

EFFECTS OF RHEUM EMODI ON HALOPERIDOL INDUCED PARKINSONISM BY USING DESPIRE SWIM TEST

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ABSTRACT

In this study, effects of rheum emodi (100, 200, and 400 mg/kg, p.o.) were studied using in vivo behavioral parameters like catalepsy, locomotor activity, muscles coordination and its effects in rats. The experiment was designed by giving haloperidol to induce catalepsy and Parkinson's disease-like symptoms. The increased cataleptic scores (induced by haloperidol) were significantly ($p < 0.001$) found to be reduced, with the EERE at a dose of 200 and 400 mg/kg. Significantly induced motor dysfunction (muscle rigidity and hypolocomotion). Daily administration of EERE (400 mg/kg) significantly improved motor performance and also significantly attenuated oxidative damage. In the present study, we evaluated anti-Parkinson's activity of Ethanolic extract of Rheum emodi (EERE) leaves in haloperidol induced catalepsy in experimental animal models. Thus, the study proved that Rheum emodi treatment significantly attenuated the motor defects and also protected the brain.

KEYWORDS: Rheum emodi, haloperidol induced, catalepsy, locomotor activity, neuroprotective.

INTRODUCTION

PD is the progressive neurodegenerative disorder caused as a consequence of degeneration of dopaminergic neurons in substantia nigra pars compacta (SNpc) in the mid brain.^[1] PD affected person shows motor symptoms such as rigidity, tremors at rest and gait disturbances as well as non motor symptoms such as sleep disturbance, olfactory dysfunction, cognitive disturbance and CNS depression^[2] There is no effective cure for PD since currently available drugs can't stop and reverse the progression of the disorders and produce more side effects such as depression, confusion, hallucination, insomnia, anxiety, myocardial infarctions and hepato toxicity^[3] Therefore the recent research mainly focused on searching newer drugs from plants having effective therapeutic properties against progression of disorder with less side effects. Neuroleptic-induced catalepsy has long been used as a model for the extrapyramidal side effects (EPS), such as parkinsonian-like catalepsy associated with antipsychotic use in humans. Evidences indicate that haloperidol induces catalepsy in animals, and this behavior response has long been used as a model for EPS effects.^[4-5] the present investigation was aimed to evaluate the protective capacity of herbal drug rheum emodin against haloperidol induced Parkinson's disorder in rat.

Several studies reveal that rheum emodi is rich in various flavonoids and has high antioxidant property. Recently,

this plant showed acceptable result such anti-inflammatory, antibacterial antiulcer activity,^[6-15] In the present study, an attempt was made to evaluate antiparkinsonian activity of rheum emodin in haloperidol-induced catalepsy.

Plant Profile

Rheum emodin is commonly used worldwide herb and often known as "the wondrous drug" because of its extensive medicinal uses. It consists of dried rhizome and root of some rheum species and their hybrid of family polygonaceae. The genus Rheum consists of approximately 60 perennial species distribution around the world; several species are used in medicine, some for culinary purpose and few others are grown as ornament; about ten species occur in India.^[16]



Figure 1: Rheum. Emodi.



Figure 2: Rhizome of Rheum Emodin.

Plant description

Rheum emodi or Himalayan rhubarb is known by various vernacular names in different geographical regions or system.^[17-23]

Table 1: Vernaculars or Rheum emodi.

Language/System	Name
English	Indian Rhubarb or Himalayan Rhubarb
Sanskrit	Gandhini, Revatchini Hindi Dolu , Pita
Kashmiri	Pumbehakh
Tamil	Nattu-ireval-chini, Nattu-manjal-chinni-kizhangu
Telgu	Nattu-revalchini
Arabic	Reward
Persian	Rewardchini
Ayurvedic	Amlaparni, pitamuuli, Gandhini Revatikka
Siddha	Revalchinikattai, Nattirevaichini
Unani	Revandchini

Classification

Kingdom - Plantae
 Clade - Angiosperm
 Clade - Eudicots
 Clade - Core eudicots
 Order - Caryophyllales
 Family - Polygonaceae
 Genus - Rheum
 Species - R. emodi

