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Neuroprotective potential of ethanolic leaf extract of *Aristolochia tagala* (Charm) against lipopolysaccharide-induced neurotoxicity in Wistar rats

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ABSTRACT

Introduction: *Aristolochia tagala, contains various essential phytoconstituents and is a potential medicinal plant used traditionally to treat various ailments such as neurodegenerative diseases. The present study aimed to evaluate the neuroprotective effect of ethanolic leaf extract of Aristolochia tagala against the lipopolysaccharides (LPS) induced behavioral changes in rats.*
Methods: *The in-vitro antioxidant potential was evaluated by 1-diphenyl-2-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assay methods. For in-vivo studies, the animals were pre-treated with ethanolic leaf extract of Aristolochia tagala (EEAT) at 200 and 400 mg/kg of b.w for 30 days, and neurotoxicity was induced with a single intraperitoneal injection of LPS 1 mg/kg, b.w on day 31. The neurotoxicity was evaluated with a chain of behavioral tests such as Radial arm maze and Forced swim tests. At the end of the study, rats were sacrificed, the brain hippocampal region was removed, and the levels of acetylcholinesterase, nitric oxide, and protein were measured.*
Results: *The IC50 value in the DPPH method was 38.58±1.24 µg, and the total antioxidant activity of EEAT was found to be 700.66 ± 16.37 µmol Fe (II)/g extract. In behavioral tests, animals treated with EECC at 200 and 400 mg/kg showed a neuroprotective effect in Radial arm maze and Forced swim tests. Both doses reduced acetylcholinesterase, nitric oxide, and protein levels ($P < 0.001$), respectively.*
Conclusion: *The present study results showed the promising neuroprotective effects of ethanolic extract of leaves of Aristolochia tagala and its action against the LPS-induced cognitive impairment in rats.*
Keywords: *Neuroprotective; lipopolysaccharides; Aristolochia tagala.*

INTRODUCTION

Neurodegenerative diseases are gradual deteriorations of the neurological system, particularly the brain, that usually develop slowly and manifest symptoms later in life(Berman and Bayati, 2018). Neuroinflammation is a key factor in the initiation and progression of neurodegeneration and neuronal loss in neurodegenerative diseases like Alzheimer's, Parkinson's, and multiple sclerosis. Neuroinflammation, a brain defence mechanism, initially protects the brain by inhibiting pathogens and promoting tissue repair. When triggered by infection, injury, or chronic stress, inflammatory mediators impair immune system equilibrium, impacting memory, brain plasticity, and neurogenesis(Okun *et al.*, 2012). However, prolonged inflammatory responses, involving microglia and astrocytes, can inhibit regeneration and lead to neurodegenerative diseases(Kwon and Koh, 2020).

Many of these illnesses are closely associated with aging and are significantly more likely to occur in adults over the age of 65. The World Health Organization (WHO) predicts that the number of persons over 65 with neurodegenerative diseases will at least double over the next 30 years(Srinivasan *et al.*, 2021). This suggests that the number of persons with these disorders will increase at a comparable rate. There is currently no cure for neurodegenerative illnesses, and the treatments available have side effects and are ineffective. Current medications for neuropsychiatric diseases, such as depression, do not adequately treat one-third of patients, known as treatment-resistant patients. So, seeking for novel remedies is critical. Medicinal herbs appear to be an effective substitute and complement existing treatment regimens because they have been utilized for health purposes for thousands of years.

Various substances were tested to build a suitable animal model for neurodegenerative illnesses based on their effect on behavior patterns. It includes scopolamine, ethanol, colchicine, heavy metals, lipopolysaccharide, streptozotocin, and okadaic acid. Bacterial infections trigger innate immune responses, activating TLR4 and NF-κB in microglia and macrophages. This leads to cytokine expression and nitric oxide production(Parajuli *et al.*, 2012). These pathways serve as crucial for immune cells to degrade bacteria, which has a negative impact on neuronal death.

