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ANTIEPILEPTIC ACTIVITY OF HYDROETHANOLIC EXTRACT OF *THALICTRUM FOLIOLOSUM* ON MAXIMAL ELECTROSHOCK (MES) AND PENTYLENETETRAZOLE (PTZ) INDUCED SEIZURE IN RATS

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ABSTRACT

The present study was an attempt to investigate of antiepileptic activity of hydroethanolic extract of *Thalictrum foliolosum* (HEETF) belonging Ranunculaceae family. *T. foliolosum* is well known plant which is being used to Indian traditional medicine for treating different ailments like malaria, epilepsy, neurotonic and jaundice etc. Wistar albino Rats either sex (n=6), the animals treated with different doses of HEETF extracts 300, 400, 500 mg/kg ip. Injection of Diazepam 4mg/kg ip as positive control, whereas other group injected vehicle as normal saline 1ml/100gm for 7th days. On the 7th day administered PTZ 110mg/kg ip were injected. Determine behavioral responses of all animals when PTZ were administered evaluated antiepileptic response using following criteria, like onset of convulsion, duration of convulsion, protection against GTCS and mortality. Effects of HEETF on MES Induced Epilepsy, at doses of 300, 400 and 500 mg/kg were protect animals from seizures and significantly 300 mg/kg, 400 mg/kg and 500 mg/kg have shown 49.8%, 66.4% and 83% protection respectively. Intraperitoneal administration of lower dose of hydroethanolic extract of *T. foliolosum* (200 mg/kg) had no potent effects on generalized tonic-clonic seizures (GTCS), while injection of 300 & 400 mg/kg caused significant increase in GTCS latencies (p<0.01). In this study diazepam, (4 mg/kg) 1 hrs prior to PTZ, significantly increased GTCS latency. *T. foliolosum* at test doses protection against mortality following PTZ administration. Experimental finding reveal that hydroethanolic extract of *T. foliolosum* could be a useful protective antiepileptic agent. However further study still needed to be causes on exposure of extract to human beings.

Keywords: Pentylene tetrazole, Phenytoin, Meprobamate, MES, *Thalictrum foliolosum*, Antiepileptic, Pilijari, GTCS, Diazepam.

INTRODUCTION

Epilepsy is a brain disorder including unpredictable and recurrent tendency of seizures that interrupt normal brain functioning [1]. Epilepsy is one of the most common neurological clinical- pathological disorders that affect about 15% of the general population of India. Which have abnormal electrical discharge of neuronal circuit due to abnormal sensory, motor and psychomotor experiences in central nervous system, seizures occur because a group of excessive cortical neurons discharge, abnormal in ascending as well as

descending tract of corticospinal pathways [2]. Seizure can be controlled with current therapies however the treatment option which are available now a days for treating seizure is only preventive not curable. Anti-seizure drugs have low neuroprotective activity or their side effects which are the outcomes of long therapy overcome their therapeutic benefits. Therefore considerations now focus on neuroprotective effects of various components. Considerable numbers of clinical and epidemiological studies reveal that about one third of adult patients (20-

30%) are suffering from epilepsy but they do not respond to drug therapy or surgical treatment hence comes under resistant type epilepsy [3]. Anything that disturbs the normal homeostasis or stability of neurons can trigger hyper excitability and seizures.

Experimental animal models are beings used in order to explore basic mechanisms underlying epilepsy and to invent new anticonvulsant drugs, since intracellular recording of intact human brain. There are many etiological factors like liver disease [3], chronic injury at central nervous system due to severe glucose deficiency, major electrolyte disturbance, severe hypokalemia and other medical condition may involve in the epilepsy like genetic mutations, chromatic brain injury [4], intracerebral artery pressure elevation due to high blood pressure, genetic predisposition to seizures has been seen in many ways of primary generalized epilepsy. Mental retardation of patient, cerebral trauma, necrotic plaques in the CNS, or strokes are at an enhance risk tool for seizures. Mental retardation frequently has been determined by the intelligence quotient [5]. In the elderly, the cause of focal neuronal injury is typically depending on stroke, neurodegenerative disorders. For example Alzheimer's disease and other conditions like excitotoxicity in neuronal circuit many neurotransmitters are involve in abnormal function of the brain.

With over 10 billion of neurons and an estimated connection of synapse and axonal transportation in the human brain basis for uncontrolled automaticity complexity unlike myocardial tissue where electrical signal spared through a syncytium of cells proper functioning of the brain requires distinct isolation electrical signal and thus demand of a fighter level of regulation [6]. A seizure can present with a variety of symptoms and result from of a various cause.

