthria, respiratory and gastrointestinal disturbances, motor function difficulty, fixed postures and difficult in swallowing can often occur alongside PD. There may be feelings of embarrassment and guilt, shame, depression, social withdrawal and the patient may find it difficult to maintain the personal and professional relationships.

Historical background:

Dyskinesia was first named and classified in 1964. By the early 1960s, symptoms associated with dyskinesia were apparent in approximately 30 percent of psychiatric patients treated with antipsychotic medications, linking the development of the condition to these drugs. The development of dyskinesia is commonly linked to metoclopramide use. The drug metoclopramide (sold today under the brand name Reglan, among others) was developed in Europe in the mid-1960s and become available for use in 1982. In early 2009, the Food and Drug Administration issued a warning about metoclopramide, informing the public of research that suggests the use of metoclopramide is the most common cause of drug induced movement disorders. A 2004 study found that older women treated with metoclopramide were at increased risk for developing symptoms of dyskinesia, additionally, men, infants and children are also commonly affected by metoclopramide induced dyskinesia.

Risk Factors:

1 Age

Statistics from the National Alliance on Mental illness show that the elderly are much more likely to develop PD than those in a younger age category. Part of the reason for this may be that the deugs which cause the disorder are more frequently prescribed to other individuals, such as those drugs used in the treatment of gastrointestinal problems, including heartburn, nausea, vomiting, gastric acid reflux,and gastroparesis in diabetic patients. One study published in the American Journal of Psychiatry in 1998 indicated that patients who are over the age of 50 and taking typical antipsychotics are three to five times more likely to develop T. Proposed hypothesis include interactions with drug induced changes in the receptors of striatum and age related degenerated changes in the nigrosriatal system. While PD has been reported in children and adolescents, it is considered to be uncommon but adequate epidemiological studies are few.

2 Gender

Gender differences in the prevalence of PD have reported by number of researchers. Some reports suggest that women tended to have more severe PD and a higher prevalence of spontaneous dyskinesia than men. Post menopausal women accounts for the group of individuals most often diagnosed with disorder. Again, part of the reason for this may be that they are the group most likely to be given the drug for long term use. Further, estrogen has been suggested to have a protective anti-dopaminergic effect and the decline in the levels of this hormones at menopause may account for the reported increased prevalence in older women.

3 Mental retardation

Individuals who are mentally handicapped or who suffer from some sort of organic brain dysfunction or atrophy are more likely to develop the disorder than those with healthy brains. Patients receiving long term treatment with drugs such as prochlorperazine and metoclopramide hydrochloride are also at risk of PD. Although, metoclopramide hydrochloride is marketed for

gastrointestinal disorders and nausea, its mechanism of action is through dopamine receptor blockade, therefore it acts as antipsychotic at higher doses and can produce all acute extrapyramidal symptoms.

4 Substance abuse

People with a history of alcoholism or drug abuse seem to be more prone to develop the disorder when prescribed the drugs associated with PD. Studies have been reported a positive correlation between smoking and PD. Reports found a positive association between higher antipsychotics doses and smoking but not between PD and smoking. The use of other psychoactive substances especially chronic use of high dose of amphetamines may lead to PD.

5 Neuroleptics dose and duration of treatment

A large, long term, prospective study of PD shows a significant positive correlation between its onset and both total treatment duration and total drug dose. Prospective studied correlating blood levels of neuroleptics agents with PD have not yet been done. In cross sectional evaluatons, blood levels of these drugs were higher in patients with PD than in those without the disorder but this was not confirmed in a subsequent investigation.

6 Drug type

At the present time, there are no compelling data from prospective clinical studies that any currently available antipsychotic drug has a lower risk of PD, with the possible exception of clozapine.

7 Other drugs

Anticholinergic and antiemetic drugs are known to be exaggerate PD or make latent PD become manifest, but there is no convincing evidence they are risk factors for PD.

8 Genetic factors

Genetic predisposition to PD has been suggested from family studies that show concordance for PD among first degree relatives of patients with PD also treated with antipsychotics. As the hypothesis for the pathophysiology posits a state of dopamine receptor hypersensitivity from chronic antipsychotic treatment, an obvious candidate gene is the D_2 receptor gene. Chen et al. reported a significant association between the dopamine D_2 receptors gene and PD. Dopamine D_3 receptor polymorphism has been implicated as a vulnerability factor in some reports but not in all.