Occurrence and distribution

Rheum emodin is a stout herb, endemic to the Himalayan region distributed in the temperate and subtropical region from Kashmir to Sikkim at an elevation of 2800-3000m in India. It grows in the alpine zone on rocky soils, moraines and cervices.^[24-27]

Botanical description

Rheum emodin Wall. ex Meissn, is a leafy perennial herb 1.5-3.0 m in height. Roots very stout. Radical leaves long petioled, very large, often 60 cm in diameter, orbicular or broadly ovate obtuse, base cordate 5-7 nerved, papillose beneath, subscaberulous above; petiole 30-45 cm, very stout, scaberulous. Panicle is 0.6-0.9 m, papillosely puberulous, fastigiately branched and leafy with erect strict branches; flowers small 3 mm diameter, dark

purple or pale red, in axillary panicles. Fruit ovoid-oblong, 13 mm long, purple, base cordate, apex notched, wings narrower than the disk. Roots and rhizomes are the main parts used as drug and are collected in October to November. Root of Indian Rhubarb is darker, inferior in aroma, coarser and untrimmed, is not decorticated. Fresh rhizome is 6 to 12 inches long, and the freshly fractured surface is dull orange to yellowish brown.^[28]

Phytochemistry

Rheum emodin possess a number of phytoconstituents and these are: anthraquinones, anthrones, stilbenes, oxanthrone ethers and esters, flavonoids, lignans, phenols, carbohydrate and oxalic acid. The most common constituents of Rheum emodi are anthraquinone and stilbene. Anthraquinones include rhein, chrysophanol, aloe-emodin, emodin, physcion (emodin monomethyl ether), chrysophanein and emodin glycoside. Stilbene includes picetannol, resveratrol and their glycosides Different derivatives of oxanthrone include oxanthrone ether (revandchinone-4), oxanthrone esters (revandchinone-1 and revandchinone-2), and revandchinone-3 .Other complex compounds have also been reported, including torachryson 8-O-b-D-glucoside, sulfated anthraquinone glycoside sulfemodin 8-O-b-D-glucoside b-asarone and rhein 11-O-b-D-

glucoside Tannins are also present in rhubarb Journal of Pharmacognosy and Phytochemistry which includes hydrolysable tannins, containing ester or glycosidic bonds composed of gallic acid, glucose and other monosaccharides and condensed tannins, derived primarily from the flavone derivatives catechin and leucocyanidin.^[28-32]

Pharmacological actions and traditional uses

Rheum emodin is considered as purgative, stomachic, and astringent tonic. It also possesses aperient, emmenagogue and diuretic properties. Root is regarded as expectorant and appetizer. Anti-inflammatory, anti-dysentery and alexentric actions have also been ascribed. Rheum emodin is widely used in various traditional systems such as, Unani, Ayurveda, Chinese etc. Rheum emodi is used as a purgative and astringent tonic. Its primary action is of mild purgation; but it has also astringent property, so that its secondary effect is to confine the bowels, hence it is well fitted for use in simple diarrhoea, but not in constipation or any affection in which a continuous aperients action is necessary. It is useful remedy in ailments of children. For the errors in diet of children and for the diarrhoea set up by undigested food, it is best given combined with sodium bicarbonate or magnesia. Rhein (4, 5-Dihydroxyanthraquinone-2-carboxylic acid, is the only anthraquinone that is absorbed into cerebrospinal fluid of TBI (Traumatic Brain Injury) patients after Rheum Emodin administration. Thus, rhein derived from Rheum Emodin is responsible for most of the observed neuroprotection. Rheum emodin forms an important ingredient of a large variety of compound. Combined with ginger it may be given in the form of pills in cases where the bowels are sluggish. Root is regarded as panacea in local home remedies and is used in stomach problems, cuts, wound, and muscular swelling, tonsillitis and mumps. Some persons chew the root, and to them this is a very good way of taking it. Powdered roots are used for cleaning teeth and sprinkled over ulcers for quick healing. Ethno-medically leaf-stalk, leaves and flowers are consumed as vegetables after cooking In Assam its leaves consumed as vegetables and cultivated for this purpose. Leaves are also dried and stored for consumption along with other foods, or made into a preserve. It is however stated that cook stalks act as a powerful Purgative. Besides the medicinal uses, it is also used for coloration of textile and wooden material.

