Original Research Paper Medical Science

Effect of *Ficus Racemosa* Linn on Haloperidol Induced Parkinsonism in Wistar Rats

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ABSTRACT

The present study is carried out to evaluate the anti-parkinsonism effect of Ficus racemosa Linn ethanol leaves extract(250mg/kg and 500mg/kg body wt) by measuring various neurological and behavioural parameters against Haloperidol(1mg/kg,i.p) induce Parkinsonism. Parkinsonism caused due to depletion of dopamine level in substansia nigra. Treatment with Ficus racemosa Linn reversed the haloperidol induced Parkinsonism significantly, when compared to standard drugs, i.e. combination of L-dopa & Carbidopa (100mg+25mg/kg). From the above studies it could be predict that the symptoms of Parkinsonism may be due to alteration in dopaminergic system, which play important role in the protection from Parkinsonism. The result of the study implies that Ficus racemosa Linn is a potent source to protect the brain from dopamine depletion level due antioxidant property

KEYWORDS Ficus racemosa, Haloperidol, Ethanolic extract, Dopaminergic system, L-dopa & Carbidopa

INTRODUCTION

Parkinson's disease (PD) is one of the major neurodegenerative disorders that affect the nerve cells in the part of the brain controlling muscle movement. Peoples with Parkinson's disease often experience difficult walking, trembling, muscles rigidity, problems with balance and slowed movements. These symptoms usually Parkinson's disease develops after the age 60. Despite many approaches and efforts, to date no researchers have been successful in developing a cure or a modality to check the disease, and most of the therapies only provide functional relief. Evidence suggests that immense oxidative stress, free radical formation[1] Genetic susceptibility [2] programmed cell death [3] are known to cause the disease. The another unknown factor which might be exogenous (or) endogenous [4]. The pathology of the disease is based on depigmentation and cell loss in the dopaminergic nigrostraital tract of the brain, with decrease in the dopamine (DA) concentration the striatum[5]. Haloperidol is an neuroleptic drug or antipsychotic that produces parkinsonism in humans or catalepsy in animals. It has been attributed to cause the blockade of dopamine receptors[6]. Several brain regions appear to be involved in the expression of neuroleptic induced catalepsy.

Ficus racemosa is a medium tall tree with quite rich green foliage that provides good shade. It is popularly known as "Country fig" in English and "Atti" in Tamil. The leaves, bark and fruits of ***F. racemosa*** are employed in native medicine to treat several diseases[7]. Experimental studies have demonstrated its anti-inflammatory, hepatoprotective and hypoglycemic effects[8,9,10].

Preparation of ethanolic extract

The leaves of ***Ficus racemosa Linn*** were collected, shadow dried and coarsely powder . The shade dry coarsely leaves of ***Ficus racemosa Linn*** were extraction by soxhlet apparatus and extracted with ethanol at 55°C for 18 h. The extract was air dried at 25-30°C and weighed. For oral administration, extract was dissolved in 10 mL Phosphate Buffer Saline (PBS) at different concentrations. To make the extract soluble in PBS, 1% tween 80 was used.

Preliminary Phytochemical Screening

Ficus racemosa Linn ethanolic leave extracts were subjected to preliminary phytochemical chemical screening for their presence or absence of active phytochemical constituents by the following methods (Kokate 2007 and Khandelwal 2004).

Experimental Animals

Wistar albino rats (150-200 g) of both sexes were obtained from the Osmania university animal house. Before and during the experiment, rats were fed with standard diet. After randomization into various groups, the rats were acclimatized for a period of 7 days under standard environmental conditions of temperature, relative humidity, and dark/light cycle. Animals described as fasting were deprived of food and water for 16 h ad libitum. All animal experiment were carried out in accordance with the guidelines of CPCSEA and study was approved by the IAEC.