Lipopolysaccharide (LPS) is a cell wall component of gram-negative bacteria that acts as a TLR4 ligand, triggering immunological responses to infections. Microglia cells are significantly more susceptible to LPS than other glial cells, resulting in learning and memory deficits. Intraperitoneal injection of LPS increases the release of pro-inflammatory cytokines, leading in neuroinflammation, hippocampal apoptosis, cognitive impairment, learning difficulties, as well as beta-amyloid plaque formation in the hippocampus(Rock *et al.*, 2004).

Plants that contain a variety of secondary metabolites have been effectively employed as a treatment for a variety of illnesses, including neurological disorders. The current interest in and demand for herbs is a global phenomenon(Khazdair *et al.*, 2019). Medicinal plants and plant-derived chemicals have potential to prevent, treat, or delay neurological disorders. Plants including *Ashwagandha, Ginkgo biloba, Centella asiatica, Ginseng,* and *Baccopa monnieri*, as well as extracts containing chemicals like curcumin, flavonoids, celastrol, resveratrol, trehalose, sesamol and lycopene, have been shown to have antioxidant and neuroprotective benefits.

Aristolochia tagala, a sun-loving shrub in Aristolochiaceae family, is found in semi-evergreen forests, open lowlands, and thickets. Commonly known as Valiya arayan and has a long history of medical use. *Aristolochia tagala L.* (Aristolochiaceae) has been traditionally used to treat various ailments including cholera, bowel issues, ulcers, leprosy, skin diseases, menstrual issues, and snakebites. This plant has been reported for the following pharmacological activities such as anti-inflammatory(Battu *et al.*, 2011), an anti-cancer agent (Hadem *et al.*, 2014)., nephroprotective (Tripatara *et al.*, 2012), and larvicidal actions (Baskar *et al.*, 2011). In conjunction with this, the objective of the current study aimed to evaluate the neuroprotective effect of leaf extracts of *Aristolochia tagala*, against LPS induced
neuroinflammation in Wistar rats.

Drugs and chemicals

LPS, 1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin- Ciocalteu reagent, and dexamethasone were purchased from the Sigma-Aldrich Chemicals Private Limited, Bangalore, India.

Plant materials

Fresh *Aristolochia tagala* leaves were taken from Trivandrum, Kerala in October 2019. The plant materials were identified and certified by the Botanical Survey of India, Tamil Nadu, at Agri University in Coimbatore, Tamil Nadu. A voucher specimen (ATKA-1) was placed with the Department of Pharmacology at Vinayaka Mission's College of Pharmacy in Salem, Tamil Nadu for future reference.

Extraction of plant materials

The leaves were dried in the shade at ambient temperature for 10 days before being coarsely crushed and stored in an airtight container for later use. Approximately 500 g of coarsely crushed leaves were subjected to continuous hot percolation with various solvent systems of increasing polarity, including pet ether, chloroform, acetone, ethanol, and aqueous solution. Preliminary phytochemical analyses were carried out to determine the presence of phytoconstituents such as alkaloids, flavonoids, glycosides, phenols, saponins, sterols, tannins,

proteins, and carbohydrates in the extracts (Arul *et al.*, 2005; Manivannan *et al.*, 2012).

Animals

In this study, adult male Wistar rats of 45-day-old weighing 120 grams was used. The animals were obtained from Sri Venkateshwara Enterprises, Bangalore, India, a supplier listed by the Committee for the Control and Supervision of Experiments on Animals (CCSEA). The animals were housed in well-ventilated polypropylene cages with 12 hours of light and 12 hours of darkness, 25°C temperature, and 55-65% humidity level. Rats were fed a standard pellet diet and had free access to water.

Animal Preparation

The animals were chosen at random, identified, and housed in cages for at least at least 5 days before dosing to acclimate to laboratory conditions. Animals were fasted for 12 hours prior to each test. The Institutional Animals Ethical Committee (P.col/28/2021/IAEC/VMCP) approved the experimental protocols. All investigations were conducted in the early morning to adhere to CPCSEA norms and ethical guidelines for examining pain in conscious animals. The typical orogastric cannula was used to give drugs orally in experimental animals.

