

# CORILAGIN ATTENUATES THE PARKINSONISM IN JAPANESE ENCEPHALITIS VIRUS INDUCED PARKINSONISM

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## Abstract

This study evaluates the protective effect of corilagin against Parkinsonism in Japanese encephalitis virus (JEV) induced Parkinson's disease. The JaGAR-01 strain of virus was used to induce JE. The virus was injected into the rats (13 days age) at the midpoint between the two ears. Adult rats, 12 weeks after the inoculation of virus, were used for the further study. Corilagin (20 mg/kg) and levodopa with dopa decarboxylase inhibitor (LEV, 10 mg/kg) were administered intraperitoneally for the duration of one week. Bradykinesia and the levels of dopamine in the brain were estimated at the end of protocol. There was a significant decrease in the motor function in the corilagin, LEV and LEV + corilagin treated groups compared to the negative control group. However, treatment with corilagin, LEV and LEV + corilagin significantly increases the level of dopamine in the brain compared to the negative control group. This study concludes that corilagin ameliorates the Parkinsonism in JEV induced Parkinsonism. Moreover, it shows a synergistic effect when treated with LEV. Data presented in the investigation supports that corilagin can be used clinically.

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## Keywords

• Japanese encephalitis virus • Parkinsonism • Corilagin • Bradykinesia • Levodopa

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## Introduction

Japanese encephalitis (JE) commonly occurs in children below the age of 6 years and the major factors that contribute to JE are neuronal plasticity, malnutrition of central nervous system and immunological factors [1]. Patients suffering from JEV infection show parkinsonism syndrome postencephalitis. The literature reveals that from the 200 acute cases of JE, 75% of patients show a mask-like face symptom, more than 40% complain about muscular rigidity and 90% of people suffer from tremors [2]. A report reveals that JE results in the development of postencephalitic Parkinsonism in Japanese encephalitis virus-infected Fisher rats [3]. In the substantia nigra, the quantity of hydroxylase-positive cells is reduced in the rats suffering from JE [4]. JEV infection causes neuropathology such as the formation of plaques, shrinkage of neurons, neuronal degeneration, perivascular cuffing and cellular infiltration after 10 days of inoculation [5]. Moreover, bradykinesia was also observed in

JEV-infected rats and treatment with levodopa improves the behavior of rats [6].

In the recent years, alternative medicine such as herbs shows great potential against chronic disorders. Corilagin is an ellagitannin isolated from the *Caesalpinia coriaria* herb [7]. Previously reported studies suggested that corilagin is a potent antioxidant, hepatoprotective, anti-inflammatory, analgesic, antihypertensive, antitumor and has carbonic anhydrase inhibitor properties [8-11]. It is reported that corilagin possesses analgesic activity by altering the glutaminergic system and anti-inflammatory activity by decreasing the production of pro-inflammatory cytokines and mediators such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), IL-1 $\beta$ , IL-6, NO (iNOS) and cyclooxygenase-2 (COX-2) on both the protein and gene level by blocking NF- $\kappa$ B nuclear translocation [12, 13]. There are several drugs available for the management of Parkinsonism, but neuroprotection is not achievable. However, corilagin is reported to possess neuroprotective effects by reducing inflammatory mediators and on the basis

of its antioxidant activity. In addition, it alters the glutaminergic system that is responsible for the tremors. Thus, the present investigation evaluates the effect of corilagin against Parkinsonism.

## Material and methods


### Animals

Albino Wistar rats (13 days old) were procured from Shanghai Medical College, China. All the animals were housed under controlled conditions as specified by the guidelines. All experimental procedures were approved by the institutional animal ethical committee of Hubei Provincial Hospital of Traditional Chinese Medicine (IAEC/HPHTCM/2016/08).

### Induction of JE

The JaGAR-01 strain of virus was used to induce JE. The brain of the infected mouse was homogenized and the supernatant separated and diluted with Hemacel (20%) in Eagle's minimum essential medium. Later, a specially

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designed two-step-thin 27-gauge needle was used to inoculate the virus intracerebrally. The virus was injected in the rats (13 days of age) at the midpoint between the two ears. Adult rats, 12 weeks after the inoculation of virus, were used for further study. However control group of rat receives only diluents. Thereafter JEV infected rats were further separated into four groups: negative control, corilagin (20 mg/kg, ip), levodopa with dopa decarboxylase inhibitor (10 mg/kg, ip) and corilagin+levodopa with dopa decarboxylase inhibitor for the duration of seven days.