Acute toxicity studies

Acute oral toxicity was performed as per Organization for Economic Co-operation and Development (OECD)-423 guidelines. Three male Wistar rats weighing between 150–200 g were used for each dose. The dose levels of 5 mg, 50 mg, 500 mg, 1000 mg, 2000 mg, and 5000 mg/kg/body weight, per os were selected. The lethal dose (LD)-50 value of the extract was determined. The animals were observed for toxic symptoms, such behavioral changes, loco-motion, convulsions, and mortality for 72 hours.

Experimental design

Measurement of Parkinsonism by Block Method[11]

The animal were divided into five groups each consisting of 6 animals each

Group1: Control animal receiving 1% tween 80 (1ml/100gm)

Group 2: Haloperidol (1mg/kg,i.p)

Group 3: ***Ficus racemosa Linn*** (250mg/kg) suspended in 1%v/v tween 80 for 15days and haloperidol (1mg/kg)

Group 4: ***Ficus racemosa Linn*** (500mg/kg) suspended in 1%v/v tween 80 for 15days and haloperidol (1mg/kg)

Group 5: Standard L-Dopa & Carbidopa (100mg+25mg/kg,i.p)1hr prior to the Challenge with haloperidol.

Severity of Parkinsonism was measured every 30minutes, there after upto for 3hrs. Parkinsonism of an individual rat was measured in a step wise manner by a scoring method.

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 – II The front paws of the rat were placed alternately on a 3cm high 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 I.

Step – III The front paws of the rat placed alternately on a 9cm high 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 step I, Step II. Thus, for an animal, the highest score was 3.5 (cut-off score) and that reflects in total catalepsy.

Behavioral assessment (Metal bar test) [12]
The animal were divided into five groups each consisting of 6 animals.

Group 1 :Controlreceiving1% tween 80(1ml/100gm)

Group 2 : Haloperidol (1mg/kg)

Group 3 : Ethanolic extract of ***Ficus racemosa Linn*** (250mg/kg) suspendedin 1%v/v Tween 80 for 15days and haloperidol (1mg/kg)

Group 4 : Ethanolic extract of ***Ficus racemosa Linn*** (500mg/kg) suspended in 1% v/v tween 80 for 15days and haloperidol(1mg/kg)

Group5 : Standard L-Dopa & Carbidopa (100mg+25mg/kgi.p)1hr prior to the Challenge with haloperidol

Procedure
A cataleptic behaviour were measured with a high bar test method. Catalepsy core was measured for 4hours at one hour intervals after haloperidol administration by gently placing both the fore paw of the rat over a metal bar (diameter 2-5mm suspended 6cm above the table top). The intensity of catalepsy assessed by counting the time in seconds until the rat brought both fore pass down to the table top, with a maximum cut off time of 3 minute. Finally, scores at different time points (0, 60,120,180 & 240 minutes after haloperidol injection) were added and expressed as cumulative catalepsy score for comparison purpose.

Statistical analysis
The data obtained were analyzed by One way analysis of variance (ANOVA) followed by Dennett's test. P-value <0.05 or was taken as the criterion of significance.

RESULTS
Ficus racemosa Linn ethanolic leaves extract showed the presence of Alkaloids , Glycosides, Steroids, flavonoids, Tannins, Saponins etc. The acute oral toxicity was done according to the OECD guidelines 423 (acute toxicity class method).There was no considerable change in the body wt before and after treatment of the experiment and no sign of toxicity were observed.

Haloperidol induced Parkinsonism significantly at a dose of 1mg/kg/ip in animals. The Haloperidol induced Parkinsonism was decreased by the treatment with ethanolic leaves extract of ***Ficus racemosa Linn***, L-Dopa & Carbidopa. The maximal decrease in Parkinsonism was observed in group V animals treated with standard drug. ***Ficus racemosa Linn*** at a dose of 500mg/kg has more significant effect (p<0.01) than 250mg/kg in the reversal of haloperidol induced Parkinsonism. The combination of L-Dopa & Carbidopa at a dose of (100mg+25mg/kgi.p) also shown significant effect in the reversal of haloperidol induced Parkinsonism which is assessed by block method (Table 1 and Fig 1)and metal bar test (Table 2 and Fig 2)

