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Research Article

STUDY OF THE PROTECTIVE EFFECT OF FORTIFIED WHEAT GRASS WITH COW URINE IN EXPERIMENTALLY INDUCED PARKINSONISM

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Abstract: Parkinson's disease (PD) is neurodegenerative disorder characterized by the progressive loss of the dopaminergic neurons in the substantia nigra pars compacta which innervates the dorsal striatum. Since the existing anti Parkinson's drugs encountered many side effects and need for prolonged treatment including questionable efficacy in the treatment, may cause Parkinson related movement problems, hallucinations and orthostatic hypotension. These reasons force the area of research to find improved treatments which will counteract the side effects and the draw backs of the existing treatment. Herbal drugs having diversified uses are always an alternative option to the synthetic drugs which are well known for their side and adverse effects. Using haloperidol induced catalepsy and muscle rigidity in rats the effects of Fortified Wheat grass (FWG) were studied. Haloperidol was administered at a dose of 1mg/kg; Fortified wheat grass with cow urine was administered at doses 150mg/kg & 300mg/kg. In conclusion fortified wheat grass with cow urine at doses of 150 and 300 mg/kg exhibited significant anti cataleptic activity, significantly reversed the haloperidol inhibited locomotor activity, restored the changes in behavioral assessment like akinesia, immobility in haloperidol administered rats, reduced the haloperidol induced rigidity. The possibility of pharmacological interactions between haloperidol and fortified wheat grass with cow urine should be further investigated in my research work.

Keywords: Parkinson's disease, *Wheat grass*, Haloperidol, Cataleptic activity, muscle rigidity.

INTRODUCTION

Parkinson's disease is a progressive neurodegenerative movement disorder that is estimated to affect approximately 1% of the population older than 65 years of age[1, 2]. Clinically the cardinal symptoms were first described by James Parkinson 1817 with most patients presenting bradykinesia, resting tremor, rigidity and postural instability[3]. A number of patients also suffer from autonomic, cognitive, and psychiatric disturbances[4]. The major symptoms of PD result from the profound and selective loss of dopaminergic (DAergic) neurons in the substantia nigra pars compacta (SNc)[5]. The pathological hallmarks of PD are lewy bodies (LBs) and dystrophic neuritis (Lewy neuritis) present in surviving neurons[6]. PD is a common neurodegenerative disorder characterized by neuronal cell loss in the substantianigra (SN) and subsequently reduced secretion of dopamine (DA). PD is the second most common neurodegenerative disease after Alzheimer's disease, affecting up to 1% of the elderly population over the age of 65. Indeed, the estimated generic risk ratio for PD is approximately 1.7 (70% increased for PD) for all ages[7, 8, 9]. The age –adjusted prevalence rate of PD revealed from a pilot study of at least 42.5% increase in the disease compared to 1966[55].

Idiopathic Parkinsonisim (IPD) is characterized by progressive and profound loss of neuromelanin containing DAergic neurons in the substantial nigra pars compacta (SNpc)[10]. Moreover, slight gliosis and neuronal loss in the locus coeruleus, dorsal vagal nucleus with variable involvement of the nucleus basalis Meynert, and other subcortical nuclei have been reported[11]. Degeneration of pigmented neuronal systems located in the brain stem, particularly in SNpc, is the most striking pathological feature of PD. This causes striatal DA deficiency and all the major motor PD symptoms.

Apart from the loss of melanized[12], nigrostriatal DAergic neurons another major pathological hallmark of PD includes the presence of intraneuronal proteinacious cytoplasmic inclusions "Lewy Bodies" (LBs). This result in the classical neuropathological finding SNpcdepigmention where the SNpcDAergic cell bodies project primarily to the putamen. However LBs are not specific for PD as they are also found in Alzheimers disease (AD). The role of LB in neuronal cell death is controversial, as the reason for their increased frequency in AD and the relationship of incidental LB to the occurrence of PD. LB's are more than 15um in diameter with an organized structure containing a dense hyaline core surrounded by a clear halo. Electron

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microscopy reveals a dense hyaline core surrounded by a clear halo.

MATERIALS AND METHODS

Animals:

Healthy adult Wister albino rats of either sex were housed in standard polypropylene cages at a constant temperature 25±20 c in a 12 hour light and dark cycle. They were fed with standard diet i.e. regular grain chow (Rayans biotechnologies Pvt. Ltd., Hyd.) with water ad libitum throughout the experiment. All experimental protocols were approved by the institutional animal ethical committee (IAEC) Reg.no:516/01A/CPCSEA under the regulation of committee for the purpose of control and supervision of experiments on animals (CPCSEA), New Delhi.