**Estimation of motor function**

Bradykinesia was evaluated by estimating the motor activity through a pole test in rats. A pole test was done as per a previously reported study. In the pole test, the time taken by the rats of both control and JEV infected groups to fall down from the rough surfaced pole to the floor was estimated before and after the drug treatment.

**Estimation of neurochemical levels**

All the animals were sacrificed at the end of the protocol (i.e. immediately after the last dose of corilagin) and the brain was isolated from each animal. The isolated brain of each rat was dissected in to several parts as per a previously reported study. Each part of the brain was stored at - 80 °C in liquid nitrogen. Ethylenediaminetetraacetic acid (0.7 mM) and perchloric acid (0.1 M) was used to homogenize the frozen brain tissues. High performance liquid chromatography (HPLC) was used to separate the catecholamines after purifying it with aluminum oxide. An electrochemical detector was used in the HPLC for the estimation of the catecholamine levels. We attached an ECD to the HPLC apparatus to make a system based on the principle of amperometry. Protein levels were determined as per a previously reported method, using bovine serum albumin as the standard.

**Statistical Analysis**

Statistical analysis and results are reported in the form of mean ± SD. One way analysis of variance (ANOVA) was performed for the comparison of results and P < 0.05 was

considered a significant value. Graph Pad Prism version 5.0 for windows (San Diego, CA, USA) was used to analyze the results.

**Result**

**Effect of corilagin on motor function**

The effect of corilagin on motor function in JEV induced Parkinsonism is shown in Fig. 1. It was observed that JEV induced Parkinsonism results in an increase in the time to fall from the rod than control group. There was significant decrease in the time to fall down from the rod in corilagin, LEV and LEV+ corilagin treated group compared to the negative control group. This data reveals that corilagin alone and in combination with LEV restores the motor function in JEV induced Parkinsonism rats.

**Effect of corilagin on the level of monoamines**

The effect of corilagin on the concentration of dopamine in various part of the brain such as cerebral cortex, midbrain and hypothalamus is shown in Table 2. There was a significant decrease in the levels of dopamine in the hypothalamus and cerebral cortex of rat brains compared to the negative control group. However levels of dopamine was found to be attenuated in corilagin, LEV and LEV+corilagin treated groups.

In addition, the effect of corilagin on the concentration of dopamine in the brain striatum is shown in Fig. 2. It was observed that treatment with corilagin and LEV alone and in combination significantly enhances the level of dopamine in the substantia nigra compared to the negative control group. These result

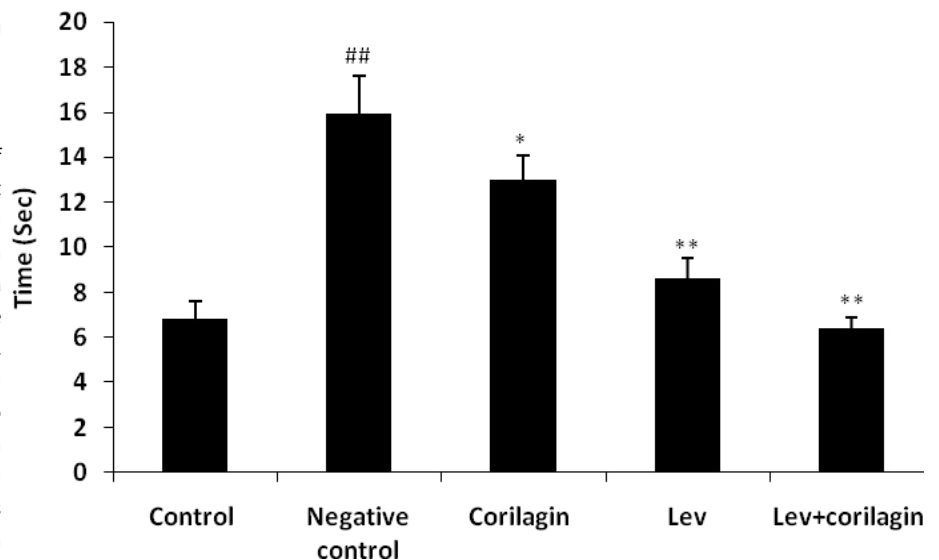


Fig. 1. Effect of corilagin on motor function in Japanese encephalitis virus induced Parkinsonism. Mean±SD (n=6); ##p<0.01 compared to the control group; \*p<0.05; \*\*p<0.01 compared to the negative control group  
Table 1. Effect of corilagin on the concentration of dopamine in various parts of the brain