MATERIALS AND METHODS

Collection and authentication of plant material

The Powder of Rhizome of Rhubarb (*rheum emodin*) had been procured from VHCA AYURVEDA LLP. Haryana.

Experimental Animal

The experiment was performed on wistar rats (weighing 150-250g), which were obtained from the animal house of Department of Pharmacology, Vidyabharati college of Pharmacy, Amaravati. All the animals were acclimatized to animal house prior to use. They are kept in cage in

animal house with 12h light: 12h dark cycle. Animals were fed on pellets and tap water ad libitum. The care and handling of rat were in accordance with the internationally accepted standard guidelines for use of animals (CPCSEA) permission and approval for animal studies were obtained from the Institutional Animal Ethics Committee (IAEC) of Vidyabharati College Of Pharmacy, Amaravati. SGB Amaravati University.

Drugs and chemicals

Standard Drug

1. Haloperidol injection IP (serenace) manufactured by RPG life science Ltd. was used to induce Parkinsonism in rats.
2. Levodopa and carbidopa tablet (syndopa 110) was used as standard drug.

Other Chemical

Saline water, Ethanol (90%), DPPH, Ascorbic acid, Hcl, 0.1N NaoH, chloroform, ferric chloride, 40% sodium hydroxide. The chemicals used and other solutions were all of analytical grade. All drugs and reagents were prepared immediately before use.

Extraction method

Extraction of plant material by maceration method

Maceration of 150 g of the powder of dried rhizome part of Rheum emodin in 350 ml of Ethanol solvents was carried out. The mixture was shaken by an electrical shaker at room temperature for 48 h. After that time, the mixture was filtered and the solvent was removed on a rotary evaporator. After drying the residue at 70 °C in an electrical oven, a yellow powder was obtained.

Solubility analysis

The solubility analysis of ethanolic extract of Rheum emodin has been carried out using different solvent. Extract was found to be soluble in water, freely soluble in hot water.

Determination of Acute Toxicity LD₅₀

The acute toxicity of prepared extract was performed using OECD guideline 425 in Following manner.

A. Selection of Animal species & housing

For the acute toxicity study the Female rats were used, as Female rats are more sensitive than Male rats. All the test animals were kept in separate cages at least 5 days before the commencement of toxicity test. Animals were maintained at 22 ± 3°C in (12:12) light & Dark cycle with free access of Food and Water.

B. Preparation of doses

During each study procedure Fresh aqueous solution of root extract of *Rheum emodin* was made and each time same volume of dose was administered by varying the conc. of the drug extract.

C. Test procedure

The required dose is administered in animal one at a time by using oral gavage. The animal (Rats) were fasted overnight but water was not withdrawn. The fasted body weight of rat is determined and Dose is calculated on body weight basis after administration of *Rheum emodin* extract the food is withheld for further 3-4 h. For limit test 2000 mg/kg dose was administered in one animal and then the animal was observed for mortality for a period of 48 h the tested rat was serviced therefore test was continue by taking 4 more animals.

In main test dose of 1.75, 5.5, 17.5, 55, 175, 550, 2000 was selected and was administered in animal one at a time. The animal was observed for any toxic symptoms initially for 1h. interval for 4 h. then periodically for up to 14 days.

D. Selection of Dose groups

1. On the basis of acute toxicity study data. It was conclude that LD₅₀ of *Rheum emodin* extract is upto 2000mg/kg.
2. Therefore the test groups were divided as 100mg (low dose), 200 mg (medium dose), 400mg (high dose).