Table-1: Effect of Ethanolic extract of ***Ficus racemosa Linn*** on Haloperido induced Parkinsonism.
A. Block method

S.No	Drug treatment	30 min	60 min	90 min	120 min	150 min	180 min
1	Tween 20	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
2	Haloperidal treated	1.52±0.1 a**	2.60±0.62 a**	3.51±0.24 a**	3.70±0.24 a**	3.38±0.20 a**	3.18±0.04 a**
3	Ficus racemosa Linn (250mg/kg)+ Haloperidal	1.38±0.18 bns	2.30±0.24 bns	2.98±0.41 b*	2.18±0.15 b**	1.22±0.61 b**	0.70±0.32 b**
4	Ficus racemosa Linn (500mg/kg)+ Haloperidal	0.51±0.42 bns	1.32±0.68 bns	1.08±0.72 b*	1.12±0.48 b**	0.72±0.62 b**	0.00±0.00 b**
5	L-Dopa +Carbidopa+ Haloperidol	0.39±0.31 b**	1.22±0.24 b**	0.92±0.48 b**	0.63±0.54 b**	0.48±0.66b**	0.0±0.0 b**

Values are mean±SEM of four samples of six observations. Statistical significant test for Comparison was done by ANOVA, followed Dennett's test.a-Group I and Group II, b-Group II Vs Group III, Group IV *p<0.05;**P<0.01; Vs nonsignificant.

Figure-1: Effect of Ethanolic extract of ***Ficus racemosa Linn*** on Haloperido induced Parkinsonism.
A. Block method

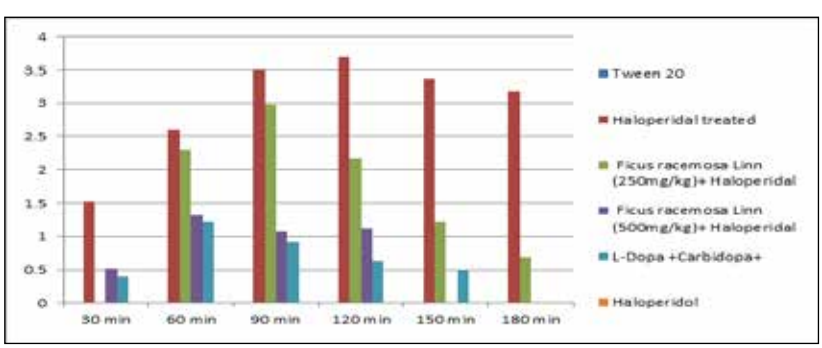
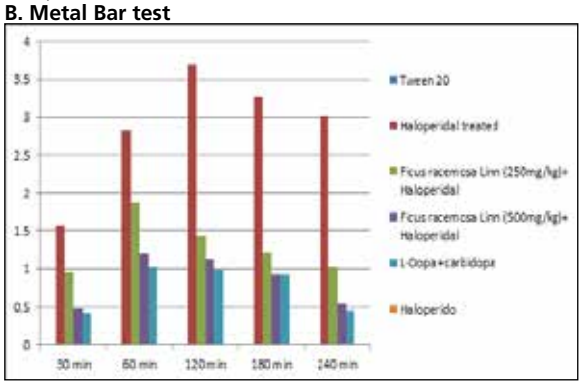


Table-2: Effect of Ethanolic extract of ***Ficus racemosa Linn*** on Haloperido induced Parkinsonism.
B. Metal Bar test

S.No	Drug treatment	30 min	60 min	120 min	180 min	240 min
1	Tween 20	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
2	Haloperidal treated	1.58±0.6 a**	2.82±0.62a**	3.70±0.18a**	3.28±0.45a**	3.02±0.20a**
3	*Ficus racemosa Linn* (250mg/kg)+ Haloperidal	0.95±0.42bns	1.88±0.42bns	1.42±0.72b*	1.22±0.24b**	1.02±0.01b**
4	*Ficus racemosa Linn* (500mg/kg)+ Haloperidal	0.48±0.32bns	1.20±0.56bns	1.12±0.45b*	0.92±0.02b**	0.54±0.28b**
5	L-Dopa +carbidopa Haloperido	0.42±0.26b**	1.02±0.18b**	0.98±0.82b**	0.92±0.55b**	0.44±0.76b**

Values are mean±SEM of four samples of six observations. Statistical significant test for Comparison was done by ANOVA, followed Dennett's test. a-Group I and Group II, b-Group II Vs Group III, Group IV *p<0.05;**P<0.01; Vs nonsignificant.