Drugs & Chemicals:

All chemicals used in this study were of analytical grade. Haloperidol, Wheat grass and Sodium CMC and cow urine were used in the present study where in Haloperidol was procured from Sigma chemical Ltd (USA) and Wheat grass has been purchased from Baba market.

Pharmacological Studies:

The various pharmacological studies evaluated in the present study were

(a) Measurement of locomotor activity using Actophotometer and Rota rod apparatus
(b) Measurement of Catalepsy in Haloperidol administered rats by Block method
(c) Behavioral assessment in Haloperidol administered rats by Metal bar test

Experimental Design:

All the rats were randomized into six groups comprising of six animals in each group as given below.

- **Group I**- control group of animals treated with 1% w/v sod CMC (1gm/100 ml)
- **Group II**- animals treated with haloperidol (1mg/ kg) I.P
- **Group III**- animals treated with FWG with cow urine (150mg/kg) P.O. +Haloperidol (1mg/ kg) I.P
- **Group IV**- animals treated with FWG with cow urine (300mg/kg) P.O. +Haloperidol (1mg/ kg) I.P

Treatment protocol:

FWG with cow urine (150 mg, 300 mg/ kg P.O) were administered in one day one hour prior to the challenge with haloperidol. Again these were administered for 15 days one hour prior to the challenge with haloperidol in acute and chronic studies respectively.

(a) Measurement of Locomotor activity using Actophotometer:

The locomotor activity can be easily measured by using Actophotometer. It operates on photo electric cells that are connected in a circuit with a counter. When a beam of light falling on photo cell is cut off by the animal, a count is recorded. An Actophotometer could have either circular or square area in which animal moves. Effect on locomotor activity was measured every 30 min for 10 min thereafter up to a total duration of 3 hr.

Measurement of Locomotor activity using Rota rod apparatus:

After turn on the Rota rod apparatus a training phase was done which consisting of 3 trails. One 60sec trail at 0rpm and two 60sec trails at 4 rpm at constant speed and all being performed per 10 min apart. Mice were placed on the rod as it was being rotated at 4 rpm constant speed. After placing the animals on the rod, acceleration button was pressed. Record the latency as each mouse falls off the rod.

(b) Measurement of Catalepsy in Haloperidol administered rats by Block method:

All the animals were treated as per given above the grouping and catalepsy was induced using Haloperidol. The effect of test compound on catalepsy after induction with Haloperidol was studied by scoring method. Severity of catalepsy was measured every 30 minutes thereafter up to a total duration of 3 hours. Catalepsy of an individual was measured in a step wise manner by a scoring method as described below. The method assessed the ability of the animal to respond to an externally imposed posture.

Step-1: the rat was taken out of the home cage and placed on a table. If the rat failed to move when touched gently on the back or pushed, score of 0.5 was assigned.

Step-2: the front paws of the rat placed alternately on a 3cm height block. If the rat failed to correct the posture within 15 seconds, a score of 0.5 for each paw was added to the score of the step-1.

Step-3: the front paws of the rat placed alternately on a 9cm height block. If the rat failed to correct the posture within 15 seconds, a score of 1 for each paw was added to the scores of the step-1, step-2.

(c) Behavioral assessment in Haloperidol Administered rats (Metal bar test):

A cataleptic behavior was measured with a high bar test method. Catalepsy score was measured for 4 hours at 1 hour intervals after haloperidol administration by gently placing both the fore [aw over a metal bar (diameter 2-5nm suspended 6cm above the table top). The intensity of catalepsy assessed by counting the time in seconds until the rat brought both

fore paws down to the table top with a maximum cut – off time 3 minutes. Finally scores at different time points (0, 60,120,180 and 240minutes after Haloperidol injection) were added and expressed as cumulative catalepsy score for comparison purpose.