Determination of in-vitro antioxidant activity

DPPH radical scavenging activity

EEAT was tested for antioxidant activity by the DPPH method(Salari *et al.*, 2019). The extract (20, 40, 60, 80, 100 µg/mL) was mixed with 3 mL of methanolic solution containing DPPH radicals (0.1 mM). After 30 minutes, absorbance was determined at 517 nm. The percent inhibition of activity was calculated by using the formula:

$$\text{DPPH scavenging effect (\%)} = \frac{A_0 - A_1}{A_0} x100$$

Where, Ao = absorbance without extract; Ae = absorbance with extract.

The results were expressed as IC50, which is the concentration of the sample required to inhibit 50 % of DPPH concentration.

Ferric reducing antioxidant power (FRAP) assay

FRAP assay is a novel method to assess the antioxidant power of the sample. The method performed in this study was described by Prasad et al(Prasad *et al.*, 2010)). It is based on the ability of antioxidants to reduce Fe+3 to Fe+2 in the presence of TPTZ (2, 4, 6-tripyridyl-s-triazine), forming an intense blue Fe+2 – TPTZ complex. FRAP solution (3 mL) mixed with 100 ml of the EEAT and incubated at 37°C for 10 minutes. Absorbance was measured at 593 nm for different concentrations (0.2, 0.4, 0.8 or 1 mg/mL) of extract in FRAP reagent. The absorbance of the samples was compared to a FeSO4 standard curve and the FRAP values were expressed as mmol Fe (II)/mg extract.

Pharmacological studies

Experimental design

Thirty healthy Wistar male rats weighing 200 g were randomly divided into 5 groups with six animals in each group. Group I served as normal control, which received 0.1 mL of normal saline orally for 30 days. Group II served as disease control, which received a single intraperitoneal dose of LPS (1 mg/kg) (21) on day 31. Group III served as positive control, which received LPS (1 mg/kg) + dexamethasone (0.5 mg/kg) (21) for 30 days. Groups IV and V respectively received LPS (1 mg/kg) + EEAT 200 and 400 mg/kg (15) for 30 days. On the last day, after the evaluation of behavioral studies, the animals were sacrificed under light ether anesthesia and the brain was dissected out immediately and subjected to biochemical studies.

Behavioral tests for learning and memory

Radial arm maze test

A radial arm maze is a method to assess the working memory of animals(Gomathi *et al.*, 2017). It consists of a wooden apparatus with eight elevated–arm radial maze which extends from the central platform having a diameter of 26 cm. Each arm is 60 cm long, 2 cm height, and 5 cm width which holds along the length of the arm. The evaluation was conducted in a well-lighted room which contains many signs. During the study, all the arms

were filled with pellets and the experiment was continued until all the food pellets were collected or 10 min passed, whichever occurred first. All the animals were trained daily to collect the pellet for 4 weeks. The trial was ended after 8 choices and the animals have to make more correct choices with fewer errors. The following parameters were checked during the study to assess the performance of the animals such as the number of correct choices, the number of errors, test duration (s), and the total number of errors before all the food pellets in the eight arms were collected.

Forced swimming test

A force swimming test (FST) was done for all groups as previously described Hosseini *et al*(Norouzi *et al.*, 2016). Each rat was compelled to swim in a cylindrical glass tank (60 cm in height and diameter of 38 cm) which was filled with water (40 cm depth) at 24±1 °C. The total duration of immobility was calculated by a single observer for 5 min. The immobility was considered when the rats made no effort to escape, except necessary movements which enabled them to keep their head above the water. The active and climbing times were also recorded for 5 min.

Biochemical studies

At the end of the behavioral studies, all rats were sacrificed under light ether anaesthesia and the rat brain hippocampal region was isolated. It was homogenized with 0.1M phosphate buffer (pH 8) at 0°C using Potter–Elvehjem homogenizer. The homogenate was then subjected to centrifugation at 10 000×g for 5 minutes at 4°C to get clear supernatant liquid and the same was used for biochemical estimations like acetylcholinesterase, nitric oxide (NO), and protein.

Estimation of acetylcholinesterase

The brain concentration of acetylcholinesterase was estimated by the method of Ellman *et al* with slight modification(Ellman *et al.*, 1961). 0.1ml of brain homogenate was mixed with 6ml of sodium phosphate buffer (pH 8), acetylthiocholine iodide (0.2 mL), and 5,5'-dithio-bis-(2-nitrobenzoic acid (DTNB, the Ellman reagent). The changes in the absorbance of the mixture were measured at 412 nm.

