

ANTICONVULSANT AND MUSCLES RELAXANT ACTIVITY OF DENDROPTHOE FALCATE LEAVES

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Abstract: To investigate the anticonvulsant and muscle relaxant activity of ethanolic extract of leaves of *Dendrophthoe falcata* in mice. The ethanolic extract of leaves of *D. falcata* (100, 300 and 500 mg/kg, p.o.) was studied for its anticonvulsant effect on maximal electroshock-induced seizures and muscle relaxant activity at the same dose level using rota rod and actophotometer in mice. Preliminary phytochemical analysis revealed presence of proteins, carbohydrates, glycosides, steroids, triterpenes, flavonoids, tannins and phenolic compounds. *D. falcata* ethanolic extract (DFEE) (100, 300 and 500 mg/kg, p.o.) significantly ($P < 0.001$) inhibited seizures induced by MES, reduced the duration of Hind limb tonic extensor phase (HLTE) and a decline in motor coordination. The ethanolic extract possesses anticonvulsant activity and muscle relaxant activity.

Key words: Anticonvulsant, Muscle relaxant, *Dendrophthoe falcata*.

Introduction: Epilepsy is a involving of neurological problem or issue described by epileptic seizures, as per ‘Chang BS, Lowenstein DH (2003), Wiebe S (April 2014)’. In epilepsy, seizures probably repeat and, have no moment fundamental reason. Disengaged seizures that are incited by a particular reason, for example, harming are not esteemed to speak to epilepsy as indicated by ‘Fisher R(2005)’.

A muscle relaxant may be a drug that affects skeletal muscle perform and reduces the tonicity. it should be wont to alleviate symptoms corresponding to muscle spasms, pain, and hyperreflexia. The muscle relaxant term is employed in to 2 major therapeutic groups: neuromuscular blockers and spasmolytics. neuromuscular blockers act by officious with transmission at the neuromuscular finish plate and haven't any central systema nervosum (CNS) activity!

Dendrophthoe falcate is known as Vanda, Bnda, and Vriksha. Hemi parasitic plants of *D. falcata* are belong to the family of (Loranthaceae) mistletoes, “Flora of China”, (2003) Flavonoids are the major constituent of *D.falcata*. Difference type of flavonoids precence in the host plant of parasite. *D.falcata* mainly contain the quercitrin “Dr.B.K. Prashanth”. *Dendrophthoe falcate* are used in the wound healing and antimicrobial. The other use like antioxidant and antinociceptive properties of its ethanolic extracts, reported by “Pattanayak and Sunita”, (2008), “Shihab et al”., (2006) The hemiparasite plants are presence of different type of medicinal properties, reported by “Mallavadhani et al”, (2006). In this study, investigate the anticonvulsant and muscle relaxant activity in ethanolic extract of leaves of *D.falcata* in rat.

Materials and methods:

Plant Material: The fresh leaves of *D. falcata* was collected from the month of December 2016 from District Allahabad, U.P. The plant material was authenticated by “Dr.G.P. Sinha”, Scientist-E\Head of Office, Botanical survey of India, CRC, Allahabad (U.P.) for future reference Accession No. 98882.

Drugs and chemicals: The standard drugs phenytoin and diazepam obtained from Central drug house (CDH) Pvt. Ltd, new Delhi. The plant extract of *D. falcate* leaves, Phenytoin and Daizepam was dissolved in polyethylene glycol.

Preparation of plant extract: Plant leaves was air-dried, crushed to a fairly coarse powder. The powder (aprox. 200 gm) leaves was packed in soxhlet apparatus and continuously extracted with ethanol (95%) at 60°C. Dried leaves powder (200gm) was placed in a stoppered flask with 1200 ml distilled water and allowed it to stand for a period of 7 day in a warm place with , soluble matter is dissolved by regular stirring. The extractive material is filter then the liquid is drainage, the residue on to produce 1000 ml. Aqueous extract and ethanolic extract was concentrate to give a semisolid mass then store in desiccators. The % yield of *Dendrophthoe falcate* ethanolic extract (95%) (DFEE) and *Dendrophthoe falcate* aqueous extract (DFAE) was found to be 10.2% and 12% respectively.