Symptoms of PD

1 Motor symptoms of PD

a. Dystonia: It implies an abnormal posture of one or more portions of the body with an inappropriate sustained contraction of muscle. A dystonia foot may be involuntary inverted or a dystonic neck (torticollis) may be apparent only in certain situations or with certain tasks. For example, foot dystonia during walking may normalize during running or walking backwards [15].

b. Tremor: Tremors are basic categories of hyperkinetic movements that originate outside conscious awareness. Rhythmicity is the hallmark of tremor implies a regular rhythm with a

relatively consistent periodicity. Tremor occurs for more than just a few seconds, attending to the rhythmicity and frequency [16].

c. Myoclonus: Myoclonus is defined as sudden lightening like jerks of a body part. The jerk, by definition, involves at least one entire muscle and displaces a body part. This is in contrast to fasciculation or myokymia which occur in only a segment of muscle and does not result in movement across joints [17].

d. Chorea: Chorea is a rapid, flowing movements of a part or parts of the body that are random. Patients may perform simple voluntary movements with a choreiform flourish. For example, voluntary touching of the nose may be preceded by a wide circling movement of the hand before the finger lands precisely on the target [18].

e. Bradykinesia: Bradykinesia is a hypokinetic disorder mainly responsible for the degeneration of the substantia nigra. Bradykinesia has many different aspects including prolonged reaction time to initiate a movement, prolonged time to arrest a false movement, prolonged time to change a motor pattern and weakness, at least to the extent of rapid fatigue on prolonged tasks [19].

f. Tic Disorders: Tics are simple or complex motor acts occurring in response to an urge to perform the movement. Usually they are manifested as a single type or a few repeated type or a few repeated types that are stereotyped. These stereotyped movements may be simple or complex. Shrugging, blinking, grimacing and grunting are examples of simple tics. More complex tics might99 include kicking, squatting or vocalizing words [20].

g. Athetosis: Athetosis is a slow, writhing involuntary movement of a distal limb. On close analysis, the appearance is actually that of chorea combined with dystonia. When the choreiform component is prominent, the term “choreoathetosis” may be applied. Athetosis and choreoathetosis are terms that conventionally have most often been used in cerebral palsy [21].

h. Akathisia: Akathisia implies an inner restlessness, which if severe, provokes movement as a means of relief. This may occur as a consequence of administration of dopamine antagonist drugs either early, as a reversible adverse event, or later as a tardive disorder. The motor responses to akathisia take on a variety of forms, but the usual appearance is in ability to sit still [22].

2 Non motor symptoms of PD

a. Cognitive Dysfunction: Cognitive dysfunction is the loss of intellectual functions such as thinking, remembering, and reasoning of sufficient severity to interfere with daily functioning. Patients with cognitive dysfunction have trouble with verbal recall, basic arithmetic, and concentration. Cognitive dysfunction is a category of mental health dysfunction that primarily affect learning, memory, perception, and problem solving, and include amnesia, dementia, and delirium [23].

b. Anxiety: Anxiety is a psychological and physiological state characterized by somatic, emotional, cognitive, behavioural components. Anxiety can create feeling of fear, worry, uneasiness, and dread. It is also associated with feelings of restlessness, fatigue, concentration problems and muscle tension [24].

c. Depression: PD can cause depression in upto 80% of patients. Depression is a common psychiatric symptom. Women are more prone to develop depression and such susceptibility might be related to 5-hydroxytryptaminergic dysregulation [25].

d. Pain: Pain is a non-motor symptom that substantially affects the quality of life of at least one-third of patients with PD. Interestingly, patients with PD frequently report different types of pain.

Pain, defined as an unpleasant or distressing sensory experience, has been recognized as feature of PD since the first descriptions of the disorder [26].

e. Sensory Dysfunction: Dysfunction of Sensory Integration is a neurological disability in which the brain is unable to accurately process the information coming in from the senses. An individual with sensory integration dysfunction would have a decreased ability to organize sensory information as it comes in through the senses [27].

