

Original Article

Anticonvulsant activities of *Quercus infectoria* (galls) and *Trewia nudiflora* (seeds) in Nepalese varieties

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ABSTRACT

Background and objectives: Epilepsy is defined as the heterogeneous, synchronized abnormal electrical discharge from the brain area. The numerous synthetic antiepileptic drugs currently available are ineffective to many patients and plant-derived drugs could be an alternative. The aim of this study was to evaluate anticonvulsant activities of *Quercus infectoria* (galls) and *Trewia nudiflora* (seeds) found in Nepal.

Materials and methods: Anticonvulsant activity was carried out using Isoniazide-induced clonic convulsion (INH 250 mg/kg) using rat model. Onset and duration of clonic convulsion, percentage protection and death were observed.

Results: *T. nudiflora* showed a dose dependent hindrance on the onset of clonic convulsions when compared with the control group. Treatment with higher doses of *T. nudiflora* (400 mg/kg) showed significant increase ($p < 0.01$) in latency of clonic convulsions whereas, lower dose showed significant increase ($p < 0.05$) in latency of clonic convulsion. The extract of *T. nudiflora* protected 25% of animals from convulsion at a dose 400 mg/kg. However, *Q. infectoria* did not display dose

dependent delay on the onset of clonic convulsion, *Q. infectoria* (100 and 400 mg/kg) showed insignificant increases in latency of clonic convulsions. The extract of *Q. infectoria* protected the 25% of animals when used at a lower dose of 100 mg/kg. Positive control group treated with diazepam at a dose 4 mg/kg showed 100% protection from clonic seizures and death. *Q. infectoria* (100 mg/kg) *T. nudiflora* (400 mg/kg) and Diazepam (4 mg/kg) showed significant decrease ($p < 0.01$) in seizure duration (s). *Q. infectoria* (400 mg/kg) and *T. nudiflora* (100 mg/kg) showed significant decrease ($p < 0.05$) in seizure duration compared to control group (Normal saline 10 ml/kg). Study reports the antiepileptic effects *Q. infectoria* and *T. nudiflora*.

Conclusion: The methanolic extract galls of *Q. infectoria* and seeds of *T. nudiflora* showed protective effect on Isoniazide induced convulsion.

Keywords: Anticonvulsant, Clonic convulsion, *Q. infectoria* and *T. nudiflora*

INTRODUCTION

Epilepsy is defined as the infrequent, unexpected, excessive, quick and an abnormal coordinated electrical depolarization (local discharge) in grey matter of the central nervous system [8]. About 1% of the world's population has epilepsy. After stroke it is the second most common neurologic disorder. Even though standard therapy permits control of seizures, millions have uncontrolled epilepsy [1-2].

The generation of epileptic seizure is due to the abnormalities of some neurotransmitter, reduction in inhibitory neurotransmitter (GABA) and over production of excitatory neurotransmitter (aspartate, acetylcholine, glutamate). If the motor cortex is involved epileptic seizure may cause convulsion. If the parietal or occipital cortex is involved, the epileptic seizures may include visual, auditory and olfactory hallucination. Although the modern drugs are effective for the treatment of epilepsy, it is not effective in all patients. Hence it is necessary to search new treatment approach [3]. About 450 million people in the entire world have suffered mental, neurological, or behavioral problems at some time in their life [4]. Extensive research on plants and their derivatives has taken place in recent years that could provide some new alternative treatments and therapeutic uses for diseases of the central nervous system [5]. Literature review reveals that a number of plants such as *Acorus calamus*, *Crocus sativus*, *Embllica officinalis*, *Ginkgo biloba*, *Hypericum perforatum*, *Matricaria recutita*, *Panax ginseng*, *Passiflora incarnata*, etc. have been reported to demonstrate antiseizure activity [6]. Several classes of phytoconstituents such as alkaloids, lipids, terpenes, triterpenoids, flavonoids and coumarins have been reported to acquire anticonvulsant activity [7-11]. The main objective of our study is to find the alternative antiepileptic drugs from natural sources. In this study anticonvulsant activities of two plants *Quercus infectoria* (galls) and *Trewia nudiflora* (seeds) Nepalese varieties were carried-out.

MATERIAL AND METHODS

It is an experimental study done by collecting plants from field by which extract are

prepared and experimented. The details is discussed in following headings.

