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In-vivo Assessment of Neurotoxicological Effect of Mugwort Leaves on Induced Rat Model of Multiple Sclerosis

Neelamma G*[1], Shaik Harun Rasheed[2], Kausar Sulthana[2], Nagarani B[2], Srilekha P[3]

[1]Department of Pharmacognosy, Srikrupa Institute of Pharmaceutical Sciences, Velikatta, Kondapak, Siddipet, Telangana, India
[2]Department of Pharmaceutics, Srikrupa Institute of Pharmaceutical Sciences, Velikatta, Kondapak, Siddipet, Telangana, India
[3]Department of Pharmaceutical Analysis, Srikrupa Institute of Pharmaceutical Sciences, Velikatta, Kondapak, Siddipet, Telangana, India

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ABSTRACT

Multiple sclerosis (MS) is a demyelinating ailment in which the loss of myelin destructs conduction along the affected axons typically resulting in the conduction block. The motive of this protocol is to carry out the neuroprotective activity of Artemisia vulgaris (Mugwort) by induced demyelination of ethidium Bromide in wistar rats. The leaf methanolic extract was treated at 100mg/kg and 200mg/kg weight administered through oral route and continued for 28 days in demyelinated rats. Demyelination was affected by administering intracranial injection of toxin (ethidium Bromide) at the dose of 1μg/0.03ml of PBS per kg body weight. The potency of the extract was analysedanalyzed in the terms of their behavioral study on the first, second and fourth week. The animals were immolate after 28 days and subjected to histopathological assessment. The unearthing from behavioral histopathological and biochemical studies evince that the methanol extract of Artemisia vulgaris have potential protective effect on the ethidium bromide induced demyelinated rats.

*Corresponding Author

Name: Neelamma G
Phone: +91-8099925838
Email: nanisony2012@gmail.com

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INTRODUCTION

Demyelinating disease (MS) is a condition in which the nerve of the CNS degenerate (Yang and Wang, 2001) myelin which provides a covering for nerves improves the nerve conduction of impulses and also important for maintaining the health of nerves in multiple sclerosis inflammation causes the myelin sheath damage. MS pathogenesis characterized by the presence within the CNS, of mononuclear inflammatory infiltrates inducing sporadic demyelination, axonal loss and astroglial scarring (Martino *et al.*, 2002; Lassmann *et al.*, 2001). The ethidium bromide is an intercalating toxin extensively induced demyelination in CNS (Graça *et al.*, 2001). Demyelination follows the disappearance of neuro glial cells (Pereira *et al.*, 1998; Bondan *et al.*, 2000) the degeneration of astrocytes makes a breach in the glial limiting membrane through which Schwann cells invade the CNS and repair the lost myelin sheath (Graca and Blakemore, 1986; Fernandes *et al.*, 1997).

Mugwort is often known as Artemisia Vulgaris belonging to family Astraceae. It is natural to

the temperate regions of Europe, North Africa, and Asia. It is wide spread throughout the world. It is an aromatic herbaceous perennial plant. The plant is widely distributed in different habitats from 0-1800m above sea level (Valant-Vetschera and Wollenweber, 2001) in traditional herbal medicine the Mugwort aerial parts are used as Anti-epileptic, Anti-depressant, Anti-spasmodic and Anti-helmintic (Duke *et al.*, 2002). The leaves are also said to be appetizer, diuretic, haemostatic and stomachic (Duke and Ayensu, 1985). Mugwort leaves contain phytoconstituents like flavonoids, polyphenolic compounds having free radical scavenging activity. The motive of this study is to assess the neuro protective effect of mugwort leaves on ethidium bromide induced demyelinated rats.

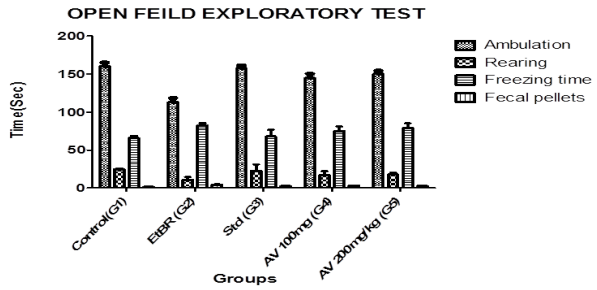

Figure 1: The effect of AV methanol leaf extract 200mg/kg and ethidium bromide on the open field exploratory test