Etiology

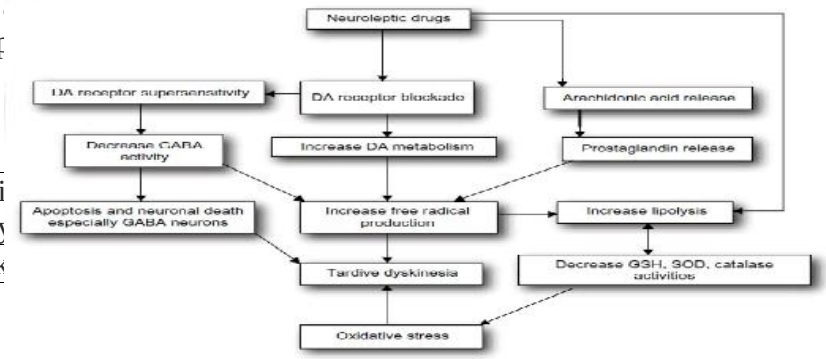
1 Drugs

PD can be caused by long-term treatment with dopamine antagonists. It can also be caused by both high-potency and low-potency traditional neuroleptics, including long-acting depot formulations (e.g., deaconate and enanthate). Greater D_2 dopamine receptor blockade at the trough levels of the neuroleptics may be associated with a greater degree of PD. Amisulpride has been associated with PD [28], but in general, newer atypical antipsychotic agents, including olanzapine and risperidone (and its metabolite paliperidone' appear to carry less risk of PD[29].

The antiemetic metoclopramide, a potent D_2 dopamine receptor antagonist, may cause PD, particularly in elderly patients. PDs have also been reported with the use of antihistamines, fluoxetine, amoxapine (a tricyclic antidepressant), and other agents (see table below 1)

Table-1. Medications responsible for dyskinesia

Category	Agents
Antipsychotic neurolep	Butyrophenones: droperidol, haloperidol,
Newer atypical anti agents (sporadically	erazine ulpride
PD= dysk	



Pathophysiology of PD

Inspite of the enormous frequency of occurrence of PD, relatively known about the primary neurological mechanisms responsible for its development. Supersenstivity of striatal dopamine receptors were previously thought to be the mechanism involved in the development of PD and this supersenstivity may be a normal consequence of neuroleptic treatment and does not explain why PD develops and persists only in some patients [30]. It now seems that several neurotransmitters may be affected. These include dopaminergic, noradrenergic pathway and serotonergic pathway. Abnormalities in various neurotransmitter in caudate putamen and substantia nigra which are basically involved in the neuropathology of PD.

Figure:1 Illustration presenting the different mechanisms of manifestation of oxidative damage by neuroleptic and cause PD.

1 Dopamine system

Although the pathophysiology of PD is not well understood, it is hypothesized that central dopamine plays a role in the pathogenesis of the condition. It is also hypothesized that acute

movement disorder result, in part, from the blockade of dopamine receptors by dopamine antagonists.

Several mechanisms have been proposed by which PD may develop including the following:

1. Striatal dopamine receptor supersenstivity.
2. Chronic dopamine blockade may result in upregulation of dopamine receptor responsiveness.
3. Compensatory supersenstivity of dopamine receptor may develop after long term blockade; long term blockade of dopamine D_2 antagonists (e.g. neuroleptics) may produce PD.
4. When dopamine D_2 receptor blockade is reduced (even slightly), an exaggerated response of the post synaptic dopamine D_2 receptor (even to low concentrations of dopamine) may result.
5. Striatal disinhibition of the thalamocortical pathway from imbalance of D_1 and D_2 receptors may be involved.
6. Neurodegeneration secondary to lipid peroxidation to excitotoxic mechanisms may be responsible [31].

Although the dopamine D_2 receptor has traditionally been implicated in the pathogenesis of PD, there is evidence to indicate that in some individuals, the dopamine D_3, D_4 and D_5 receptors are involved. Support for the hypothesis that PD may result from blockade of postsynaptic dopamine receptors in the basal ganglia and other parts of the brain exists in the form of the beneficial effects of increasing doses of neuroleptics for some patients with PD. Thus, dopamine antagonists may mask PD [32].

(a) Dopamine receptor supersenstivity:

Chronic blockade of dopamine receptors by haloperidol in animals may lead to an upregulation of dopamine receptors and increased their sensitivity toward the dopamine or dopaminergic agonists.

(b) Enhanced dopamine metabolism:

Chronic blockade of dopamine receptors may also result in an increase in metabolism of dopamine following its release. An increase in dopamine metabolism may lead to the generation of free radicals and thus enhance oxidative stress.

(c) Free radical generation:

Generation of excessive free radicals and oxidative stress by chronic haloperidol administration may damage different neurons such as GABAergic or dopaminergic systems and produce PD-like symptoms in animals.