Chemicals and equipments

Pure diazepam was obtained from Asian Pharmaceutical Private Limited Nepal. Normal physiological saline purchased from Claris Pharma Laboratory India. UV Spectrophotometer (UV180) was obtained from Shimadzu Japan. Rotatory evaporator used was Rotavapor 218/219 from Buchi Switzerland.

Collection of plants

Medicinal plants (*Q. infectoria* and *T. nudiflora*) used traditionally were collected from the different regions of the Nepal. A voucher is deposited at the Herbarium in the museum of material medica, Karnali Academy of Health Sciences; Jumla, Nepal. Collected plants were carefully transported, dried and allowed for the extraction.

Short introduction and details of two medicinal plants used in this study is presented here.

***Quercus infectoria* (galls/gall oak)**

It (Fagaceae) is a small tree or shrub native to Greece, Nepal, Iran, Syria, South China, Malayasia, India and minor Asia. There are about three hundred species distributed in north temperate region; Indo-malayan region and pacific coast. Around twenty species are native to India. These are deciduous or evergreen trees, rarely shrubs [12-13]. Leaves are petioled, serate, lobed or pinnetifid, rarely entire. Flowers appear with or before leaves. Through the attack of insect species *Adleria* (cypnis) gallae-tincotoriae oliv, galls are formed on the tree (shoot) which constituents the drug. Several pharmacological experiment of the galls of *Q. infectoria* shows as astringent, antitremorine,

antidiabetic, a local anaesthetic, antipyretic, anticandidicidal, antibacterial and antifungal properties [12-13]. Flavanols as one of the major compounds from this plant was reported to inhibit cell proliferation *in vitro* [14]. Ethanolic extract of *Q. infectoria* galls possess antioxidant activity and abrogate oxidative stress-induced functional alterations murine macrophages [15]. It is distributed at 2700-4300 m, east to west of Nepal.

*Q. infectoria**T. nudiflora*

Figure 1: Photos of selected medicinal plants

Trewia nudiflora

It (Euphorbiaceae) consists of the dried seeds of *T. nudiflora*, known as *Belar* in Nepali and Rhino-apple in English. It is a deciduous tree, 12-20 m tall. Leaves are simple, opposite, stalked, ovate, 8-20 cm long, 3-8 cm broad, entire base usually cordate, acute [15-16]. Flowers are dioecious, male flowers are yellow in drooping panicles 10-23 cm long, appearing before new leaves; female flowers are green, solitary, on axillary stalks. Fruits are green, globose, and somewhat succulent. Plant is cooling, alexiteric and used in swelling, bile and phlegm [14-16]. Juice of the root is given in case of stomach complaints such as indigestion, externally in gouty rheumatism diarrhea, and dysentery. Plant contains Pyridine alkaloid-N- methyl-5-carboxamide-2-pyridone [16]. Seed contain

an alkaloid –Ricinidine. Leaves contain an alkaloid-Nudiflorine. Bark contains Tarxerone and β -Sitosterol [16-18]. It is distributed at 150- 1800 m, (damp places) east to west of Nepal.

Preparation of extracts

The plant species *Q. infectoria* and *T. nudiflora* were washed with distilled water, cut into small pieces and dried in a ventilated oven at 40 °C for 120 h. Each dried sample (100 g) was dried and ground crude drugs were allowed for the maceration with methanol, 500 ml at room temperature for 48 h. The residue is again extracted with 500 ml methanol for 48 h. The methanol extracts were evaporated to dryness using a Rotatory evaporator.

Experimental animals

Albino wistar rats of either sex, 8-10 weeks old weighing about 100-150 g were used in experiment. All the rats were normally housed in groups of four per cages (polypropylene) and were maintained under standard condition {12 h light/dark cycle; 25±3 °C, 45-65 percent (%) humidity and had free access to tap water and food *ad libitum*. All the animals were acclimatized to laboratory condition for a week before the beginning of experiment. Each rat was used for one pharmacological screening experiment only. Animal handling, care, and experimental design were conducted according to the guidelines of the Institutional Review Committee, Nepal Health Research Council (Ref. no. 327/2021).

Determination of anticonvulsant activity

In this study 24 rats were used. They were divided in to six groups of 4 animals each. Group I served as the control and received normal saline (10 ml/kg p.o). Group II received diazepam (4 mg/kg i.p, dissolved in

1% acacia gum in normal saline) as a positive control (reference), and group III and group IV were treated with 100 and 400 mg/kg (p.o) methanolic extract of *Q. infectoria* respectively. Group V and VI were treated with methanolic extract of *T. nudiflora* 100 and 400 mg/kg (p.o) respectively. Both the extracts were dissolved in normal saline (0.9% NaCl w/v).