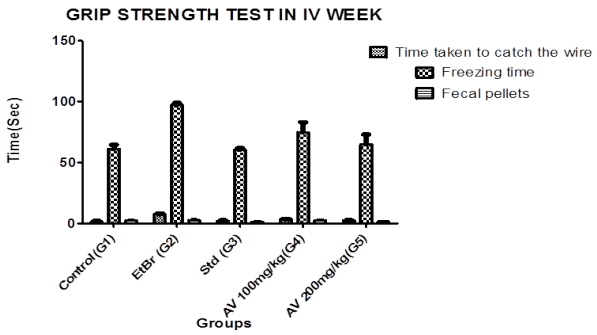

Figure 2: Grip strength test

MATERIALS AND METHODS

Plant Material

The fresh aerial part of mugwort leaves were collected in Telangana. The plant was identified and authenticated.

Preparation of Extract

Collected aerial parts of the plant were shade dried and coarsely powdered. The powder was extracted by using methanol. The extract was evaporated under reduced pressure by rotary vacuum evaporator.

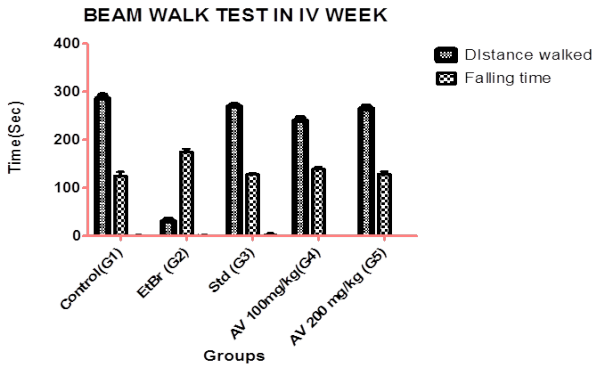

Figure 3: Beam walk test

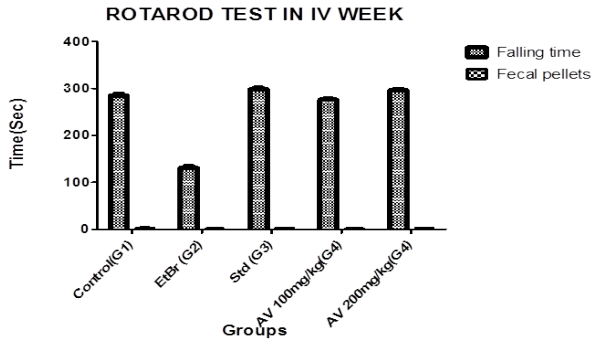

Figure 4: Rota rod test

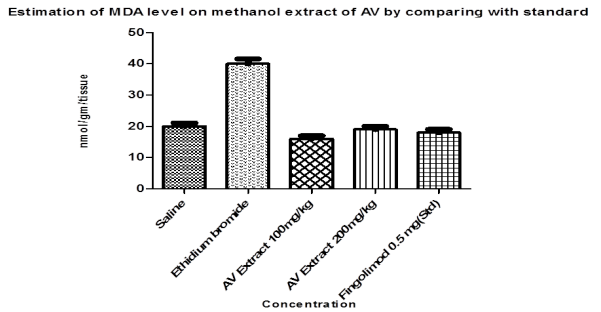

Figure 5: Effect of MDAactivity in cerebral cortex following methanol leaf extract of AV (200mg/kg)administration with toxin ethidium bromide

Collection and Maintance of Animal

Wistar rats weighing about 250 to 300g were collected for the experiment the animals were kept in cages under normal laboratory conditions. The animals were fed on standard balanced diet. The experimental animals were handled according to the guidelines of animal ethical committee Proposal No. JSSCP/IAEC/PH.D/PH.COG/03/2016-2017/JSSAHER.