Estimation of nitric oxide

The brain concentration of nitric oxide was estimated by the method of Green *et al* (Green *et al.*, 1982). In this method, equal amounts of brain homogenate and Griess reagent (1% sulphanilamide, 2% H_3PO_4, 0.1% N-(1-naphthyl) ethylenediamine-HCl) were allowed to react at room temperature for 5 min. The resulting bright reddish-purple-colored azo-dye was measured spectrophotometrically at 540 nm.

Estimation of Protein

Protein concentrations of the brain homogenates were determined by the standard method of estimation explained by Lowry *et al*(Lowry *et al.*, 1951).

Results

Percentage yield and phytochemical screening

The percentage yield of the extracts was calculated and found to be 2.6, 1.3, 1.61, 11.8, and 6.2 %w/w for pet ether, chloroform, acetone, ethanol, and aqueous extracts, respectively. The glycosides, carbohydrates, phenols, saponins, terpenoids, tannins, and flavonoids were present in the acetone, ethanol, and aqueous extracts. Alkaloids and terpenoids were present in chloroform extract. Gums and fixed oils were present in petroleum ether extract. Thus, the phytochemical analysis confirmed the presence of bioactive compounds, and this might serve as a potential source in the treatment of neurological disorders. Hence, based on the percentage yield and phytochemical results, ethanolic extract of leaves of *Aristolochia tagala* was selected for its neuroprotective studies.

In-vitro antioxidant studies

DPPH radical scavenging activity

The DPPH free radical scavenging activity of the EEAT was carried out. The extracts were tested at concentrations of 20, 40, 60, 80, and 100 µg/mL. The EEAT showed 88.24% inhibition of the DPPH radical at 100 µg/mL concentration, whereas the standard (ascorbic acid) revealed 92.3% inhibition at the same concentration. The extract showed the DPPH radical scavenging activity even at the lowest concentration of 20 µg/mL. The DPPH

radical inhibition was increasing and concentration dependent as that of ascorbic acid as the standard compound. The IC50 values of the EEAT and ascorbic acid were found to be 38.58±1.24µg/mL and 8.6 ±0.97 µg/mL, respectively.

Total antioxidant activity by FRAP method

In-vitro total antioxidant activity for EEAT was estimated by FRAP method using ascorbic acid as standard. The EEAT showed the FRAP activity of 700.66 ± 16.37 µmol Fe (II)/g extract, whereas ascorbic acid showed 1048.67±17.77µmol Fe (II)/g.

Radial arm maze test

The effect of EEAT on working memory was determined using the radial arm maze method. In this, the total number of errors in entry and exploration time was checked to assess its effects and the results were shown in table.13 and Fig 8. The total number of errors in the entry was significantly increased in LPS treated animals as compared to the control group $P < 0.001$. But dexamethasone tended to reduce the total number of errors in entry. However, there was a significant ($P < 0.001$) dose-dependent reduction of a total number of errors in the entry on animals treated with EEAT at 200 mg/kg,b.w when compared with those treated with a high dose of 400 mg/kg,b.w. In exploration time, there was a significant decrease was observed in EEAT at 400 mg/kg,b.w treated animals, as compared to 200 mg/kg, b.w treated animals.

Similarly, dexamethasone also reduced the exploration time While, LPS treated animals showed increased exploration time as compared to control group animals ($P < 0.001$). The EEAT at high doses (400mg/kg,b.w) significantly reduced the total number of errors in entry and exploration time ($P < 0.001$) and ameliorate the LPS induced working memory deficit.

Table 1. Effect of ethanolic leaf extract of *Aristolochia tagala* (EEAT) on working memory and Depression.