The animals were pretreated with diazepam or extract 45 min prior to administration of chemoconvulsant, Isoniazide (250 mg/kg, i.p). Animals that did not convulse within 120 min were considered as protected. The number of rat protected in each group was expressed as a percentage. In the Isoniazide treated group, the animals were monitored for 120 min, and the percent protection was determined. In unprotected animals, the latency to first convulsion and the duration of convulsion were recorded. The animals were observed for mortality for 24 h after administration of Isoniazide [19].

Statistical analysis:

All the values and data were expressed as mean \pm SEM, (n= 4). The data were analyzed by one way analysis of variance (one way-ANOVA) followed by post-hoc Dunnett's test using SPSS version 16. p-value less than 0.05 were considered statistically significant.

RESULTS

Table 1: Anticonvulsant activity

Group no.	Treatment Group	Onset of seizure (s)	Seizure duration (s)	Protection (%) 2 h	Mortality (%) 24 h
I	INH+Saline (10 ml/kg)	1695 \pm 155.64	101.50 \pm 7.89	0	100
II	INH+ <i>Q. infectoria</i> (100 mg/kg)	2895 \pm 407.21	24.00 \pm 8.61	25	0
III	INH + <i>Q. infectoria</i> (400 mg/kg)	2610 \pm 241.86	31.70 \pm 10.00	0	0
IV	INH + <i>T. nudiflora</i> (100 mg/kg)	3225 \pm 216.85	29.00 \pm 9.88	0	0
V	INH+ <i>T. nudiflora</i> (400 mg/kg)	3540 \pm 60.00	25.50 \pm 8.99	25	0
VI	INH+Diazepam (4 mg/kg)	Protected	Protected	100	0

All the values represents mean \pm SEM, n=4 rats per group

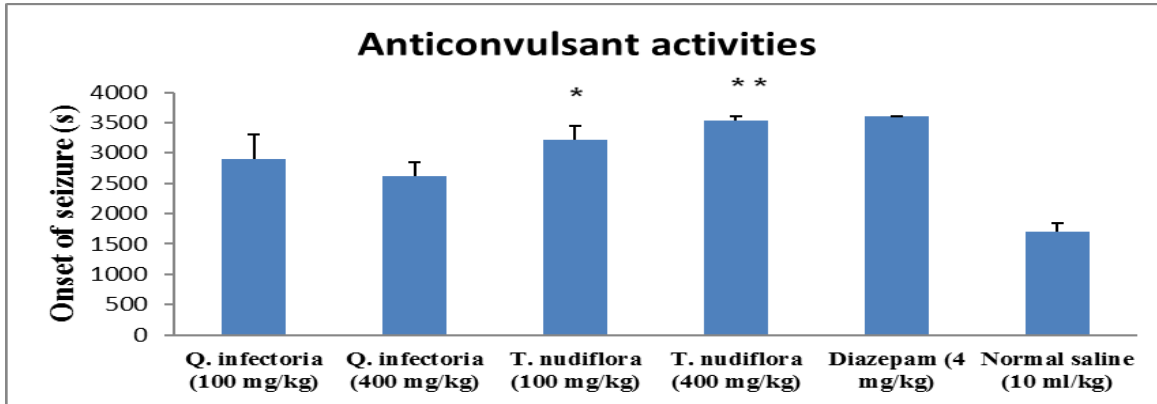
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Isoniazide produced clonic followed by tonic seizures were seen in all the animals used. *T. nudiflora* showed a dose dependent hindrance in the onset of clonic convulsions while *Q. infectoria* does not showed dose dependent delay in the onset of clonic

convulsion when compared with the control group. Onset of clonic convulsion, duration of clonic convulsion, percent protection and percent mortality are shown in Table 1. Treatment with higher doses of *T. nudiflora* (400 mg/kg) showed significant increase ($p <$