(d) Enhanced prostaglandin production: Chronic haloperidol treatment may lead to enhanced production of inflammatory prostaglandins that, in turn, can increase the oxidative stress in the brain and exaggerate PD-like symptoms.

All these proposed mechanisms have been illustrated in Figure.1. Chronic administration of haloperidol for 21 days in rodents has shown to produce Vacuous chewing movements (VCMs), Tongue protrusions (TP) and facial jerking. Neurochemical analysis has revealed that chronic administration of haloperidol (1 mg/kg) reduces the level of norepinephrine, dopamine and serotonin in the striatum region of the rat brain when identified through in-vivo micro dialysis studies. Chronic administration of haloperidol has been associated with an increased expression of inflammatory markers such as TNF- alpha (tumour necrosis factor-alpha) and NF-kB (nuclear factor kappa-light chain-enhancer of activated B cells) that may lead to neurotoxicity. A study

conducted in our laboratory has shown that chronic administration of haloperidol significantly increased the levels of TNF-alpha and NF-kappaB p65 subunit in rat striatum [33].

6 Animals models of PD: Various models of PD are

(a) Haloperidol-induced PD
(b) Reserpine-induced PD
(c) Chlorpromazine-induced PD
(d) Isoniazid-induced PD
(e) Primate model of PD
(f) Resperidone induced PD

1 Haloperidol-induced PD

Haloperidol marketed under the trade name Haldol among others, is atypical antipsychotic medication. It is used in the treatment of schizophrenia, acute psychosis, mania, delirium, tics in Tourette syndrome, chorea, nausea and vomiting in palliative care, intractable hiccups, agitation and severe anxiety.

Haloperidol interferes with the effects of neurotransmitters in the brain which are the chemical messengers that nerve manufacture and release to communicate with one another. Haloperidol blocks receptors for the neurotransmitters (specifically the dopamine and serotonin type 2 receptors) on the nerves. As a result, the nerves are not “activated” by the neurotransmitters released by other nerves. Haloperidol was approved by the FDA in 1967. Haloperidol may result in a movement disorder known as Dyskinesia which may be permanent [34]. Neuroleptic malignant syndrome and QT-interval prolongation may occur. Its use in the elderly with psychosis due to dementia results in an increased risk of death. When taken during pregnancy it may result in problem in the infant. Haloperidol is used in the control of the symptoms of:

1. Schizophrenia [34]
2. Acute psychosis, such as drug-induced psychosis caused by LSD, psilocybin, amphetamines, ketamine, phencyclidine and psychosis associated with high fever or metabolic disease
3. Hyperactivity, aggression
4. Hyperactive delirium (to control the agitation component of delirium)
5. Otherwise uncontrollable, severe behavioural disorders in children and adolescents
6. Agitation and confusion associated with cerebral sclerosis
7. Adjunctive treatment of alcohol and opioid withdrawal
8. Treatment of severe nausea and emesis in postoperative and palliative care, especially for palliating adverse effects of radiation therapy and chemotherapy in oncology
9. Treatment of neurological disorders, such as tic disorders, Tourette syndrome and chorea
10. Therapeutic trial in personality disorders, such as borderline personality disorder

Haloperidol (1 mg/kg) if administered chronically for approximately 21 days in rodents has been shown to produce PD like symptoms. The symptoms are more severe in aged rats as demonstrated by significant increase in hyperkinetic motor activities, Vacuous chewing movements (VCMs), Tongue protrusions (TP), facial jerking, and development of dopamine supersenstivity as compared to young adults.

OBJECTIVE OF THE WORK:

The present study was aimed to study behavioural and biochemical abnormalities in haloperidol induced dyskinesia in rats.

MATERIAL AND METHODS:

Experimental model: Haloperidol induced dyskinesia (PD)
Experimental protocol: n=6
GROUP 1- Vehicle control GROUP 2- Haloperidol treated (1mg/kg) i.p

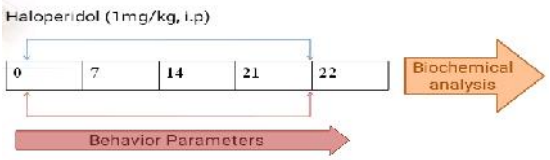
EXPERIMENTAL ANIMALS:

Male wistar rats, weighing 180-250 g (4-6 months old) were bred in the central Animal House of I.S.F. College of Pharmacy, Moga, Punjab and were used in the study. Animals were acclimatized to laboratory conditions at room temperature prior to experimentation. Animals were kept under standard conditions of a 12 h light/dark cycle with food and water at libitum in plastic cages with soft bedding. All the behavioural assessments were carried out between 9:00 and 15:00 hrs. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) with the registration no. ISF/IAEC/73/5/2012 and were carried out in accordance with the guidelines of the Indian National Science Academy (INSA) for the use and care of the experimental animals.