Experimental Design

The wistar rats were grouped in to five each with a minimum of five animals and the administration of respective dosing was done regularly between 9 a.m

Table 1: Concentration of Malondialdehyde (MDA)

Sample Name	Concentration	nmol/gm/tissue
Methanol extract of Artemesia vulgaris leaves	Saline (0.9) %	25±1.06
	Ethidium Bromide (3μl)	40±1.53
	Extract 100 mg	29±1.21
	Extract 200 mg	21±0.95
Fingolimod (Standard drug)	Dose 0.5 mg	18 ± 1.02

Table 2: Effect on Superoxide Dismutase

Name of the sample	Concentration	mg/tissue
Mugwort leaf methanol extract	0.9% saline	31±1.31
	0.3 μL of Ethidium Bromide	48±1.25
	100 mg	36±1.02
	200 mg	28±1.06
FTY 720 (Standard drug)	0.5 mg	23 ± 1.22

Table 3: Effect mugwort leaf methanolic extract on TNF – α

Sample Name	Concentration	Pg/ml
Mugwort leaf methanol extract	0.9% saline	14.4±0.096
	0.3 μL of Ethidium Bromide	19.9±0.126
	Extract 100 mg	15.5±0.198
	Extract 200 mg	12.53±0.099
FTY 720 (Standard drug)	0.5 mg	10.5 ± 0.116

Table 4: Effect of Mugwort leaf methanol extract vs IL-6

Sample Name	Concentration	Pg/ml
Mugwort leaf methanol extract	0.9% saline	23.6±0.145
	0.3 μL of Ethidium Bromide	36.9±0.106
	Extract 100 mg	22.5±0.954
	Extract 200 mg	16.6±1.24
FTY 720 (Standard drug)	0.5 mg	15.1 ± 0.954

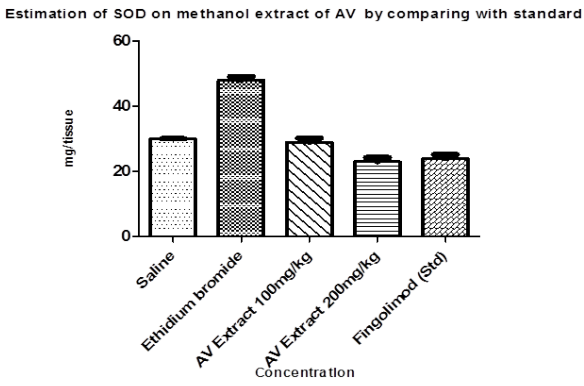

Figure 6: SOD activity incerebral cortex following methanol leaf extract of AV (200mg/kg)administrationwith toxin ethidium bromide

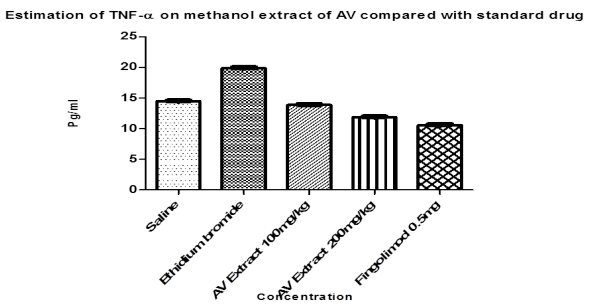

Figure 7: TNF-α activity in cerebral cortex following methanol leaf extract of AV(200mg/kg)administration with toxin ethidium bromide

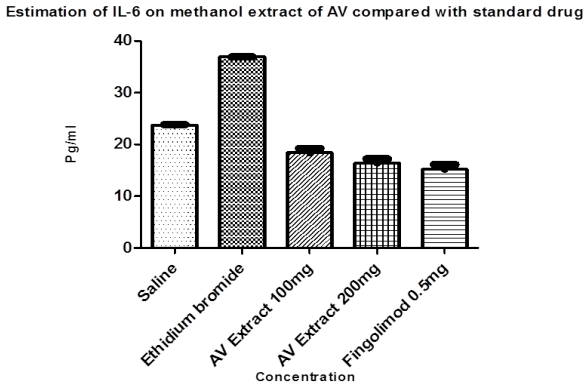

Figure 8: IL-6 activity incerebral cortex following methanol leaf extract of AV (200mg/kg)administration with toxin ethidium bromide