Sr. No.	Group	Concentration of Dopamine (ng/mg protein)		
		Cerebral cortex	Midbrain	Hypothalamus
1	Control	6.38±1.26	2.62±0.43	4.72±0.84
2	Negative control	2.17±0.83##	3.45±0.53	2.94±0.32##
3	Corilagin	3.24±0.62*	3.29±0.42	3.67±0.72**
4	Lev	4.16±0.92**	3.11±0.59	2.96±0.53##
5	Lev+corilagin	5.42±1.12**	3.08±0.61	3.89±0.63**

Mean±SD (n=6); ##p<0.01 compared to control group; \*p<0.05; \*\*p<0.01 compared to negative control group

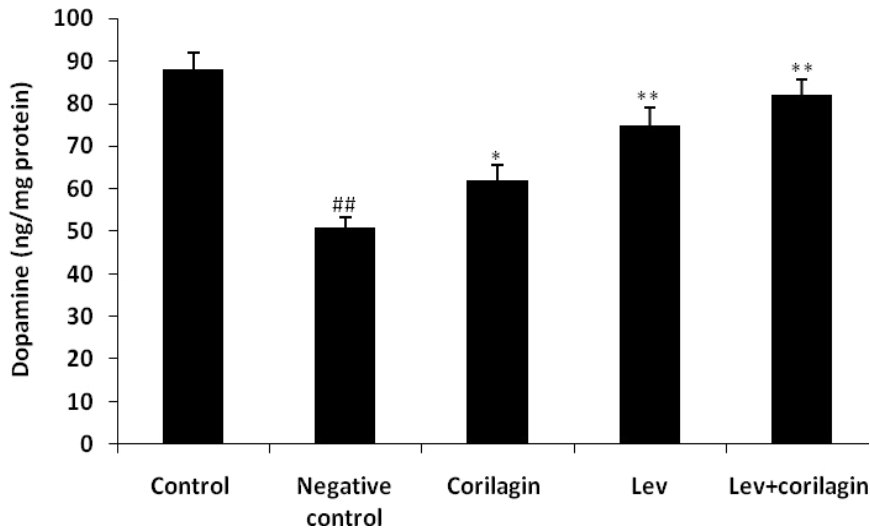


Fig. 2. Effect of corilagin on the concentration of dopamine in the brain striatum. Mean±SD (n=6); ##p<0.01 compared to the control group; \*p<0.05; \*\*p<0.01 compared to the negative control group

suggest that treatment with LEV+corilagin shows synergistic effects in the management of JEV induced Parkinsonism.

## Discussion

A study reveals that Japanese encephalitis results in development of lesions in the substantia nigra, causing post encephalitis Parkinsonism, and JEV infection causes neuropathology of the brain, confirming the parkinsonism in a rat model too [14]. There are several factors that contribute to the pathogenesis of Parkinsonism such as infections and environmental and genetic factors [15].

Parkinsonism induced by viral infection is well proven in the literature [16]. Hence, our study used the JEV infection induced Parkinsonism in a rat model and evaluated the protective effect of corilagin by estimating the motor function and level of dopamine in the brain.

The literature suggested that the marked bradykinesia in JEV infected rats could be treated with LDOPA to attenuate these behavioral changes [17]. Our study reveals that treatment with corilagin significantly attenuates the motor dysfunction in rats with Parkinsonism and a synergistic effect was found in the rats treated with LEV+ corilagin.

Alterations in the levels of dopamine in

brain is due to degeneration of dopaminergic neurons, developing into Parkinsonism. The literature reveals that JEV infection leads to the development of postencephalitis Parkinsonism [3]. Moreover, alterations of motor function in Parkinsonism occurs due to a decrease in the level of dopamine in different areas of the brain. A reported study on JEV infected rats also reveals that the level of dopamine decreases in the hypothalamus and cerebral cortex of the brain [18]. Corilagin is reported to have a strong antioxidant and anti-inflammatory property on the basis of which it possesses neuroprotective effects and thus it could attenuate the parkinsonism by enhancing the level of dopamine in the brain. However, results from this study has shown that treatment with corilagin significantly improves the level of dopamine in the brain of rats compared to the negative control group and also shows synergistic effects when treated with LEV. The present study has a limitation that this work needs to further study on the molecular and genetic levels for a better understanding of the mechanism of action of corilagin.

## Conclusion

This study concludes that corilagin ameliorates the Parkinsonism in JEV induced Parkinsonism. Moreover corilagin shows a synergistic effect when treated with LEV. The data presented in the investigation supports that corilagin can be used clinically.

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