Preliminary phytochemical study: Preliminary study was performed in the ethanolic extracts of the leaves of *Dendrophthoe falcate* was presence of various phytoconstituents such as alkaloids, glycosids, flavonoids, steroids, fixed oil, phenolics, and tannins.

Animals: Male Swiss albino mice, weighing 20-25gm healthy, adult Albino Wistar rats weighing 150-210 gm of either sex were purchased from the CDRI Lucknow used for study. The animal was placed in individual cage, and maintain the environmental condition (12 hours day and night cycle; $23\pm 2^{\circ}\text{C}$, $50\pm 5\%$, relative humidity), they were fed of standard animal capsule diet and were ad libitum. The experimental protocol was accepted by the "Institutional animal Ethical Committee" (IAEC) bearing RegdNo.SIP-IAEC/008/09/16.

Acute toxicity study: Acute toxicity studies was carryout on Wistar rats/albino mice according to OECD – 425 guidelines. The animals were kept fasted for 6 hours with free administer to water. The ethanolic extract of leaves of *D. falcata* was administered orally at a dose of 45 mg/kg. If mortality were done in 2 animal out of 3 animal to show the drug is toxic in nature. Therefore, no mortality was shows, then the procedure was continous carryout with higher dose such as 100, 150, 300, 600, 1200, 3000, 4000 and 5000 mg/kg body weight. Toxic observation such as atypical motor activity, changes in water/ food intake, respiration are affected and sedation are common, were observed at 6 hours and mortality for 24 hours. The LD_{50} was assess in Wistar rats/albino mice and toxicity was found to be 5400 mg/kg.

Assessment of Anticonvulsant Activity:

Maximal electroshock model: MES model are used for the determination of the anticonvulsant activity. The electrode are used for the flow of electric current to stimulate the rat. The current shock 50 MA for 0.2 second delivered to rat then induced the phase of seizure.

- Group I was treated with polyethylene glycol 10 ml/kg body weight.
- Group II was treated with Phenytoin 25 mg/kg (i.p.) body weight.
- Group III was treated with 100 mg/kg (orally) of DFEE, dissolved in polyethylene glycol.
- Group IV was treated with 300 mg/kg (orally) of DFEE, dissolved in polyethylene glycol.
- Group V was treated with 500 mg/kg (orally) of DFEE, dissolved in polyethylene glycol.

Assessment of Muscle relaxant Activity:

Rota rod test: The motor coordination activity was perform by using of rotarod . The test was carryout on five groups of five mice each. Mice was traind on the rotarod for 5 minute before treatment, the speed of rotarod was 25 rpm. The *D. falcata* extract were administered 100, 300, 500 mg/kg after 60 minute and administration of diazepam 4 mg/kg by intaperitoneal.

Actophotometer: In the locomotor activity, every animal (rat) kept in the actophotometer apparatus individually for 10 minute time interval. The basal activity score for all the animals was noted.

- Group I was treated with polyethylene glycol 10 ml/kg body weight.
- Group II was treated with Daizepam 4 mg/kg (i.p.) body weight.
- Group III was treated with 100 mg/kg (orally) of DFEE, dissolved in polyethylene glycol.
- Group IV was treated with 300 mg/kg (orally) of DFEE, dissolved in polyethylene glycol.
- Group V was treated with 500 mg/kg (orally) of DFEE, dissolved in polyethylene glycol.