Fig-2: Effect of Ethanolic extract of ***Ficus racemosa Linn*** on Haloperido induced Parkinsonism.
B. Metal Bar test



DISCUSSION

In the present study anti-parkinsonism effect of ***Ficus racemosa Linn*** ethanolic leaves extract were studied in haloperidol induced of Parkinson's disease in rats. ***Ficus racemosa Linn*** at the dose of 500mg/kg, p.o, exhibited a more pharmacological effect than 250mg/kg when compared to L-Dopa &Carbidopa (100mg+25mg/kgi.p). Dopamine depletionis in brain considered as a cardinal feature in causing a Parkinsonism in humans (or) in animal models. The enhancement of dopamine concentration as in ***Ficus racemosa Linn*** treatment might have restored the alteration in loco motor activity, exploratory behavior. Extract of ***Ficus racemosa Linn*** having dopamine antioxidant potential property might have afforded protection in the haloperidol induced Parkinson's disease. Several studies have reported on exacerbation of neuroleptic induced catalepsy by enhanced serotonergic neurotransmission in the CNS.

The two main dopamine pathways in the brain are the nigrostraited pathway and meso limbic pathwa. It can be hypothesized from this study that ***Ficus racemosa Linn*** ethanolic leaves extract inhibits the symptoms of haloperidol induced Parkinsonism in rats. The mechanisms by which the amelioration takes place may be attributed to one(or) more pharmacological/biochemical mechanism Viz. ethanolic leaves extract of Ficus racemosa Linn may enhance the bioloavailability of circulating dopamine by upregulation of dopaminergic signaling.

Antioxidants may play an important role in the prevention of Parkinsonism disease and combat against oxidative stress induced progressive neurodegeneration by reactive oxygen species. ***Ficus racemosa Linn*** has been found that phytoconstituents like alkaloids, polyphenols, saponins, flavonoids and tannins responsible for its antioxidant property.

It has been demonstrated that the cataleptic effects of the haloperidol are apparently mediated by dopamine receptors localized post synaptically on strial neurons [11]. It is well established that the administration of haloperidol leads to an increase in the oxidative stress in the brain [12]. However, in the presence of free radical-quenching agent, the induction of the antioxidant enzymes is minimized. So, overall decrease in cataleptic scores and SOD activity in the drug treated groups indicates the ability of the drug extract to combat oxidative stress in brain tissue and reduce the severity of catalepsy induced by haloperidol. The altered balance of the antioxidant enzymes caused by the decrease in CAT, GSH, SOD, activities may be responsible for the inadequacy of the antioxidant defenses in combating ROS mediated damage. The decreased activities of SOD and CAT might be a response to increased production of H2O2 and O2 by the auto-oxidation [13]. It has been suggested that antioxidant enzymes play an important role in maintaining physiological levels of oxygen and hydrogen peroxide by hastening the dismutation of free oxygen radicals and eliminating organic peroxides and hydroperoxides.

CONCLUSIONS

From the above studies it could be predict that the symptoms of Parkinsonism may be due to alteration in dopaminergic system, which play important role in the protection from Parkinsonism. The levels altered by haloperidol were restored significantly by the administration of ***Ficus racemosa Linn***250mg/kg and 500mg/kg ethanolic leaves extract. The result of the study implies that ***Ficus racemosa Linn*** is a potent source to protect the brain from dopamine depletion level. The results also imply that the Anti- Parkinsonism effects of ***Ficus racemosa Linn*** might be due to its antioxidant property.

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