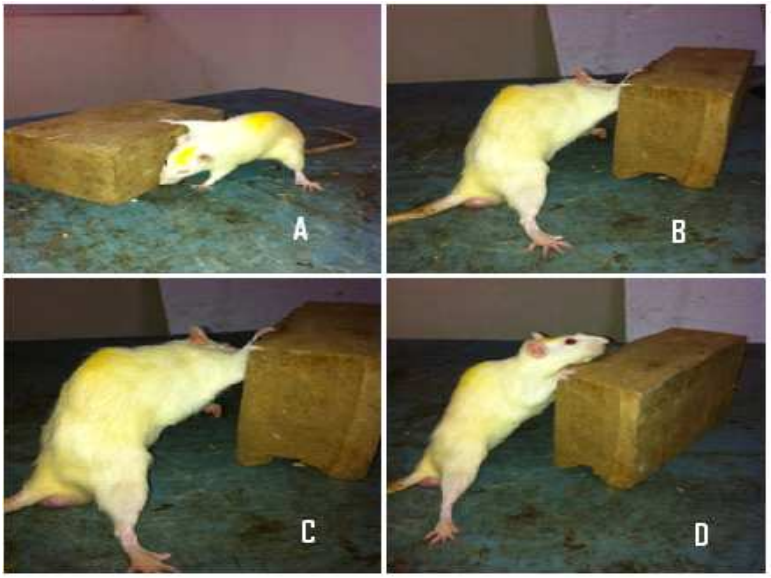
RESULTS AND DISCUSSION

The present study demonstrates the neuro-pharmacological effects of fortified wheat grass with cow urine in haloperidol model of Parkinson's disease in rats.

Measurement of Locomotor activity: Haloperidol inhibited the locomotor activity at the dose of 1mg/kg/i.p. Fortified wheat grass with cow urine at doses of 150, 300 showed the results in ameliorating the haloperidol inhibited locomotor activity. Fortified wheat grass with cow urine at a dose of 300 mg/kg exhibited significantly high protection against haloperidol decreased locomotor activity in rats. Results are expressed in tables 1, 2, 3 & 4.

Measurement of Catalepsy in Haloperidol administered rats by Block method: Haloperidol induced catalepsy in rats is used to screen the drugs for their anti parkinsonism effects. In the present study fortified wheat grass with cow urine was screened for its effects in haloperidol induced catalepsy in rats. Haloperidol induced catalepsy (rigidity, akinesia) at a dose of 1 mg/kg for i.p. The inhibition of haloperidol induced catalepsy was increased by the treatments of fortified wheat grass with cow urine. The fortified wheat grass with cow urine at doses of 150, 300 mg/kg showed the results in ameliorating the symptoms of haloperidol induced catalepsy. Fortified wheat grass with cow urine at the both the doses 150 mg, 300 mg exhibited significantly high protection against haloperidol induced catalepsy in rats. Results are expressed in table 5 & 6.

Image-1: Images A, B, C & D are different stages of catalepsy in rats induced by Haloperidol (Block method)



Behavioral assessment in Haloperidol Administered rats (Metal bar test): Haloperidol causes the changes in behavioral assessment like immobility, rigidity at a dose of 1mg/kg /i.p. The inhibition of haloperidol induced immobility, rigidity was increased by the treatment of fortified wheat grass with cow urine. The fortified wheat grass with cow urine at doses of 150, 300 mg/kg showed the results in ameliorating the haloperidol inhibited mobility. Fortified wheat grass with cow urine at a dose of 300 mg/kg exhibited significantly high protection against haloperidol induced immobility in rats. Results were expressed in table 7 & 8

Image-2: Images A, B, C & D are different cataleptic behavioral states in rats (High Metal Bar test)

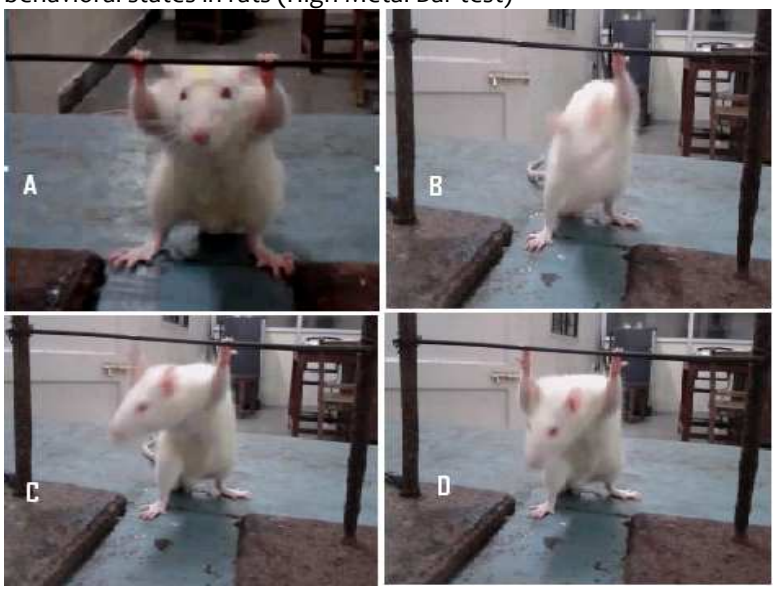


Table 1: Effect of fortified wheat grass with cow urine on locomotor activity in Haloperidol administered rats using Actophotometer - Acute Study