Statistical analysis:

Result was analyzed by one way ANOVA followed by Tukey's multiple comparison test using Graph pad prism was applied for determining the statistical significance between different group. The result was judged significant, if $P < 0.05$, $P < 0.01$ and $P < 0.001$

Results:

Physiochemical parameter: Phytochemical parameter such as ash values, extractive value, and loss of drying were determined on the powdered leaves of *Dendrophoe falcata* and results were presented in below the **Table-1**

S.No.	Parameters	Weight (in gm)
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1.	Ash value Total ash value Acid soluble ash value Acid insoluble ash value Water soluble ash value Water insoluble ash value	8% 5% 3% 7.5% 7%
2.	Extractive value Water soluble extractive value Alcohol soluble extractive value	9% 7%
3.	Loss on drying	4%

Preliminary phytochemical screening of leaves of *Dendrophoe falcate*: Preliminary study was performed in the various extracts of the leaves of *Dendrophoe falcate* and the presence of various phytoconstituents such as alkaloids, glycosids, flavonoids, steroids, fixed oil, phenolics, and tannins were determined and the results were presented in **Table-2**

Phytoconstituents/Extracts	Ethenol	Aqueous
Corbohydrates	+	+
Alkloides	+	-
Glycosides	-	-
Flavonoids	+	+
Steroids	+	+
Phenolics and Tannins	+	+
Proteins	-	-

'+' = Presence - = Absences

Anticonvulsent activity(MES Model)

Maximal electroshock produced extension seizures In all the control animals. For duration of 35 ± 2.54 sec. DFEE at 100, 300 and 500 mg/kg significantly ($p < 0.01$ and $p < 0.001$) reduced the duration of the seizures as compared to control group. Phenytoin completely inhibited the MES-induced tonic seizures in all the animals. **Table-3**

Group	Treatment/ Dose(mg/kg)	Time(in sec.) in various phase of convulsion				
		Flexion	Extensor	Clonus	Stupor	Recovery /Death

1	10 ml/kg	29.2±1.9235*	35±2.5495*	41.4±3.9115	52.4±5.3197*	R
2	25ml/kg	30.4±13.0115	30.6±15.4854**	24.6±11.0815	48.6±15.4369*	R
3	100mg/kg	30.6±1.1401	33.4±2.5099**	31.2±8.4083	31.4±5.5045***	R
4	300mg/kg	23.8±1.7888*	23±1.5811*	23.4±1.3416	22.4±1.1401*	R
5	500mg/kg	52.4±2.0736**	54.2±2.1679*	54.8±1.4832	53.4±2.4083	R

The data was represented as Mean± SD 5 rats per group *p<0.05, **p<0.01, ***p<0.001.

Musclerelaxent activity

The ethanolic extract decreased the locomotor activity in rat at all doses. The mean time duration on rotating rod was significantly decreased ($P<0.001$) in animals treated with DFEE, indicating muscle relaxant activity and decline in motor coordination.

Table for muscle relaxant activity on Actophotometer(Model-1) Table-4

Treatment/dose mg/kg	Locomotor activity count/10 minute	
	Before treatment	After treatment
Control	994.8±46.1919**	844.8±68.1997***
Daizepam (4mg/kg)	633.2±18.8334*	204.6±29.9048***
DEEF(100mg/kg)	850.6±42.0689	462.8±156.9497***
DEEF(300mg/kg)	446±80.8300*	155.6±77.4390
DEEF(500mg/kg)	882±64.2238*	650.6±306.3287***

The data was represented as Mean± SD 5 rats per group *p<0.05, **p<0.01, ***p<0.001

Table for Relaxant activity on Rota rod (Model-2) Table-5

Treatment/dose mg/kg	Rota rod activity count/10 minute	
	Before Treatment	After Treatment
Control	22.8±4.3243*	8.6±2.6076
Daizepam (4mg/kg)	19.6±2.7018*	5.2±1.9235**
DEEF (100mg/kg)	18.2±5.4954*	9.4±1.9493