S. No	Groups	Dose	Radial arm maze		Forced swim test		
			Number of errors	Exploration time (S) taken to reach the pellet	Immobility	Active time	Climbing time
1	Control	1 ml	3.33±0.42	13.17±0.48	39.33±0.88	188.33±1.58	113.83±1.10
2	LPS	1mg/kg	8.83±0.70[a]	31.16±0.16[a]	136.17±1.62[a]	113.16±1.01[a]	61.16±0.90[a]
3	LPS+Dex	0.5mg/kg	4.16±0.87[b]	19.33±0.80[b]	40.83±0.74[b]	174.83±0.87[b]	99.16±1.35[b]
4	LPS+EEAT	200mg/kg	7.16±0.40[b]	26.33±0.33[b]	87.26±0.94[b]	151.5±0.88[b]	72.33±0.98[b]
5	LPS+EEAT	400mg/kg	6.5±0.67[b]	24.17±0.74[b]	68.33±0.88[b]	161.17±0.47[b]	88.67±0.80[b]

Values are expressed as mean± SEM of 6 animals. Data were analysed by one-way ANOVA followed by Tukey's multiple comparison tests. a $P<0.001$, indicates that Group II (negative control) was compared with group I(control). b $P<0.001$ indicates that Group III, IV, V, VI, and VII were compared with group II. ***Forced swim test***

The effect of EEAT on depression was determined using the forced swim test method. In this, the immobility time, active time, and climbing time was measured to assess the LPS induced depression in animals and the results were shown in table.1.The immobility time was significantly increased in LPS treated animals as compared to the control group $P < 0.001$. But dexamethasone tended to reduce the immobility time. However, there was a significant ($P < 0.001$) dose-dependent reduction of immobility time on animals treated with EEAT at 200 mg/kg,b.w when compared with those treated with a high dose of 400 mg/kg,b.w.

In, active and climbing time, there was a significant decrease was observed in EEAT at 400 mg/kg,b.w treated animals, as compared to 200 mg/kg, b.w treated animals. Similarly, dexamethasone also reduced the active and climbing time, While, LPS treated animals showed increased active and climbing time, as compared to control group animals ($P < 0.001$). The EEAT at high doses (400mg/kg,b.w) significantly reduced the immobility time and active and climbing time ($P < 0.001$) and ameliorate the LPS induced depression deficit.

Biochemical studies

Acetylcholinesterase enzyme

The results of the inhibitory effect of EEAT on acetylcholinesterase enzyme were shown in table 2. In LPS treated neurotoxic animals, the inhibition levels of acetylcholinesterase were high which denotes the

neurodegenerative effects. Treatment with dexamethasone significantly reduced the inhibition levels as compared to LPS treated animals. However, EEAT treated groups at 200 and 400mg/kg, b.w showed a considerable decrease in inhibitory when compared to disease control animals' $P < 0.001$. This indicates the neuroprotective effect of EEAT when compared to disease control animals.

Table 2. Effect of ethanolic leaf extract of *Aristolochia tagala* (EEAT) on Biochemical parameters

Groups	Dose	Biochemical parameters		
		Ache (µg/min/mg protein)	NO (µmol/g tissue)	Protein (g/dl)
Control	1 ml	63.33±0.88	0.86±0.04	5.03±0.18
LPS	1mg/kg	106.83±0.60 [a]	5.78±0.34 [a]	10.25±0.38 [a]
LPS+Dex	0.5mg/kg	67.17±0.90 [b]	1.391±0.11 [b]	6.02±0.15 [b]
LPS+EEAT	200mg/kg	96.5±0.99 [b]	3.99±0.19 [b]	9.03±0.18 [b]
LPS+EEAT	400mg/kg	81.50±0.76 [b]	2.26±0.09[b]	8.2±0.13 [b]

Values are expressed as mean± SEM of 6 animals. Data were analysed by one-way ANOVA followed by Tukey's multiple comparison tests. a $P<0.001$, indicates that Group II (negative control) was compared with group I(control). b $P<0.001$ indicates that Group III, IV, V, VI, and VII were compared with group II.

Nitric oxide

The effect of EEAT on nitric oxide level was shown in table 2. There was a significant increase in NO levels was observed in LPS treated animals as compared to the control animals' $P < 0.001$. Dexamethasone treatment considerably reduced these levels near to normal. Treatment with EEAT at 200 mg/kg and 400 mg/kg, b.w also reduced the elevated levels of nitric oxide significantly in a dose-dependent manner.