Groups	Treatment	Counts/Minute					
		0 min	30 min	60min	90 min	120 min	150 min
G I	Vehicle control	68.8	54.33	53.4	53.0	53.8	50.4
G II	Haloperidol (1mg/kg)	64.25	21.25	15.25	6.78	1.75	0
G III	FWG (150 mg/ kg) + Haloperidol(1mg/kg)	93.5	68.7	45.3	21.24	29.2	39.5
G IV	FWG (300 mg/ kg) + Haloperidol (1mg/kg)	109.2	69.3	49.8	24.6	31.8	42.94

Table 2: Effect of fortified wheat grass with cow urine on locomotor activity in Haloperidol administered rats using Actophotometer - Chronic Study

Groups	Treatment	Counts/Minute					
		0 min	30 min	60min	90 min	120 min	150 min
G I	Vehicle control	136.16	86.33	72.16	83.3	83.83	74.16
G II	Haloperidol (1mg/kg)	83.5	38.33	20	10.16	9.33	8.66
G III	FWG (150 mg/ kg) + Haloperidol(1mg/kg)	160	101.4	77	61.2	31.4	22.2
G IV	FWG (300 mg/ kg) + Haloperidol (1mg/kg)	161	111	84.4	66.77	44.25	43.25

Fig.1: Effect of fortified wheat grass with cow urine on locomotor activity using Actophotometer

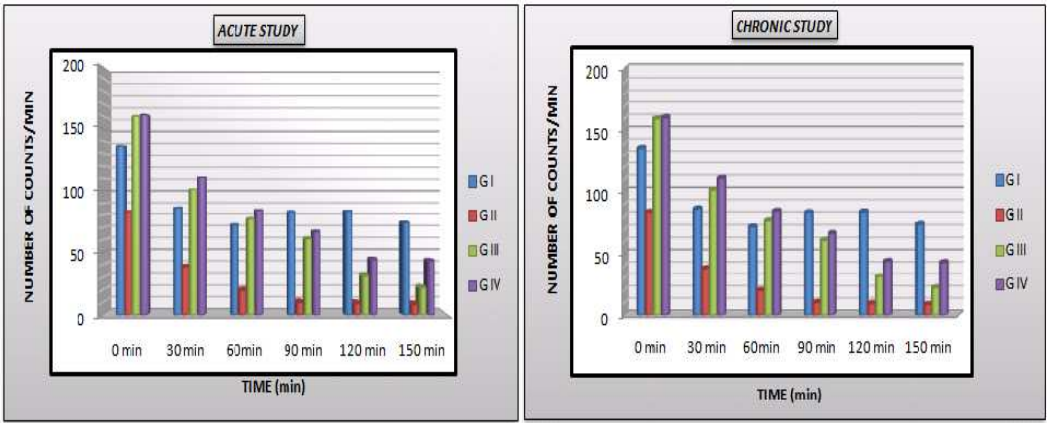


Table 3: Effect of fortified wheat grass with cow urine on locomotor activity in Haloperidol administered rats using ROTA ROD apparatus - Acute Study

Groups	Treatment	Fall off time (sec) at different time points					
		0 min	30 min	60min	90 min	120 min	150 min
G I	Vehicle control	>300	>300	>300	>300	>300	>300
G II	Haloperidol (1mg/kg)	67	26.5	18	16.16	14	11.33
G III	FWG (150 mg/ kg) + Haloperidol(1mg/kg)	193.2	84.2	34.4	19.4	32.4	61
G IV	FWG (300 mg/ kg) + Haloperidol (1mg/kg)	225.5	81.2	69	66.4	70.6	79.6

Table 4: Effect of fortified wheat grass with cow urine on locomotor activity in Haloperidol administered rats using ROTA ROD apparatus - Chronic Study