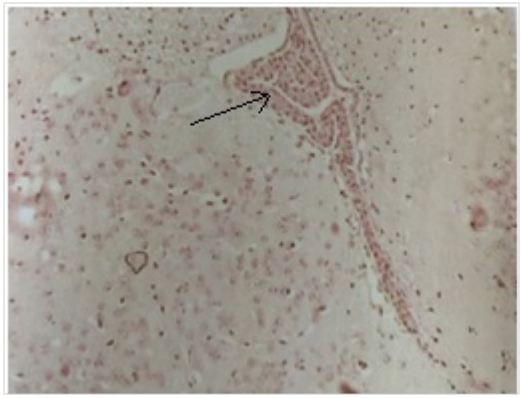

Figure 9: Control Group

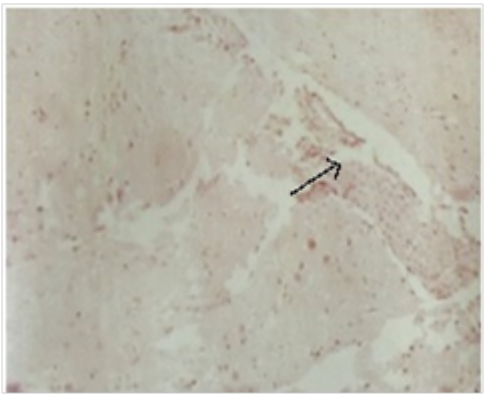

Figure 10: Induction Group

to 10 a.m with respective group.

Group I: solvent control group

Group II: toxin induced group (ethidium bromide 1μg in 0.03ml PBS through intra cerebral route)

Group III: ethidium bromide and Anti MS drug (FTY720)

Group IV: ethidium bromide and plant extract at the dose of 100mg/kg

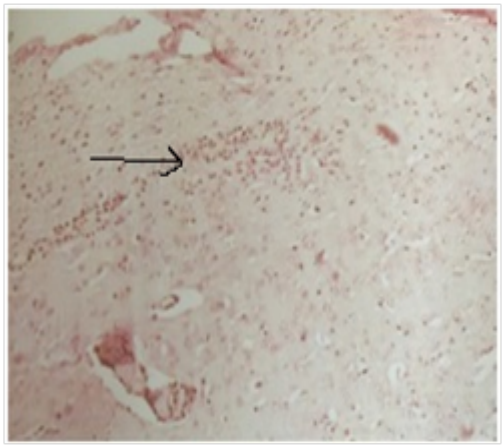

Figure 11: AV Extract 100mg/kg

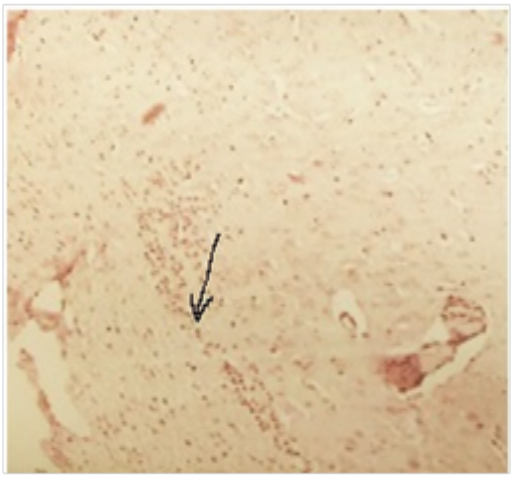

Figure 12: AV Extract 200mg/kg

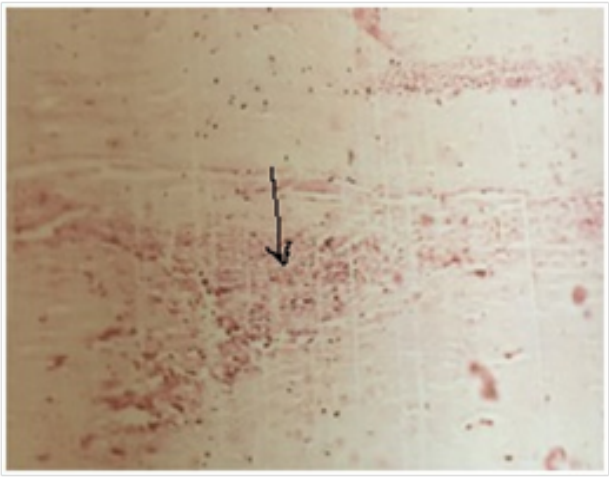

Figure 13: Fingolimod (Standard drug)

Group V: ethidium bromide and plant extract at the dose of 200mg/kg

Behavioral studies

Behavioural and locomotor measurements are important parameters that help to define the phenotype of animals with neuromuscular disorders. These assessments were done in open field, rotarod, grip strength and beam walk test.