Pentylenetetrazole come under the categories as CNS stimulant epileptogenic properties have been used to find out antiseizure phenomenon of herbal drugs with high efficacy and to identify pharmaceuticals entities that may control seizure susceptibility [7]. Basic mechanism of PTZ is that a non-competitive antagonize by GABA, the antagonize action of PTZ on inhibitory neurotransmitter as GABA can lead excitotoxicity of neuronal cells and spontaneously depolarization of after administration of PTZ rats are observed for seizure episode ranking and determine of latencies and onset of convulsion and duration of convulsion as well as general tonic clonic seizure was determined with the help of PTZ

Thalictrum foliolosum DC authorized name in The Plantlist and synonyms is *Thalictrum dalingo* Buch – Hem ex DC identified by Botanical survey of India Dehradun, Uttarkhand, have worldwide distribution in both tropical and temperate regions including Asia, Africa but leads mainly in mountain area of the South-West of Uttarakhand in India where it is known under the local name of Pilijari in Garhwal region and Nagaguining

Meadow rue and Mamira in Kumaun region [8]. HO where it is widely distributed in climatically moderate zones of Northern Hemisphere [9], it is a slow growing, dump forming, rhizomatous perennial plant a traditional herb which found wildy in the forest region of eastern and western India. It is a sub erect, tall prickly herb, heavily branched which rise up to 0.9-1.2 m in height [9]. Stem is branched smooth and pale due to this reason it is known as Pilijari [10]. The cladodes are thread like and soft many frequent, 5-20 filiform, straight, ascending or recurred or erect 8-12 mm long. The spines are straight thorns measuring 1-1.5 cm in length. Traditionally used for epilepsy [11], jaundice [12], while antipyretic [13] antimalarial [14] Diuretics, antivenom, dyspepsia, febrifuge [15] and as antimicrobial agent are scientifically validated [16].

The main target of the study was evaluation of antiepileptic potential of *T. foliolosum* by using Maximal Electroshock seizures and Pentylenetetrazole (PTZ) Induced seizure in wistar albino rats.

METHODOLOGY

Drugs and Chemicals

Diazepam and PTZ was purchased from Yarrow Chem product Mumbai, India. The solvent and other chemical was used of analytical grade. The whole plant *T. foliolosum* was collected during September to October 2015 from the wild region of Rudraprayag, Uttarakhand, India, and authenticated by botanist Professor S. K. Srivastava Botanical Survey of India, with a specimen number 115900.

Preparation of Hydroethanolic extract

The whole plant shade dried at room temperature and extracted with 40:60 distilled water with ethanol 95% v/v for 48 hrs using hot soxhlation method and extract was dried at 50 °C on water bath the percentage yield formula [17]. The formula is Percentage yield = $(M_2/M_1) \times 100$; where M2 is the mass of the semi-solid portion of ethanol extract and M1 is the mass of dried leaves prior to extraction. M2 = 40.79 g and M1 = 620.13 g. Substituting the given values: Percentage yield = $(81.23 \text{ g} / 720.12 \text{ g}) \times 100 = 11.28\%$. Therefore, the percentage yield of *T. foliolosum* hydroethanolic extract is 11.28. A preliminary phytochemical Screening was done by methods as described by Joshi and sati [18].

Animal

Wistar albino rats (120-180) of either sex obtained from, Devsthal Vidyapeeth college of Pharmacy, Lalpur, Rudrapur, Uttarakhand was procured from IVRI Bareilly. Rats are acclimatize for 10 days, under a room temperature of $24 \pm 2^\circ\text{C}$ relative humidity 45-55% with 12:12 hrs light and dark cycle. The animals had free accesses to food (Ashirivad food industry Mohali, Chandigarh) and water ad libitum. The animals had

habituated to laboratory condition for 48 hrs prior to the experimental protocol to minimize the non specific stress. The institutional animal ethics committee of Devsthal Vidyapeeth College of pharmacy Rudrapur, Uttarakhand, India, approved the experimental protocol in accordance with the guideline provided by committee for purpose of control and supervision of experimental on animals (CPCSEA) with the registration no 1452/PO/a/11/CPCSEA.

LD₅₀ Determination

Acute toxicity study [12] Research Laboratory, Department of botany, KV Phandarkar College Maharashtra as per the procedure given in OECD Guideline No. 420 on male wistar albino rats (150-200 gm). The whole plant of *T. foliolosum* at the dose of 2 gm/kg body weight was given to 6 animals and was continuously observed for 14 days for mortality and general behavior. No death was observed till the end of this study. The plant extract was considered safe up to the dose of 2 gm/kg body weight. From the results, test extract dose of 300, 400 and 500 mg/kg body weight was chosen for the efficacious studies.