INDUCTION OF PD

Haloperidol at a dose of 1mg/kg s.c was administered chronically to the rats for a period of 21 days to induce PD. All the behavioural assessment was carried out every week and the last behavioural quantification was done 24 hours after the last dose of Haloperidol.

EXPERIMENTAL DESIGN



PARAMETERS ASSESSED

Behaviour Parameters:

Rota rod: The rota rod performance test is a performance test based on a rotating rod with forced motor activity being applied, usually by a rodent. The test measures parameters such as riding time (seconds) or endurance. Some of the functions of the test include evaluating balance, grip strength and motor coordination of the subjects; especially in testing the effect of experimental drugs or after traumatic brain injury [36].

In the test, a rodent is placed on a horizontally oriented, rotating cylinder (rod) suspended above a cage floor, which is low enough to injure the animal, but high enough to induce avoidance of fall. Rodents naturally try to stay on the rotating cylinder, or rota rod, and avoid falling to the ground. The length of time that a given animal stays on this rotating rod is a measure of their balance, coordination, physical condition, and motor planning. The speed of the rota rod is mechanically driven, and may either be held constant, or accelerated.

Narrow beam walking:

Fine motor coordination can be assessed by using a beam walking or beam balance test. This test essentially examines the ability of the animal to remain upright and to walk on an elevated and relatively narrow beam. Performance on the beam is quantified by measuring the time it takes for the mouse to traverse the beam and the number of paw slips that occur in the process.

Biochemical Estimation

Lipid peroxidation:

Principle: Oxidative stress that occurs in the cells, as a consequence of an inequity between the prooxidant /antioxidant systems, causes injure to biomolecules such as nucleic acids, proteins, structural carbohydrates, and lipids. Among these targets, the peroxidation of lipids is basically damaging because the formation of lipid peroxidation products leads to spread of free radical reactions. The general process of lipid peroxidation consists of three stages: initiation, propagation and termination.
Procedure: The quantitative measurement of lipid peroxidation in liver was performed according to the method of Will's, 1965. The amount of malondialdehyde (MDA), a measure of lipid peroxidation was assay in the form of thiobarbituric acid reacting substance (TBRAS). Briefly, 0.5 ml of supernatant and 0.5 ml Tris HCL was incubated at 37°C for 2 hrs. After incubation, 1 ml of 10% Trichloroacetic acid (TCA) was added and centrifuged at 10,000 × g for 10 min. To 1 ml of supernatant 1ml of 0.67% thiobarbituric acid was added and the tubes were kept in boiling water for 10 min. After cooling 1ml double distilled water was added and absorbance was measured at 532 nm using a spectrophotometer. TBRAS were quantified using an extinction coefficient of 1.56 × 105 M-1cm-1 and expressed as n mole of malondialdehyde per mg protein.

Reduced Glutathione

Principle: Glutathione, or GSH, is an antioxidant found in every cell in the body. It is widely known for controlling free radicals. The majority of glutathione in the body is present in its reduced form because this is the only way it can perform its critical role. Glutathione protects cells from the free radicals produced through oxidation. It can only do this by remaining in its naturally reduced state so that it is readily available to neutralise free radicals by bonding with them. As GSH bonds it converts to its oxidized form, called glutathione disulphide. Then an enzyme glutathione reductase reverts it back to its reduced state. The ratio of reduced GSH to oxidized GSH within the cells can be used to measure cellular toxicity. In healthy cells, 90% of the GSH should be in its reduced form.
Procedure: Reduced glutathione was estimated according to the method of Elman, 1959. About 1 ml supernatant was precipitated with 1 ml of 4% sulfosalicylic acid and cold digested at 4°C. The sample was centrifuged at 12,000 g for 15 min at 4°C. To 1 ml of this supernatant, 2.7 ml of phosphate buffer (0.1m, pH 8) and 0.2 ml of 5,5 dithiobis (2-nitrobenzoic acid {DTNB}) were added. The yellow colour developed was read immediately at 412 nm using a spectrophotometer. Results were calculated using molar extinction coefficient of chromophore (1.36 × 104 m - 1cm-1) and expressed as percentage of control.