Open Field Exploratory Behavior Test

This test is mainly focused to assess the anxiolytic and anxiogenetic activity of experimental animals. When these animals are exposed to the novel environment there is an immobilization of the disabled animal when compared to the healthy one. Dropped level of the anxiety is the reason for the immobilization and enhanced anxiety results in the better locomotion and performance (Ennaceur, 2014; Jaiswal and Bhattacharya, 1992).

Grip Strength

This test was carried out by determining the motor coordination of the animals using the string suspension task. The rats were allowed to hold a cotton string using forepaws and were shown the performance to move across the string was determined with hindpawshind paws and attempt is to climb on to string Ohkawa *et al.* (1979).

Beam Walk Test

This test is used to determine the motor coordination and the balance of experimental animal. This clearly examines the capacity of an animal to walk on the beam (Jawhar *et al.*, 2012).

Rota rod Test

This parameter is used to determine the motor performance and learning. In this the experimental animals were kept on the instrument, which is rotated from 4 to 40 rpm across the trail duration 300 sec. Trails are negligible when animals fell down from the instrument. Decreased fall time of the experimental animal is a clear indication of good motor coordination (Crusio *et al.*, 2013).

Histo pathological assessment

Histopathological study was carried out by separating the cerebral cortex from the mid brain and immersed in 10 percent formalin and were move forwarded by using conventional method, entrenched in paraffin, cut at 4-5 μm and heamotoxylin and eosin used as a staining agent. These tissues were focused using a light microscope. One part of the tissue was observed for Histopathological study whereas the other is retained for biochemical investigation.

Biochemical parameters

After behavioral examination the experimental animals were sacrificed by dislocation of cervical region, brain was dissected our and washed with normal saline and examined for biochemical parameters such as antioxidant and anti-inflammatory activities.

Anti-oxidant Activity

Determination of LPO

Lipid peroxidation levels in the brain regions were determined by the appropriate procedure. In this method 15 gram of trichloroacetic acid mixed with 100 ml of 0.25 N HCl. 15 mg of thiobarbituric acid was renederd in 4 ml of trichloroacetic acid in HCl mixture. To 0.1ml of homogenate, 0.4 ml trichloroacetic acid- thiobarbituric acid – Hydrochloride mixture was added and placed with boiling water bath for 20 min. Then let it cool to the room temperature naturally with the addition of 1ml of n-Butanol then make it centrifuge for 10 min. The supernant liquid measured at 532 nm spectrophotometrically (Ohkawa *et al.*, 1979).

Superoxide Dismutase (SOD)

The hippocampus homogenates was assessed using a method based on the potentiality of the enzyme to restrain the auto oxidation of pyrogallol. 1 ml of Tris HCl Buffer containing EDTA was mixed with 5 μl of homogenate supernant and was placed in the spectrophotometer. Then 50 μl of pyrogallol is mixed to this solution and the spertophotometrical absorbance was noted at 420 nm (Gao *et al.*, 1998).

Anti-inflammatory Activity

Pro inflammatory mediators like TNF- α, IL-6 were measure out by using ELISA kit. Mainly this kit is used with regard to its accuracy, specificity, inter and intra assay precision, and only a minute quantity of tissue is used to carry out the method.

Statistical Analysis

The statistical data is demonstrated as mean $\pm$ SD were evaluated by one way analysis of variance followed by 't' test. Values of P$\geq$0.01 were considered. Graph pad prism version of 5.0 was used for graphical and statistical evaluation.

RESULTS AND DISCUSSION

Behavioural Tests

The protective effect of mugwort leaf methanol extract was determined on ethidium bromide induced demyelinated rats. The results reveal that there is an enhancement of muscle strength and motor coordination. The protective effect of

mugwort leaves at a dose of 200 mg/kg has shown significant protective effect in managing the motor neuron. The active constituents in the leaves of mugwort has the neutralization effect on the toxins. The results are mentioned in Figures 1, 2, 3 and 4.

Biochemical parameters

In this examination, the antioxidant activity is determined with the help of tissue originated from the cerebral cortex. It has been clearly determined that the mugwort leaf methanolic extract dose at 200 mg/kg has been revealed the protective effect in comparison with the toxin induced group. The results are mentioned in (Tables 1 and 2) and (Figures 5 and 6).