Determination of Antiparkinsons Activity

The Antiparkinson's potential of ethanolic roots extract of *Rheum emodin* has been carried out by using haloperidol induce model in wistar rat of either sex weighing 150-250 gm.^[33]

Induction of experimental Parkinsonism

Haloperidol causes dysfunctioning of various neurotransmitters such as acetylcholine, GABA, and serotonin. Pathology of haloperidol induced catalepsy underlying increased oxidative stress. Haloperidol, an antipsychotic drug, blocks central dopamine receptor in striatum. It also produces a behavioral state in rats in which they fail to correct externally imposed postures (called catalepsy); thus, keeping the above fact in mind, the haloperidol induced catalepsy model was selected. The method was followed for the anticataleptic activity. Wistar rats weighing 150-250g taken and haloperidol at a dose of 1mg/kg i.p was administered chronically to the rats for a period of 14 days to induce PD. All the behavioural assessment was carried out on 4th day, 8th day and 14th day of the study and the last behavioural quantification was done 24 hours after the last dose of Haloperidol.^[34]

Behavioural assessment

Motor coordination

1. Despair Swim Test

Each animal was introduced into a pool (45 cm long; 22 cm wide diameter, and 20 cm high) filled with 10 cm deep water. The animals were allowed to make rotations.

The number of rotations made per 3 min was recorded.^[35]



Fig. 10: Despire Swim Test.

Experimental design

Rats were divided into six group with six animal in each. The first group received an oral dose of vehicle (10 ml/kg), second group received inducing agent (haloperidol 1mg/kg), third group received standard drug (levodopa+ carbidopa 10 mg/kg) along with haloperidol (1mg/kg), and fourth, fifth, and sixth group received extract of *Rheum emodin* at 100mg, 200mg, 400mg/kg.

Treatment protocol

Briefly, the animal were divided into six groups (n = 6) and treated with respective test solutions as given below.

1. Group 1 (Vehical control) - Vehicle (distilled water 10 ml/kg)po
2. Group 2 (Negative control) - Haloperidol (1mg/kg) ip
3. Group 3 (Positive control) - levodopa+carbidopa (10mg/kg)+ Haloperidol(1mg/kg)ip
4. Group 4 (low dose) - EERE (100mg/kg) po + Haloperidol (1mg/kg)ip
5. Group 5 (Moderate dose) - EERE (200mg/kg) po + Haloperidol (1mg/kg) ip
6. Group 6 (Highest dose) - EERE (400mg/kg) po + Haloperidol (1mg/kg) ip

Statistical analysis All the data were expressed as mean \pm standard error of the mean. Statistical significance between more than two groups was tested using one-way ANOVA followed by the Dunnet's test.

Observation Table

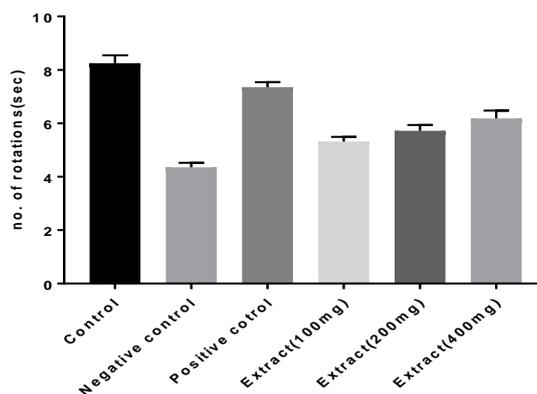
Effect of Ethanolic extract of rheum emodin(EERE) on Motor co- ordination using Despire Swim Test in haloperidol induced catelepsy.

Group	Treatment and doses	4th day No. Of Rotation/3min.	8 th day No. Of Rotation/3min.	14 th day No. Of Rotation/3min.
1.	Control (vehicle)10ml/kgpo.	8.250 ± 0.2952	9.850 ± 0.1910	10.983 ± 0.3609
2.	Negative control Haloperidol (1mg/kg i.p.)	4.350± 0.1708##	5.717 ± 0.2358##	6.483 ± 0.2372##
3.	Positive control (levodopa+ carbidopa), 10 mg/kg,i.p.) + Haloperidol (1mg/kg i.p.)	7.350 ± 0.1910	8.833 ± 0.2445	9.398 ±0.4849
4.	EERE (100mg/kg) + Haloperidol (1mg/kg i.p.)	5.317±0.17400*	6.317 ± 0.4377*	7.383 ± 0.3701*
5.	EERE (200mg/kg) + Haloperidol (1mg/kg i.p.)	5.717±0.2182***	6.917 ± 0.3027***	7.850 ± 0.2997***
6.	EERE (400mg/kg) + Haloperidol (1mg/kg i.p.)	6.183±0.2903****	7.433±0.4080****	8.550±0.2202****