Groups	Treatment	Fall off time (sec) at different time points					
		0 min	30 min	60min	90 min	120 min	150 min
G I	Vehicle control	>300	>300	>300	>300	>300	>300
G II	Haloperidol (1mg/kg)	210.16	179.5	177.6	130	72.15	35.33
G III	FWG (150 mg/ kg) + Haloperidol(1mg/kg)	>300	194	149.6	123.6	117.8	114
G IV	FWG (300 mg/ kg) + Haloperidol (1mg/kg)	>300	197	158.25	155.25	137.25	131.25

Table 5: Effect of fortified wheat grass with cow urine on haloperidol induced catalepsy in rats (Block Method) - Acute Study

Groups	Treatment	Fall off time (sec) at different time points				
		30 min	60min	90 min	120 min	150 min
G I	Vehicle control	0.0	0.0	0.0	0.0	0.0
G II	Haloperidol (1mg/kg)	1.5	2.833	3.33	3.5	3.16
G III	FWG (150 mg/ kg) + Haloperidol(1mg/kg)	1.0	1.852	1.33	1.0	0.5
G IV	FWG (300 mg/ kg) + Haloperidol (1mg/kg)	0.5	0.10	0.65	0.5	0.0

Fig.2: Effect of fortified wheat grass with cow urine on locomotor activity using Rota rod apparatus

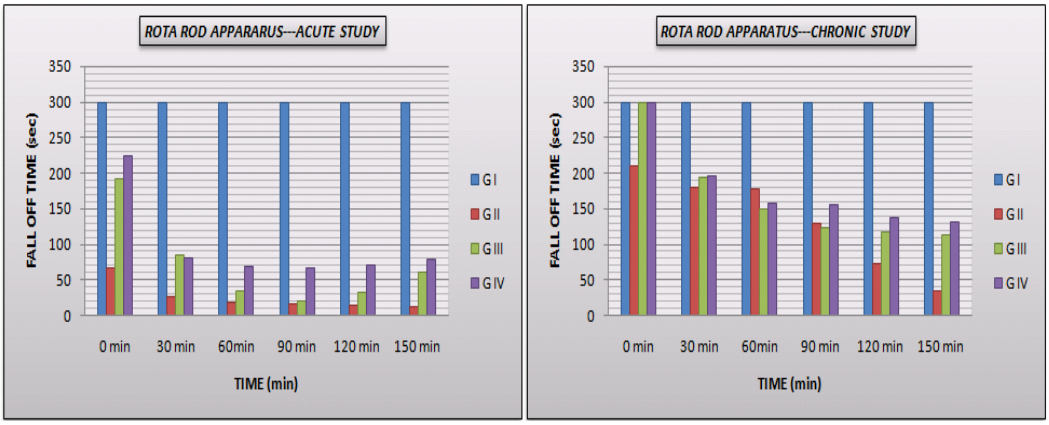


Table 6: Effect of fortified wheat grass with cow urine on haloperidol induced catalepsy in rats (Block Method) - Chronic Study

Groups	Treatment	Fall off time (sec) at different time points				
		30 min	60min	90 min	120 min	150 min
G I	Vehicle control	0.0	0.0	0.0	0.0	0.0
G II	Haloperidol (1mg/kg)	1.33	2.83	3.08	3.33	3.0
G III	FWG (150 mg/ kg) + Haloperidol(1mg/kg)	0.65	0.10	0.65	0.5	0.0
G IV	FWG (300 mg/ kg) + Haloperidol (1mg/kg)	0.5	0.10	0.50	0.0	0.0

Fig.3: Effect of fortified wheat grass with cow urine on haloperidol induced catalepsy by Block method