Total protein

The effect of EEAT on protein level were shown in table 2. LPS treatment significantly increased the protein level in the disease control group compared to normal animals. However, pre-treatment with dexamethasone, EEAT at 200 and 400mg/kg, b.w significantly reduced these elevated levels as compared to disease control animals.

DISCUSSION

The present study investigated the neuroprotective effect of ethanolic leaf extract of *Aristolochia tagala* against LPS-induced neurotoxicity. *Aristolochia tagala* (Cham) is an evergreen perennial shrub, commonly called as Dutchman's pipe belonging to the family of Aristolochiaceae having broad therapeutic properties.

It was reported that free radicals are one key factor for neuronal death in many neuro-disorders such as seizure disorders, schizophrenia, cerebral ischemia, Parkinson's disease, Alzheimer's disease, etc(Uttara *et al.*, 2009).

In this study, the *in-vitro* antioxidant potential of EEAT was evaluated by DPPH and FRAP assay methods and it showed promising antioxidant activity in a dose-dependent manner. In FRAP assay, the ability to reduce Fe^{3+} to Fe^{2+} was also significantly higher at concentrations 0.2–1 mg/mL. It was reported that the phenolic compounds were directly related to the antioxidant property(Awika *et al.*, 2003).

This study, mainly focussed on the effects of EEAT on LPS-induced behavioral changes in the rat's brain, and the behavioral changes were measured by working memory, and depression by subjecting it to the Radial arm maze test and forced swim tests respectively.

Spatial working memory is a critical cognitive function in which information is retained and active for a short time after it has been received. The radial-arm maze test is one of the common methods to evaluate working memory in rodents(Cassel *et al.*, 1998). This study was assessed by measuring the number of errors in the entry to arms and exploration time to reach the food pellets. The results of the study depict that the LPS-treated animals showed more number errors and an increase in the exploration time when compared to the control group animals. This shows the memory impairment changes in disease control animals. However, the EEAT-treated animals showed a significant reduction in the number of errors in entry and exploration time when compared with the LPS-treated animals. these results confirmed that the treatment of EEAT improves the LPS-induced impairment of working memory.

Forced Swim Test is the most extensively used pharmacological model for measuring antidepressant(Cryan *et al.*, 2002). When rodents are trapped in an unavoidable situation, they acquire immobility.

In the forced swimming test, the animals were allowed to swim in a relatively small space from which they have no option of escaping. They swiftly establish a state of despair behaviour marked by a low incentive for escaping, as seen by the increased periods of immobility. In this experiment, the immobility demonstrated by mice when subjected to unavoidable stressors such as forced swimming is supposed to reflect a state of hopelessness or decreased mood, which is thought to reflect depressive illnesses in people. It was observed from the results that LPS injection produced abnormal behaviours in FST in terms of the increased number of entries and time spent in the closed arm. While, pre-treatment with EEAT at 200 and 400 mg/kg,b.w showed a significant increase when compared to LPS. This suggests the protective effects of EEAT against LPS induced behavioural stress.

The central cholinergic system has a major role in neuro-cognition. Significant reduction in acetylcholine leads to marked behavioural changes that were indicated in many neurological diseases(Kumar and Kulkarni, 1996). In the present study, LPS induced neurotoxicity causes behavioural changes by escalating the acetylcholinesterase enzyme level. The reduction in the acetylcholinesterase inhibition level due to the treatment with EEAT for 30 days suggests the ameliorating effects of EEAI and EEAT against cholinergic changes.

Nitric oxide has important physiological activities and is linked to a variety of neurological illnesses, including Alzheimer's disease and Parkinson's disease. Numerous evidence indicated the excitotoxicity produced by nitric oxide. These activated neurons kill a large number of neurons and thus elevated nitric oxide acts as a key mediator in neurological diseases(Schulz *et al.*, 1995). Pre-treatment with EEAT resulted in a significant reduction in the levels of nitric oxide and this may be due to its antioxidant potential. While dexamethasone reversed these levels near to normal.

Elevated levels of proteins are commonly associated with acute and chronic inflammatory conditions. In our study, LPS treated animals showed high levels of protein in the hippocampus region of the rat brain. Pre-treatment with EEAT resulted in a significant reduction in the levels of protein, and this may be due to its antioxidant potential. While dexamethasone reversed these levels near to normal.