RESULTS:

Behavioural Parameters

1. Effects of haloperidol administration on rotarod activity in rats.

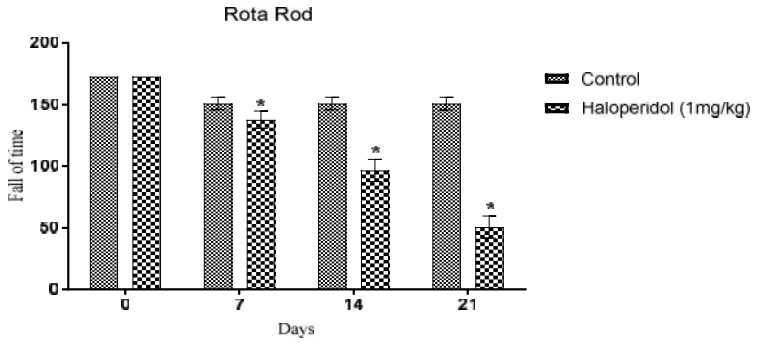


Fig. 2: Effect of haloperidol administration on rotarod activity in rats. Data expressed as Mean ± SD; *p<0.05 vs. Control.

5.1.2. Effect of haloperidol administration on transfer latency in narrow beam walk in rats.

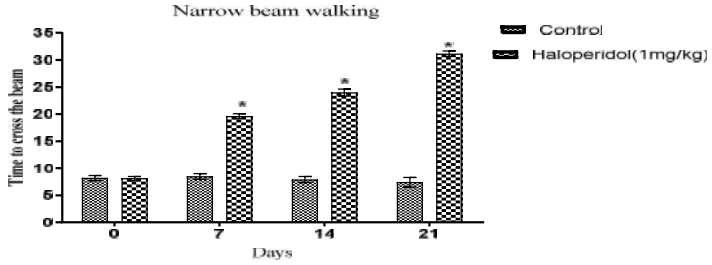


Fig. 3: Effect of haloperidol administration on transfer latency in narrow beam walk in rats. Data expressed as Mean ± SD; *p<0.05 vs. Control.

5.1.3. Effect of haloperidol administration on no. of foot slips in narrow beam walk in rats.

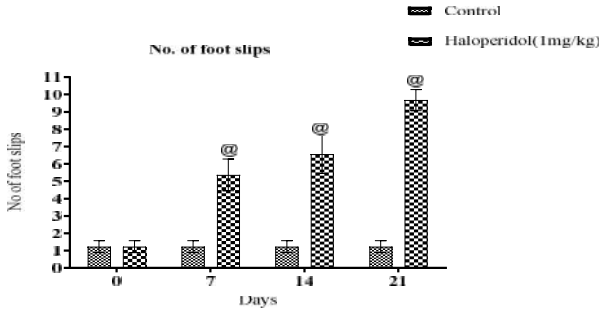


Fig 4: Effect of haloperidol administration on number of foot slips in narrow beam walk in rats. Data expressed as Mean ± SD; @p<0.05 vs Control.

Effect of haloperidol administration on facial jerking in rats.

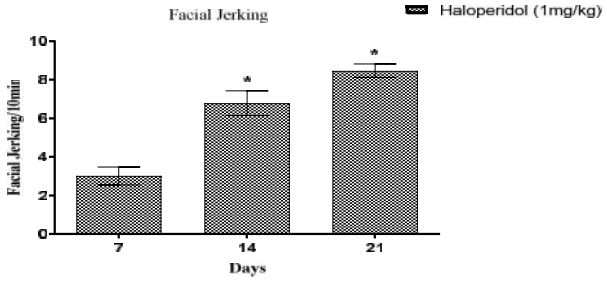


Fig 5: Haloperidol (1mg/kg i.p) treatment resulted in time-dependent increased in facial jerking as data expressed as Mean ± SD; $^{*}p<0.05$ vs 7^{th} day.

Effect of haloperidol administration on tongue protrusions in rats.