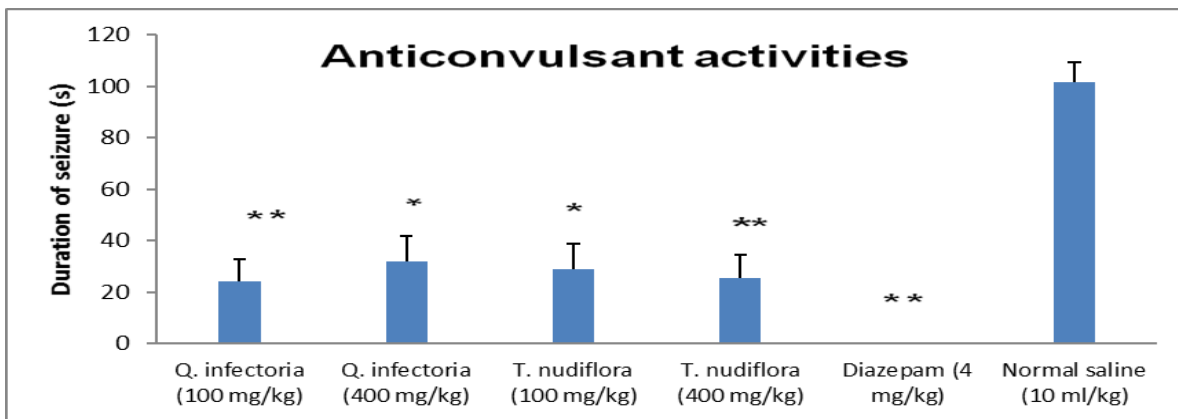
0.01) in latency of clonic convulsions where lower dose showed significant increase ($p < 0.05$) in latency of clonic convulsion. *T. nudiflora* protected the 25% animals from convulsion in dose 400 mg/kg. *Q. infectoria* (100 and 400 mg/kg) showed significant

increases in latency of clonic convulsions but statistically insignificant. *Q. infectoria* protected the 25% animals used in lower dose (100 mg/kg). Positive control group which were treated with diazepam were (100%) protected clonic seizures and death.



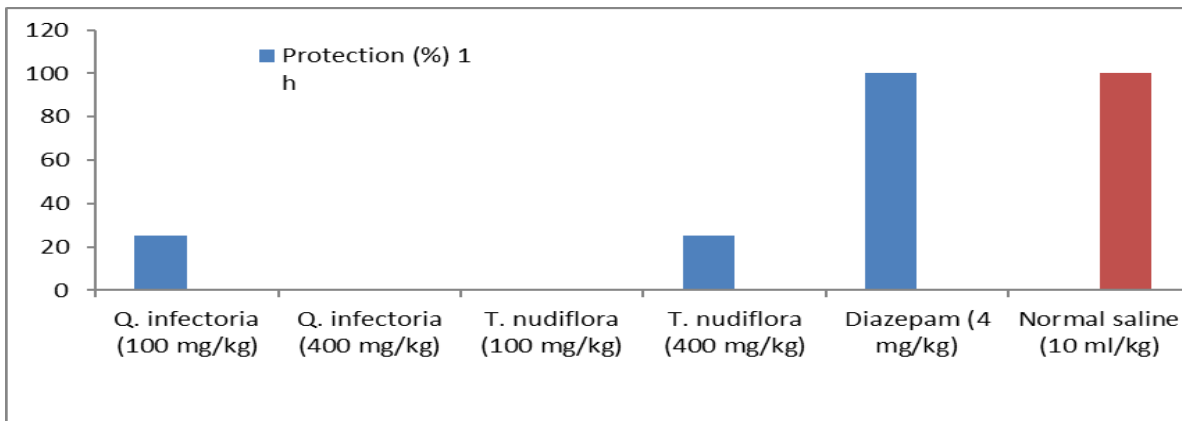
All the values represents mean±SEM, n=4 rats per group. * $p<0.05$, ** $p<0.01$ compared to normal saline group.

Figure 2: Latency of seizure (s) induces by INH in different treatment systems.



All the values represents mean ± SEM, n=4 rats per group. * $p<0.05$, ** $p<0.01$ compared to normal saline group.

Figure 3: Duration of seizure (s) induce by INH in different treatment systems.



All the values represents mean ± SEM, n=4 rats per group

Figure 4: Percent protection and percent mortality in experiment animals.

Q. infectoria (100 mg/kg), *T. nudiflora* (400 mg/kg) and diazepam (4 mg/kg) showed significant decrease ($p<0.01$) in seizure

duration (s). *Q. infectoria* (400 mg/kg) and *T. nudiflora* (100 mg/kg) showed significant decrease ($p < 0.05$) in seizure duration

compared to control group (Normal saline 10 ml/kg).