Conclusion

The results of the present study showed the promising neuroprotective effects of ethanolic extract of leaves of *Aristolochia tagala* against the LPS-induced neuroinflammation in rats. This may be due to the antioxidant properties of the flavonoids, tannins, and polyphenols present in it. In the future, isolation and characterization studies are required for further evidence of its neuroprotective activity.

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References

Arul, B., Kothai, R., Sureshkumar, K. and Christina, A.J. (2005), "Anti-inflammatory activity of Coldenia procumbens Linn.", *Pakistan Journal of Pharmaceutical Sciences*, Vol. 18 No. 3, pp. 17–20.

Awika, J.M., Rooney, L.W., Wu, X., Prior, R.L. and Cisneros-Zevallos, L. (2003), "Screening methods to measure antioxidant activity of sorghum (Sorghum bicolor) and sorghum products", *Journal of Agricultural and Food Chemistry*, ACS Publications, Vol. 51 No. 23, pp. 6657–6662.

Baskar, K., Sasikumar, S., Muthu, C., Kingsley, S. and Ignacimuthu, S. (2011), "Bioefficacy of Aristolochia tagala Cham. against Spodoptera litura Fab. (Lepidoptera: Noctuidae).", *Saudi Journal of Biological Sciences*, Saudi Arabia, Vol. 18 No. 1, pp. 23–27.

Battu, G.R., Parimi, R. and Chandra Shekar, K.B. (2011), "In vivo and in vitro pharmacological activity of Aristolochia tagala (syn: Aristolochia acuminata) root extracts.", *Pharmaceutical Biology*, England, Vol. 49 No. 11, pp. 1210–1214.

Berman, T. and Bayati, A. (2018), "What are neurodegenerative diseases and how do they affect the brain?", *Frontiers for Young Minds*, Frontiers Media SA, Vol. 6.

Cassel, J.C., Cassel, S., Galani, R., Kelche, C., Will, B. and Jarrard, L. (1998), "Fimbria-fornix vs selective hippocampal lesions in rats: effects on locomotor activity and spatial learning and memory.", *Neurobiology of Learning and Memory*, United States, Vol. 69 No. 1, pp. 22–45.