Experimental design

Effect on Maximal electroshocks (MES) induced seizures

Albino wistar rats of either sex weighing 120 to 180 gm were divided into five groups of six animals each. The first group administered vehicle as normal control (normal saline 1ml/100 g) whereas Group-II received standard drug Phenytoin, 25mg/kg ip, Group-III, IV and V received hydroethanolic extract of *T. foliolosum* (HEETF) 300, 400 and 500 mg/kg body weight ip respectively for 7 days. On the 7th day, Seizures are induced to all the groups by using an Electro convulsimeter. Maximal electroshock seizures were elicited by a 60 Hz alternating current of 150 mA intensity for 0.2 sec. Single drop of 0.9% NaCl solution with anesthetize using lignocaine was applied to the corneal electrodes prior to application in rats. This increases the contact and reduces the incidence of fatalities. The latency or duration of various phases of convulsions was observed the percentage protection was estimated by observing the number of animals showing abolition of Hindleg Tonic Extension (or) extension not greater than 90°.

Effect on Pentylene tetrazole (PTZ) induced seizures

Albino wistar rats of either sex weighing 120 to 180 gm were divided into five groups of six animals each. The first group received vehicle normal saline 1ml/100 g

as positive control whereas Group-II received standard drug Diazepam 4mg/kg intraperitoneally, Group-III, IV and V hydroethanolic extract of *T. foliolosum* 300, 400, 500 mg/kg body weight respectively for 7 days. On the 7th day, Pentylene tetrazole (PTZ) 110 mg/kg body weight, ip was administered to all the groups to induced seizure (general tonic-clonic convulsions). Animals were observed for a period of 30 min post PTZ administration. The parameters noted were mean onset time of convulsions, duration of convulsion and recovery /Death (% recovery or % of survival) due to PTZ.

Statistical Analysis

In animal study, the data are expressed as mean \pm SD. For statistical analysis data was subjected to analysis of variance (ANOVA) by using Graph Pad Instat. Values are considered statistically significant **P<0.01 (n=6).

RESULT AND DISCUSSION

Phytochemical analysis

The HEETF revealed the presence of steroids, alkaloids, reducing sugars, tannins, gums, flavonoids shown in table no.1.

In vivo antiepileptic activity

Effect of HEETF on Maximal electroshocks (MES)

Effects of HEETF on MES Induced Epilepsy at doses of 300, 400 and 500 mg/kg were protect animals from seizures and significantly (p<0.01) reduced the duration of tonic hindleg extension. With dose and concentration dependent manner whereas the standard drug phenytoin treated animals exhibits abolished tonic hindleg extension. Phenytoin treated animals have shown 100% protection against MES induced seizures whereas HEETF 300 mg/kg, 400 mg/kg and 500 mg/kg have shown 49.8%, 66.4% and 83% protection respectively shown in table no 2.

Effect of HEETF on Pentylene tetrazole (PTZ) Induced epilepsy

Intraperitoneal administration of lower dose of hydroethanolic extract of *T. foliolosum* (200 mg/kg) had no potent effects on generalized tonic-clonic seizures (GTCS), while injection of 300 & 400 mg/kg caused significant increase in GTCS latencies (p<0.01). In this study diazepam, (4 mg/kg) 1 hrs prior to PTZ, significantly increased GTCS latency. Diazepam treated animals have shown 100% protection against PTZ induced seizures whereas HEETF 300 mg/kg 76.6%, 400 and 500 mg/kg have shown 100% protection respectively shown in table no 3.

Table 1. Result of phytochemical screening of hydroethanolic extract of *T. foliolosum*

S.No	Test	Hydro- ethanol
1.	Alkaloids	++

	Dragendroff's test	++
	Mayer's test	++
	Wagner's test	++
	Hager's test	++
2.	Carbohydrates	
	Molisch test	++
	Fehling test	++
	Benedict test	++
3.	Saponins	
	Haemolysis test	++
	Foam test	++
4.	Proteins	
	Biurets test	++
	Millon's test	++
	Xanthoproteins	++
5.	Phenolic Compounds	
	FeCl ₃ test	++
6.	Tannins	
	Lead acetate test	++

Table 2. Effect of hydroethanolic extract of *T. foliolosum* (HEETF) On MES induced seizers in rats

Treatment	Flexion	Extensor	Clonus	Stupor	Recovery	%Protection
Vehicle 1ml/100gm	9.2±0.15	16.42±0.21	42.79±0.32	42.52±0.11	202.36	0%
Phenytoin 25mg/kg	4.5±0.22	0	16.69±0.21**	16.12±0.32**	98.18	100%
HEETF 300mg/kg	8.1±0.32	7.2±0.13	33.61±0.34**	33.16±0.15*	165.59	49.8%
HEETF 400mg/kg	7.2±0.26	5.21±0.32	28.56±0.22	28.52±0.14	139.85	66.4%
HEETF 500mg/kg	5.6±0.20	3.11±0.39	19.62±0.15**	19.72±0.18**	116.26	83%

Statistical significance was determined by one way ANOVA followed by dunnett test values are expressed as mean ± SD, n=6 p<0.05 ** as compared with normal control group: p<0.01 ** as compared with group II, III, IV and V respectively.