Antioxidant activity

Malondialdehyde (MDA)

Superoxide dismutase (SOD)

Inflammatory Activity

The pro inflammatory mediators such as TNF- α and IL-6 were used to measure the plant extract effect in order to inhibit the inflammation over the neurons. The results were tabulated in (Tables 3 and 4) and (Figures 7 and 8).

TNF- α

IL – 6

Histopathology

Within this investigation, group IV and V has shown significant protective activity treated with mugwort leaf methanolic extract. Group V has shown remarkable protective effect when treated with a dose of 200mg/kg mugwort leaf methanolic extract compared with ethidium bromide induced group.

The experimental rat brain was exposed to the mugwort leaf methanolic extract at a dose of 100mg/kg and 200mg/kg for a period of 28 days, the histopathological lesions were observed. Repeated treatment of the demyelinated animal to the plant extract confirms the protective activity and a chance of reduction in the lesions confirms the neuroprotective activity. The results were mentioned in (Figures 9, 10, 11, 12 and 13).

Histopathological lesions were notified in the brain of wistar rats upon exposed to mugwort leaf methanolic extract at a dose of 100mg/kg and 200mg/kg for a period of 28 days. Various observations such as vascular degenerative necrosis, degeneration of astrocytes and oligodendrocytes of have been observed when experimental animal brain in mugwort extract compared to the standard. In the present experiment the maximum severity occur in the histopathological lesions indicate that the exposure of rats repeated that plant extract causes various hazardous effect and it make them less chance for better existence. Experimental results are consistent with similar necrotic lesions observed upon exposure of rats to ethidium bromide induced demyelination. The similar study on animal histopathological changes by certain toxic xenobiotc has been reported (Savory and Garruto, 1998). Now experimental results were in comparative with results with the degeneration of neurons and degenerative diseases of neurons associated with aluminium (Singson and Bawari, 2016).

In this study the brain tissue of animal has shown as changes in histoarchitecture and necrosis in the cerebral cortex when compared to the standard. In the behaviouralbehavioral study decrease anxiolytic and anxiogenic activity is observed in open field test and increased muscle strength and muscle coordination was observed in grips strength and rota rod test. The beam test has shown increased in the distance of walk to the exposed animals. Therefore the present has given demonstration that the impairment of locomotor activity induced by ethidium bromide micro injection in various in the toxic modal of multiple sclerosis is improved by Artemisia vulgaris extract in a dose dependent manner. This finding agree with previous study showing that artemisia vulgaris extract amilatory impairment of locomotor activity in a variety of conditions (Ghadrdoost *et al.*, 2011; Dashti-R *et al.*, 2012; Hosseinzadeh *et al.*, 2011) this observed of effects may be due to the antioxidant anti inflammatoryanti-inflammatory effect of artemisia vulgaris leaf extract. Artemisia vulgaris extract contains flavanoids, polyphenols and essential oil with powerful antioxidant and anti inflammatoryanti-inflammatory activity. Which protect CNS neurons from oxidative damage and inflammation by the preservation of cell redox status and energy metabolism (Zheng *et al.*, 2007; Del-Angel *et al.*, 2007).

The outcome of the investigation states that micro injection of the Artemisia vulgaris leave extract for the 28 days in the toxic models of multiple sclerosis. Redefined the oxidative damage and inflammation induced by ethidium bromide and reinstated significantly the mentioned biochemical parameters. Moreover the experimental findings of Artemisia vulgaris leaf extract redefined the oxidative stress and inflammation induced by administration of Artemisia vulgaris leaf extract in experimental rats. It provides the possible evidence of therapeutic effect of Artemisia vulgaris leaf extract in neuro degenerative disorders. Which were identified by

oxidative stress and inflammation which are major complications.

CONCLUSION

Finally it can be concluded that the Artemesia vulgaris leaves methanolic extract that can be restored ethidium bromide induced effect in locomotor activity, oxidative stress and inflammation in the hippocampus. Thus this phytoconstituents can be used as new pharmacological activity entity to study the mechanism the oxidative study inflammation for elevating locomotor defects. In fact these findings suggest that Artemisia vulgaris leaf methanolic extract as might have effective activity in oxidative stress and inflammation could be suggestive therapy for neuro degenerative diseases like Multiple sclerosis [MS].

Conflict of Interest

None

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