Cryan, J.F., Markou, A. and Lucki, I. (2002), "Assessing antidepressant activity in rodents: recent developments and future needs", *Trends in Pharmacological Sciences*, Elsevier, Vol. 23 No. 5, pp. 238–245.
Ellman, G.L., Courtney, K.D., Andres, V.J. and Feather-Stone, R.M. (1961), "A new and rapid colorimetric determination of acetylcholinesterase activity.", *Biochemical Pharmacology*, England, Vol. 7, pp. 88–95.
Gomathi, S., Sundaram, R.S., Annapandian, V.M. and Vijayabaskaran, M. (2017), "Neuroprotective Effect of Pedalium murex Linn. Leaf against Lipopolysaccharide Induced Behavioural Disorders in Rats", *Pharmacognosy Journal*, Vol. 9 No. 6, pp. 957–962.
Green, L.C., Wagner, D.A., Glogowski, J., Skipper, P.L., Wishnok, J.S. and Tannenbaum, S.R. (1982), "Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids.", *Analytical Biochemistry*, United States, Vol. 126 No. 1, pp. 131–138.
Hadem, K.L.H., Sharan, R.N. and Kma, L. (2014), "Inhibitory potential of methanolic extracts of Aristolochia tagala and Curcuma caesia on hepatocellular carcinoma induced by diethylnitrosamine in BALB/c mice.", *Journal of Carcinogenesis*, India, Vol. 13, p. 7.
Khazdair, M.R., Anaeigoudari, A., Hashemzehi, M. and Mohebbati, R. (2019), "Neuroprotective potency of some spice herbs, a literature review", *Journal of Traditional and Complementary Medicine*, Vol. 9 No. 2, pp. 98–105.
Kumar, S. and Kulkarni, S.K. (1996), "Influence of antidepressant drugs on learning and memory paradigms in mice.", *Indian Journal of Experimental Biology*, Vol. 34 No. 5, pp. 431–435.
Kwon, H.S. and Koh, S.-H. (2020), "Neuroinflammation in neurodegenerative disorders: the roles of microglia and astrocytes", *Translational Neurodegeneration*, Vol. 9 No. 1, p. 42.
Lowry, O., Rosebrough, N., Farr, A. and Randall, R. (1951), "Protein measurement with the folin phenol reagent.", *Ournal of Biological Chemistry*, Vol. 193 No. 1, pp. 265–275.
Manivannan, E., Kothai, R., Arul, B. and Rajaram, S. (2012), "Anti-inflammatory activity of bougainvillea spectabilis linn", *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, Vol. 3 No. 1, pp. 642–646.
Norouzi, F., Abareshi, A., Anaeigoudari, A., Shafei, M.N., Gholamnezhad, Z., Saeedjalali, M., Mohebbati, R., *et al.* (2016), "The effects of Nigella sativa on sickness behavior induced by lipopolysaccharide in male Wistar rats", *Avicenna Journal of Phytomedicine*, Mashhad University of Medical Sciences, Vol. 6 No. 1, pp. 104–116.
Okun, E., Barak, B., Saada-Madar, R., Rothman, S.M., Griffioen, K.J., Roberts, N., Castro, K., *et al.* (2012), "Evidence for a developmental role for TLR4 in learning and memory", Public Library of Science San Francisco, USA.
Parajuli, B., Sonobe, Y., Kawanokuchi, J., Doi, Y., Noda, M., Takeuchi, H., Mizuno, T., *et al.* (2012), "GM-CSF increases LPS-induced production of proinflammatory mediators via upregulation of TLR4 and CD14 in murine microglia", *Journal of Neuroinflammation*, Springer, Vol. 9, pp. 1–12.
Prasad, K.N., Chew, L.Y., Khoo, H.E., Kong, K.W., Azlan, A. and Ismail, A. (2010), "Antioxidant capacities of peel, pulp, and seed fractions of Canarium odontophyllum Miq. fruit", *Journal of Biomedicine & Biotechnology*, Hindawi Publishing Corporation, Vol. 2010, p. 871379.
Rock, R.B., Gekker, G., Hu, S., Sheng, W.S., Cheeran, M., Lokensgard, J.R. and Peterson, P.K. (2004), "Role of microglia in central nervous system infections", *Clinical Microbiology Reviews*, Am Soc Microbiol, Vol. 17 No. 4, pp. 942–964.
Salari, S., Esmaeilzadeh Bahabadi, S., Samzadeh-Kermani, A. and Yosefzaei, F. (2019), "In-vitro Evaluation of Antioxidant and Antibacterial Potential of GreenSynthesized Silver Nanoparticles Using Prosopis farcta Fruit Extract", *Iranian Journal of Pharmaceutical Research : IJPR*, Shaheed Beheshti University of Medical Sciences, Vol. 18 No. 1, pp. 430–455.
Schulz, J.B., Matthews, R.T. and Beal, M.F. (1995), "Role of nitric oxide in neurodegenerative diseases.", *Current Opinion in Neurology*, England, Vol. 8 No. 6, pp. 480–486.
Srinivasan, P., Arul, B. and Kothai, R. (2021), "Neuroprotective effect of ethanolic leaf extract of Commiphora caudata (Wight &Arn) against lipopolysaccharide-induced neurotoxicity in Wistar rats", *Journal of Herbmed Pharmacology*, Vol. 10 No. 4.
Tripatara, P., Onlamul, W., Booranasubkajorn, S., Wattanarangsan, J., Huabprasert, S., Lumlerdkij, N.,

Akarasereenont, P., *et al.* (2012), "The safety of Homnawakod herbal formula containing Aristolochia tagala Cham. in Wistar rats.", *BMC Complementary and Alternative Medicine*, England, Vol. 12, p. 170.

Uttara, B., Singh, A. V, Zamboni, P. and Mahajan, R.T. (2009), "Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options", *Current Neuropharmacology*, Bentham Science Publishers Ltd., Vol. 7 No. 1, pp. 65–74.