Values are expressed as mean ± SEM, one-way ANOVA followed by Dunnett’s test multiple comparison test, ****indicates $P<0.0001$ when compare to Negative

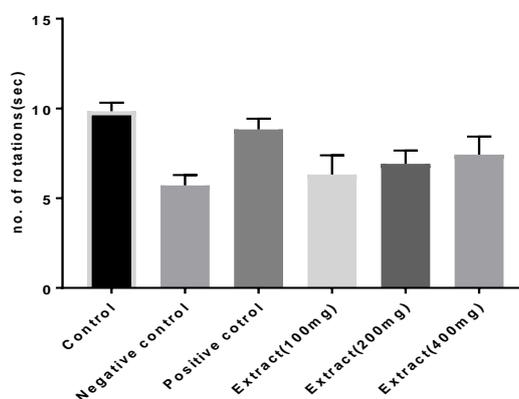
controlgroup. *** indicates $P<0.001$ when compare to negative control, * indicates $P< 0.001$ when compare to negative control group.

Day 4



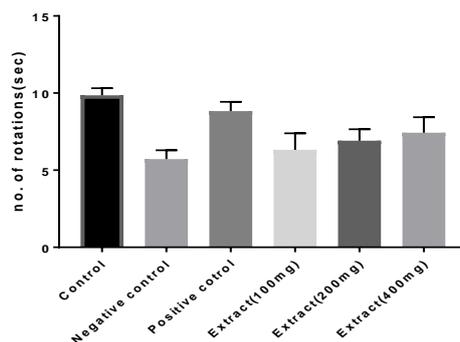
Graph 1: Effect of EERE in Motar co- ordination using Despire Swim Test in haloperidol induced catelepsy.

Day 8



Graph: 2 Effect of EERE in Motar co- ordination using Despire Swim Test in haloperidol induced catelepsy.

Day 14



Graph 3: Effect of EERE in Motar co- ordination using Despire Swim Test in haloperidol induced catalepsy.

RESULT AND DISCUSSION

In the present study, the animals which were treated for 14 days with haloperidol showed severe cataleptic responses. The pathogenesis of PD includes oxidative stress, protein accumulation like α -synuclein, mitochondrial dysfunction, apoptosis, and neuronal excitotoxicity. Among all, oxidative stress is a crucial pathological mechanism for PD.

In the present study, we evaluated the effect of EERE in haloperidol induced Parkinson disease in experimental animals. Haloperidol induced catalepsy is a widely accepted animal model of PD. Haloperidol (nonselective D₂ dopamine antagonists) provides a pharmacological model of parkinsonism by interfering with the storage of catecholamine's intracellularly, resulting in dopamine depletion in nerve endings. In this study, haloperidol (1mg/kg, i.p.) induced significant catalepsy in rats as evidenced by a significant increase in the time spent on the bar as compared to vehicle treated animals. Treatment with EERE significantly reduced the catalepsy in haloperidol treated rats in dose dependent manner. The EERE at doses of 100, 200 and 400 mg/kg showed protective effect against haloperidol induced catalepsy in bar test, indicated that this plant may have an ability to protect dopaminergic neurotransmission in striatum. EERE may also be effective in decreasing the oxidative stress in the haloperidol treated animals possibly due to its antioxidant activity. The phytochemical screening of EERE reveals presence of flavonoids and polyphenols such as Emodin and Aloemodin. Antioxidant activity of plant may be due to presence of these flavonoid and polyphenols which may be responsible for neuroprotection and preventing the oxidative stress in parkinsons diseases.

CONCLUSION

Ethanollic extract of rheum emodin significantly reduced the symptoms of Parkinsons disease may be due to antioxidant and neuroprotective activities or increase in the level of brain dopamine similar to L-dopa and carbidopa. Thus, Ethanollic extract of rheum emodin

may have therapeutic potential in the treatment of PD. Further, it is necessary to estimate the brain dopamine level and isolate the individual constituents responsible for neuroprotective potential, and also characterization of active constituents responsible for neuroprotective effect. It could be the next better, safer & cheaper herbal alternate in management of Parkinsonism and also modulation of of herbal drug with dopaminergic agonist to potentiate the activity.

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