Table 3. Effect of hydroethanolic extract of HEETF in Pentylene tetrazole induced convulsions in rats

Treatment	Dose mg/kg	No of Animal Exhibiting seizure	Onset of Convulsion (GTCS)	Duration of Convulsion	Animal Mortality	Protection against seizure
Vehicle+PTZ	1 ml/10g	6	37.5±7.61	138±40.3	5	16.6%
Diazepam+PTZ	4mg/kg	6	513.5±48.74	32.3±7.9*	0	100%
HEETF+PTZ	300mg/kg	6	259.16±24.91**	83.3±17.04	2	66.4%
HEETF+PTZ	400mg/kg	6	287.16±60.82**	78.66±16.51**	0	100%
HEETF+PTZ	500mg/kg	6	418.83±76.88**	48.5±11.041*	0	100%

Statistical significance was determined by one way ANOVA followed by Dennett test values are expressed as mean ± SD, n=6 ** p<0.05 as compared with normal control group *p<0.01 as compared with PTZ group.

Fig1. Effect of hydroethanolic extract of HEETF on onset of convulsion PTZ induced convulsions in rats

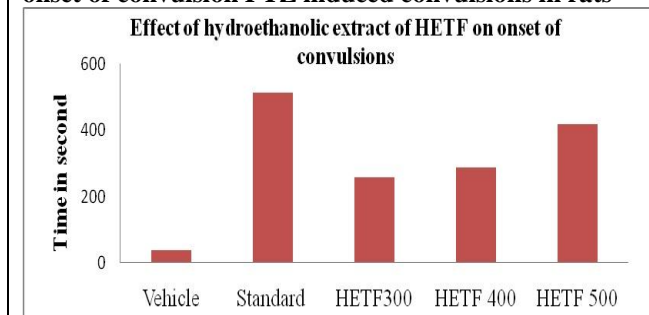
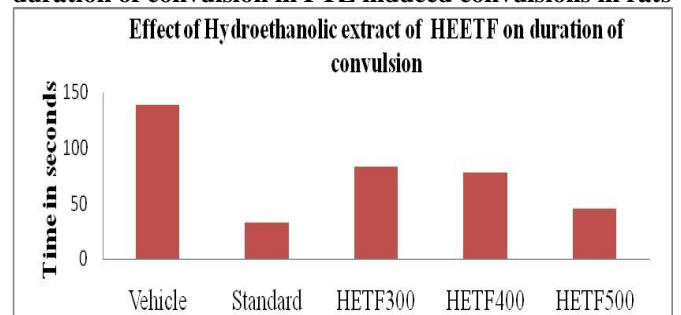


Fig 2. Effect of hydroethanolic extract of HEETF on duration of convulsion in PTZ induced convulsions in rats



DISCUSSION AND CONCLUSION

Different types of Antiepileptic drugs are available so far treat any kind of epilepsy like general Tonic-Clonic seizure Atonic etc. but there are many side effect and contraindication, so there is always a strong need for minimization of ADR and maximization of therapeutic benefit. Some time we are fail to manage epilepsy so we can use herbal medicine to manage any type of convulsion without any side effect. The MES model is the well definite screening method to find out or identified anticonvulsant activity of drugs for the generalized tonic-clonic seizures. This method is based on observation of the excitation by recurrent and spontaneously electrical impulses induced by different neuronal circuit one characteristics standard of convulsion activity In our recent study, it is found that rats are treated with HEETF significantly reduces in tonic hindleg extensor stage in MES induced epilepsy. Since, HEETF significantly prohibited generalized tonic-clonic seizures in MES method

Our result demonstrate that *T. foliolosum* have shows dose dependently affect on epilepsy using PTZ The phytochemical investigated that different constituent found in the HEETF which shows antiepileptic activity and another neuroprotective. So it can be concluded that the hydroethanolic extract of whole plant of *T. foliolosum* have shown significant antiepileptic potential with minimal toxicity.

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CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

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