DISCUSSION

The present study was designed to investigate anticonvulsant properties of some selected Nepalese medicinal plants. The anticonvulsant activity of *Q. infectoria* and *T. nudiflora* were carried out using INH as chemoconvulsant. INH induces seizures at higher doses by interfering with the synthesis of Gama amino butyric acid (GABA), through the inhibition of pyridoxal-5-phosphate, a cofactor for the glutamic acid decarboxylase (GAD), an enzyme that catalyzes the synthesis of GABA from glutamic acid [19]. Methanolic extracts of both plants exhibited the anticonvulsant effect compared to the control group.

Anticonvulsant activity of *Q. infectoria* and *T. nudiflora* were carried out using INH as a chemoconvulsant where diazepam and normal saline were used as positive and negative control respectively. Diazepam is standard anticonvulsant drugs which inhibit seizure induce by INH by potentiating GABAergic transmission [8]. According to Cevik *et al.*, INH, the first line antitubercular drug has been used for the treatment of tuberculosis since 50 years [20]. Despite it vigorous used in clinical practice, it is responsible for peripheral neuropathies and seizures, as an adverse effects. INH increased Lipid peroxidation, facilitate NO (nitrite) and Malondialdehyde (MDA) production and decreases total antioxidant capacity (TAC) levels in brain by producing free radicals, reactive oxygen species generation along with alteration in levels of enzymatic antioxidant such as SOD whereas NO, MDA and lipid peroxidation generate free radical and acts as a potential proconvulsion [20,21]. *T. nudiflora* increases the dose dependent onset of seizure

and decreases duration of seizures while *Q. infectoria* does not showed dose dependent manner but delayed onset of seizure and decreases duration of seizure.

Flavonoids have been described as a family of benzodiazepine receptor ligands with CNS depressant activities [22,23]. The presence of an essential oil, polyphenols, tannins and flavonoids in the *Q. infectoria* and *T. nudiflora* may be responsible for anticonvulsant effects [24]. Methanolic extracts of *Q. infectoria* and *T. nudiflora* at all doses delayed the latency and duration for clonic convulsions and death. The compound that can potentiate GABAergic transmission may show protection action in this INH model. It is also the mechanism for benzodiazepine and barbiturate class of anticonvulsants [8]. Similar mechanism can be drawn for *Q. infectoria* and *T. nudiflora*. Since they showed protection against INH induced convulsions. INH metabolites have been identified, as hydrazine (HZ), ammonia and oxidizing free radicals [25]. Another mechanism might be the antioxidant activity of these plants which scavenge the free radical produced during the proconvulsion and protect from refractory seizure and death from these free radicals [26]. In control group there were refractory clonus seizures until death but in case of test group there was no refractory epileptic status and death. Ascorbic acid being a powerful water soluble antioxidant, showed neuroprotective activity in hippocampus of adult rats by reducing the oxidation of lipid peroxidation and increasing the natural antioxidant such as superoxide dismutase and catalase [27]. In recent years, a great deal of attention has been given to antioxidants consumption and their role in reducing rates of chronic diseases such as epilepsy, cancer, coronary heart disease (CHD), stroke, diabetes and arthritis [28,29,30]. Treatment

with carotenoids (antioxidant) along with INH+ Rifampicin reduced lipid peroxidation and increased thiols, catalase, and superoxide dismutase in the liver and blood of rats [30]. Caffeic acid phenethyl ester reversed all harmful activity of INH, being a good antioxidant [20]. Hence these suggest that anticonvulsant activity of these two plants is due to their potential effect to potentiate GABAergic transmission, which might be due to up regulation of GAD activity and has also antioxidant and free radical scavenging property This study was the first to study and revealed anticonvulsant activity of *Q. infectoria* and *T. nudiflora* and this could be the better supplement or medication for prevention and treatment of epileptic seizure arising from down regulation of GABAergic transmission oxidative stress. This finding suggests that *Q. infectoria* and *T. nudiflora* supplementation may be used as a potential neuroprotective drug for antituberculosis therapy with INH.

CONCLUSION

The finding of the present study revealed that the methanolic extract galls of *Q. infectoria* and seeds of *T. nudiflora* showed protective effect on Isoniazide induced convulsion. This finding suggests that the further chemical constituents and mode of action of these two plants should be elucidated.

AUTHOR CONTRIBUTION:

KSK: design the idea of research and carry-out the experiment, Manuscript draft

RB: design the idea of research and carry-out the experiment, Manuscript draft

BP: analysis of the data and result, involve in experiment and writing too

RB: analysis of data and result, involve in experiment and writing too.

NT and KA: involve in experiment, wrote the manuscript.

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CONFLICT OF INTEREST: None declared

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