DEEF (300mg/kg)	21.6±1.1401*	7.8±2.8635
DEEF (500mg/kg)	19.2±2.4899*	7.2±2.8635

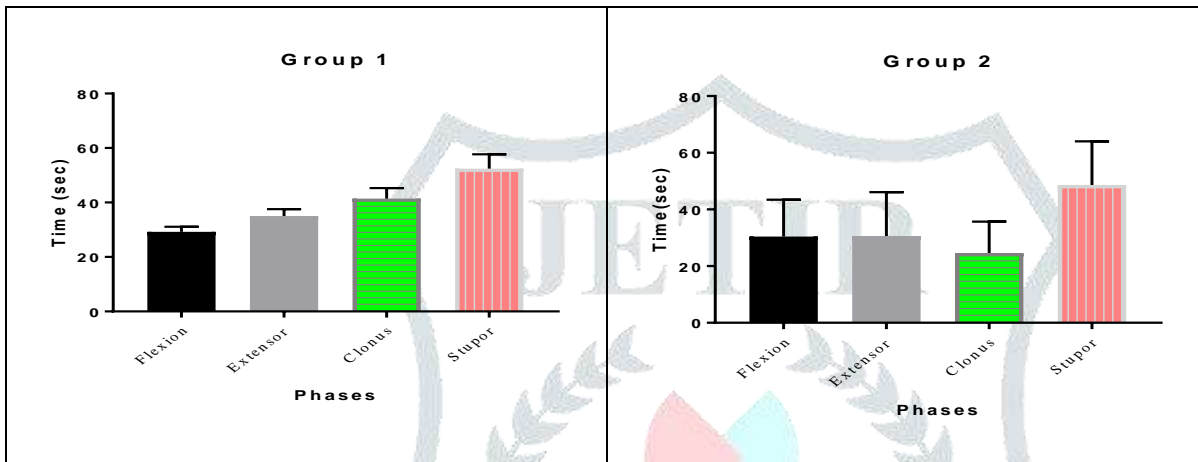
The data was represented as Mean± SD 5 rats per group *p<0.05, **p<0.01, ***p<0.001

Graph:

Anticonvulsant activity

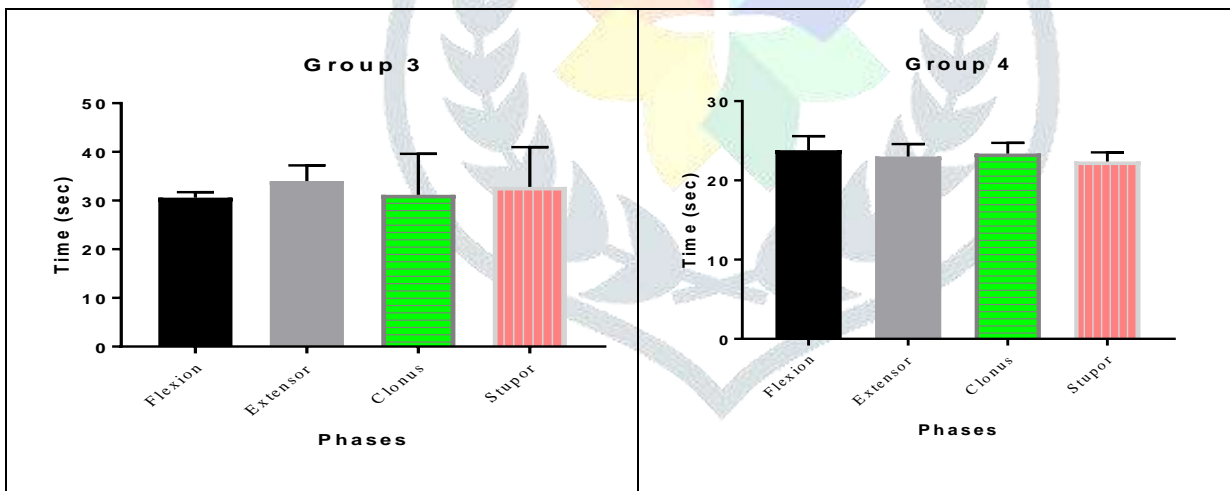
Graph-1.(Control grp)