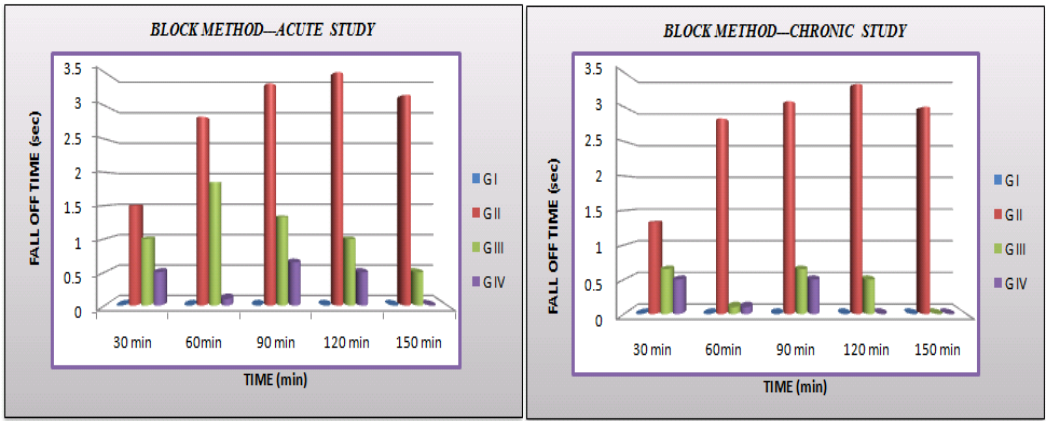


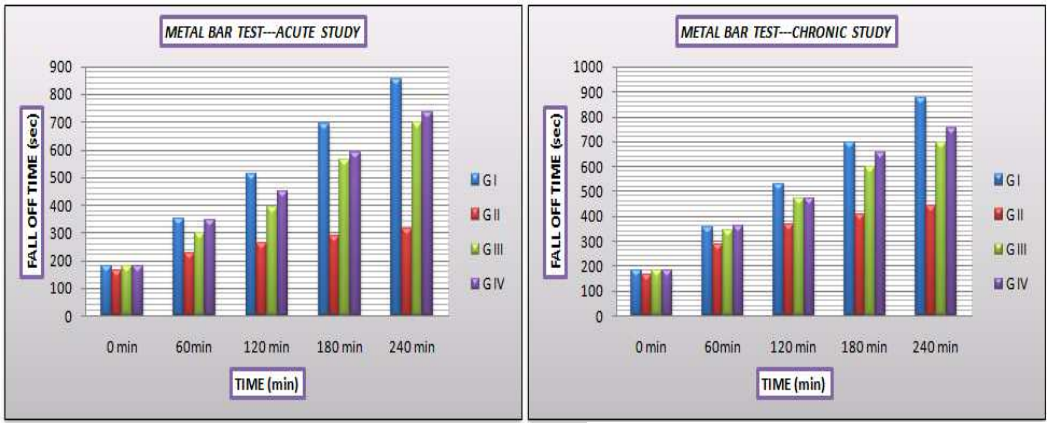
Table 7: Effect of fortified wheat grass with cow urine on behavioral assessment in haloperidol administered rats (Metal Bar Test) - Acute Study

Groups	Treatment	Fall off time (sec) at different time points				
		0 min	60min	120 min	180 min	240 min
G I	Vehicle control	180	349.2	512.6	690.6	852.16
G II	Haloperidol (1mg/kg)	161	227.1	262.16	288	313.5
G III	FWG (150 mg/ kg) + Haloperidol(1mg/kg)	178	295.75	391.5	560.7	695.75
G IV	FWG (300 mg/ kg) + Haloperidol (1mg/kg)	180	343.6	446.7	587.4	736.6

Table 8: Effect of fortified wheat grass with cow urine on behavioral assessment in haloperidol administered rats (Metal Bar Test) - Chronic Study

Groups	Treatment	Fall off time (sec) at different time points				
		0 min	60min	120 min	180 min	240 min
G I	Vehicle control	180	355.33	525.6	697	873.6
G II	Haloperidol (1mg/kg)	166	284.83	365.4	404.4	439
G III	FWG (150 mg/ kg) + Haloperidol(1mg/kg)	180	343.6	467	597.4	696.9
G IV	FWG (300 mg/ kg) + Haloperidol (1mg/kg)	180	359	469.5	656.5	749.7

Fig.4: Effect of fortified wheat grass with cow urine on behavioral assessment by Metal bar test



CONCLUSION

In the present study the effect of fortified wheat grass with cow urine on extra pyramidal symptoms such as rigidity, bradykinesia, motor coordination and depression which are the key parameters found in Parkinson's disease were studied. The results revealed that fortified wheat grass with cow urine at doses of 150 and 300 mg/kg exhibited significant anti cataleptic activity in haloperidol induced catalepsy, significantly reversed the haloperidol inhibited locomotor activity, significantly restored the changes in behavioral assessment like akinesia, immobility in haloperidol administered rats, reduced the haloperidol induced rigidity. The possibility of pharmacological interactions between haloperidol and wheat grass, fortified wheat grass with cow urine should be further investigated in my research work.

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