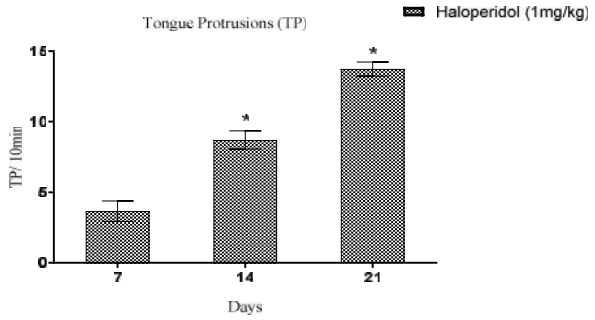


Fig 6: Effect of haloperidol (1mg/kg i.p) treatment on tongue protrusions in rats. Data expressed as Mean ± SD; $^{*}p<0.05$ vs 7^{th} day.

Biochemical Estimation:

Table 1 Effect of haloperidol treated animal on nitrite and glutathione levels in rats.

S. No	Treatment (mg/kg)	Reduced Glutathione (µ mole GSH/mg protein) (% of control)	Nitrite (µg/ml of protein) (% of control)
1.	Normal control	100 ± 5.72	100 ± 7.23
2.	Haloperidol treated (1 mg\kg i.p)	28 ±4.63	232 ±11.2

DISCUSSION:

Dyskinesia is a complex hyperkinetic syndrome of abnormal involuntary hyperkinetic movements. PD is not a disease; it is a major side effect of antipsychotic drugs. Its motor symptoms are dystonia, myoclonus, chorea, occurs due to chronic neuroleptic treatment. It involves involuntary movements of face, mouth, tongue, rarely limbs and trunk. The use of neuroleptics drugs leads to imbalance of free radicals which cause oxidative stress which might contribute to PD and other involuntary movements. Dopamine blockers block the dopamine D2 inhibitory receptors. The behavioural, biochemical and neurochemical abnormalities are easily studied in haloperidol induce dyskinesia animal model. Haloperidol is a neuroleptic and blocks the dopamine receptors which may lead to the dopamine supersenstivity, enhanced dopamine metabolism, free radical generation, increased prostaglandin production that results in PD like symptoms. The dopamine is metabolised into 3,4- dihydrophenylacetic acid along with production of hydrogen peroxide and other free radicals. The cause of generation of free radicals

and oxidative stress is due to an increased concentration of dopamine metabolism in basal ganglia. Basal ganglia is the region of brain contains copper and ferric ions, these ions react with hydrogen peroxide to form most toxic hydroxyl radical which is responsible for large production of free radicals due to increased dopamine turnover in the presence of enhanced amount of energy and polyunsaturated fatty acids. Neuroleptics and glutamatergic transmission increase the dopamine turnover leads to increased free radical production and oxidative stress. Results showed the relevance of the data obtained to the data procured from the literature after the chronic administration of haloperidol showing increased oxidative damage parameters in various areas of brain. According to recent literature the chronic use of haloperidol leads to imbalanced production and detoxification of free radicals and increase the permeability to various membranes which may be associated with initiation of orofacial movements. Chronic administration of haloperidol may also lead to decrease in brain antioxidant defence which may associate with increased oxidative metabolism which is shown by increased lipid peroxidation, nitrite concentration, reduced glutathione and reduced antioxidant enzyme like catalase and SOD in the rat striatum. The literature has many facts which shows that decreased glutathione and increased lipid peroxidation is associated with increased oxidative stress. In the present study the animals treated with chronic haloperidol showed behavioural symptoms of orofacial dyskinesia through tongue protrusion and facial jerking. The animals treated with chronic haloperidol also showed decrease in muscle co-ordination in narrow beam walk and in rota rod activity. The animals treated with chronic haloperidol showed decreased in glutathione concentration and increased in nitrite concentration as compared to control group. Our study shows relevance with literature.

CONCLUDATORY COMMENTS:

The present study was aimed to evaluate haloperidol induced behavioural and biochemical abnormalities in rats. On the basis of result obtained the salient findings may be summarized as:

1. Haloperidol treatment produces behavioural abnormalities as evidenced by increase in frequency of foot slips as well as latency to cross beam on narrow beam walk test, loss of grip strength of hind paws on rota rod test and increase in tongue protrusions and facial jerking.
2. Haloperidol treatment increased oxidative stress in rat brain as evidenced by increase in lipid peroxidation and nitrite production.

Therefore, it can be concluded that haloperidol administration to rats produces behavioural and biochemical abnormalities similar to as observed in PD patients.

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