Graph-2.(standard grp)

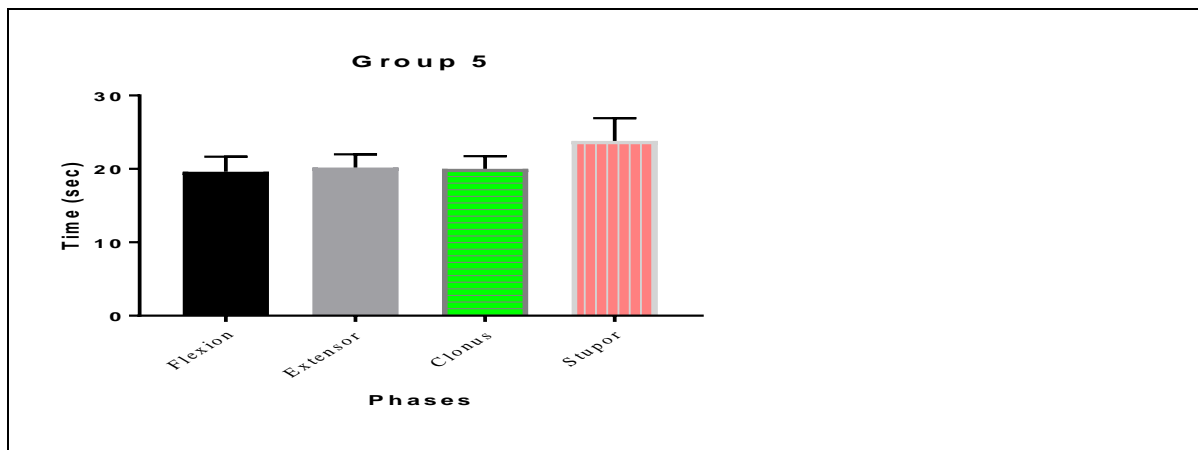


Graph-3.(Test-1)

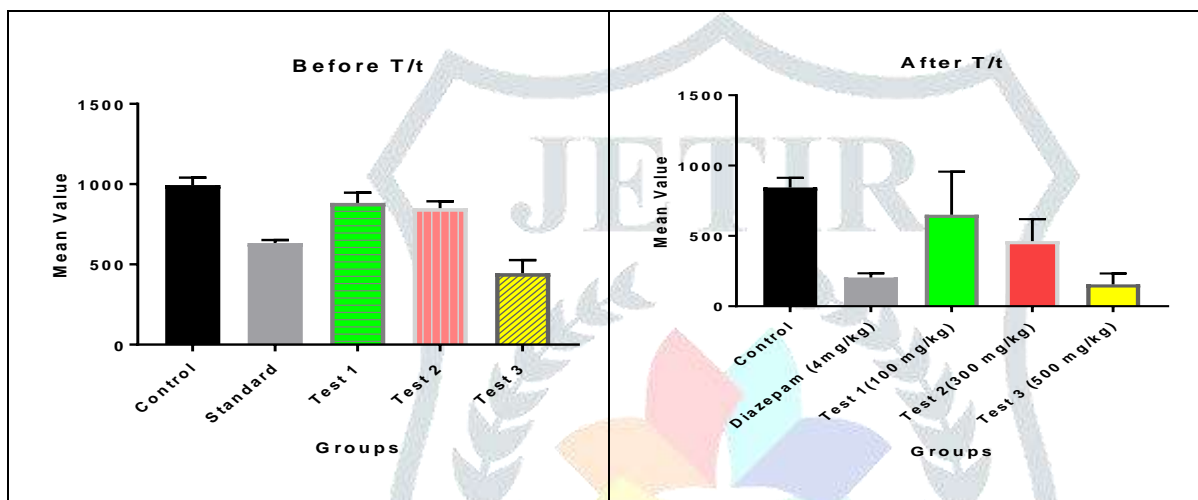
Graph-4.(Test-2)



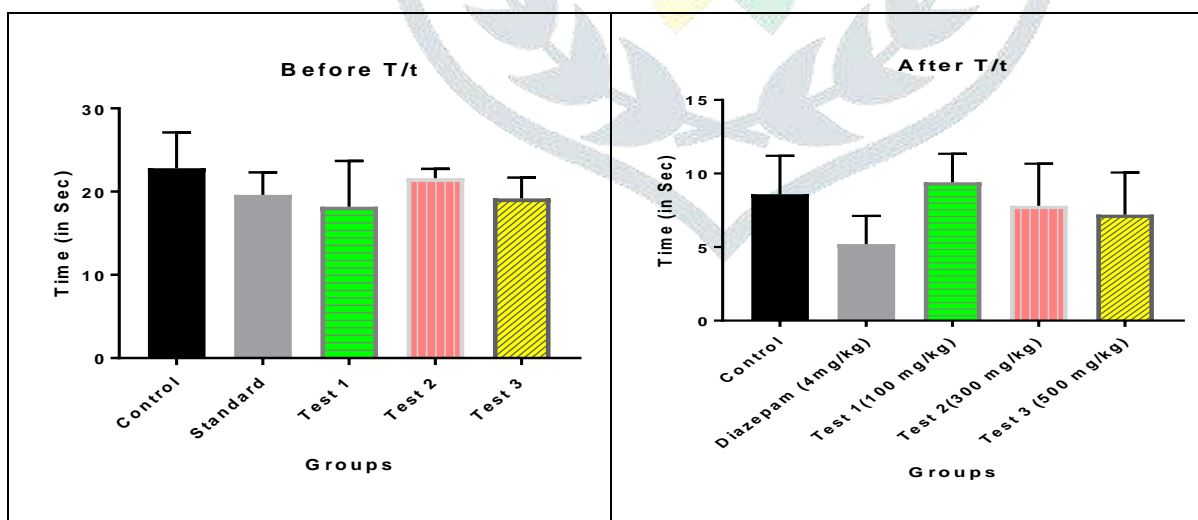
Graph-5.(Test-3)



Actophotometer activity Graph 6 & 7



Rota rod activity: - Graph 8 & Graph 9



Discussion:

After the whole analyzing process, the conclusion points of this study is, the ethanolic extract of *D. falcate* administered the dose at 100 mg/kg, 300 mg/kg, 500 mg/kg to mice, to reduce the motor coordination activity, and anticonvulsant activity observed in mice.

In this my study, the anticonvulsant and the muscle relaxant activity evaluated with the help of different type of model. MES model is applied for the evaluation of ethanolic extract of *D. falcata* efficient for the convulsion. Other applied study, the muscle relaxant activity using the rotarod and actophotometer for the evaluation the extract of *D. falcata* effective for muscle spasm.

In the evaluation process of anticonvulsant and muscle relaxant, both the study were conducted in the ethanolic extract of *D. falcata*. Ethanolic extract of *D. falcata* administered dose statistically significant ($p < 0.01$ and $p < 0.001$) are reduced the duration of convulsion. And in the other side, the muscle relaxant activity significantly decrease ($p < 0.001$) the motor coordination activity in mice.

So that, it can be discuss for both activity (anticonvulsant and muscle relaxant) are presence in the leaves of *D. falcata* and is responsible for the decline the effect of muscle relaxant and anticonvulsant, data show in the table no.3,4,5 and graph-1-5 for anticonvulsant activity and graph 6-9 for muscle relaxant activity

Different type chemical constituent such as terpenoids, and flavonoids is responsible for the muscle relaxant and anticonvulsant action are presence in *D. falcata* by perform the phytochemical study, show the table no.2

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