EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 10
Issue: 11
104-111
Year: 2023

STUDY OF PHYTOCHEMICALS AND ANTIEPILEPITIC ACTIVITY OF HYDROALCOHOLIC EXTRACT OF PUERARIA TUBEROSA

[*1]Roshnee Ghorse, [2]Rahim Khan, [3]Dr. Ravi Prakash, [4]Dr. Brajesh Ku. Arjariya and [5]Sheenam Mansuri

[1]Scholar Malhotra College, [2]Assistant Professor, Malhotra College, [3]Professor, Malhotra College, [4]Associate Professor, Malhotra College, [5]Assistant Professor Malhotra College.

***Corresponding Author: Roshani Geed**
Scholar Malhotra College.

Article Received on 31/08/2023 **Article Revised on 21/09/2023** **Article Accepted on 10/10/2023**

ABSTRACT
Epilepsy remains a challenging neurological condition affecting millions worldwide, necessitating innovative therapeutic approaches. Phytochemicals, bioactive compounds abundant in various plants, have drawn attention for their potential to address epilepsy. Pueraria tuberosa, known as "Indian Kudzu" or "Vidari Kanda," is a plant rich in phytochemicals, offering a promising avenue for research into its antiepileptic properties. This study delves into the phytochemical composition of the hydroalcoholic extract of Pueraria tuberosa, aiming to identify and quantify specific bioactive compounds, including flavonoids, alkaloids, saponins, and polyphenols. Furthermore, it rigorously evaluates the extract's antiepileptic activity through comprehensive in vitro and in vivo experiments. These assessments encompass seizure models, neurotransmitter level measurements, and oxidative stress marker evaluations. Understanding the phytochemical constituents of the Pueraria tuberosa extract and their potential effects on epileptic seizures holds significant therapeutic potential. This research contributes to the expanding knowledge base of natural remedies for epilepsy, offering new avenues for improving the quality of life for those affected by this challenging condition. Additionally, it aligns with the broader goal of identifying safe and effective alternatives to conventional antiepileptic drugs, addressing the unmet needs in epilepsy management.

KEYWORDS: antiepilepitic activity, hydroalcoholic, Pueraria Tuberosa, herbal.

INTRODUCTION
Epilepsy is a complex neurological disorder characterized by recurrent, unprovoked seizures, which affect millions of people worldwide. Despite significant progress in the development of antiepileptic drugs (AEDs), a substantial proportion of individuals with epilepsy continue to experience inadequate seizure control, adverse side effects, or limited access to effective medical treatment. Consequently, there is a growing interest in exploring alternative and complementary therapeutic approaches, with a particular focus on natural products derived from plants, to manage epilepsy effectively and mitigate its associated challenges.[1] Epilepsy is a group of long-term neurological disorders characterized by epileptic seizures. These seizures are episodes that can vary from brief and nearly undetectable to long periods of vigorous shaking. In epilepsy, seizures tend to recur, and have no immediate underlying cause while seizures that occur due to a specific cause are not deemed to represent epilepsy. In most cases the cause is unknown, although some people develop epilepsy as the result of brain injury, stroke, brain cancer, and drug and alcohol misuse, among others. Epileptic seizures are the result of excessive and abnormal cortical nerve cell activity in the brain. The diagnosis typically involves ruling out other conditions that might cause similar symptoms (such as syncope) as well as figuring out whether any immediate causes are present. Epilepsy can often be confirmed with an electroencephalogram (EEG).[2]

Phytochemicals are bioactive compounds found in various plant species. They have gained considerable attention in recent years due to their potential therapeutic properties, including their ability to influence the central nervous system and modulate epileptic seizures. Pueraria tuberosa, commonly known as "Indian Kudzu" or "Vidari Kanda," is a plant indigenous to India and has a rich history of use in Ayurvedic medicine. This plant is known to contain a wide array of phytochemicals, such as flavonoids, alkaloids, saponins, and polyphenols, which are recognized for their antioxidant, anti-inflammatory, and neuroprotective properties.[3]

Among the various plant extracts being investigated for their antiepileptic potential, the hydroalcoholic extract of Pueraria tuberosa has garnered significant attention. Researchers have been particularly intrigued by this

extract due to its potential as an antiepileptic agent. Numerous preclinical and experimental studies have explored the neuropharmacological effects of Pueraria tuberosa extract, with a specific focus on its ability to modulate neurotransmitter systems, attenuate oxidative stress, and protect against neuronal damage – all of which are relevant factors in epilepsy management.[4]

Understanding the chemical constituents of the hydroalcoholic extract of Pueraria tuberosa and their potential effects on epileptic seizures holds great promise.[5] This research is essential for advancing our knowledge of natural remedies for epilepsy and may provide novel therapeutic avenues for individuals living with this challenging condition. Furthermore, it contributes to the broader goal of uncovering safe and effective alternatives to conventional antiepileptic drugs, ultimately aiming to enhance the quality of life for individuals affected by epilepsy.

METHODOLOGY

Collection of Plant material

The plant has been selected on the basis of its availability and folk use of the plant. Leaves of Pueraria tuberosa were collected from local market of Bhopal in the month of September, 2021.

Drying and storage

Drying of fresh plant parts was carried out in sun but under the shade. Dried leaves of Pueraria tuberosa were preserved in plastic bags, closed tightly and powdered as per the requirements.

Extraction procedure

Following procedure was adopted for the preparation of extracts from the shade dried and powdered herbs.

Defatting of plant material

70.57 gram of leaves dried powdered of Pueraria tuberosa were shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether by soxhlation method. The extraction was continued till the defatting of the material had taken place.

Extraction by soxhlation process

Defatted dried powdered of Pueraria tuberosa has been extracted with hydroacoholic solvent (ethanol: water; 75:25v/v) using soxhlation method for 48 hrs, filtered and dried using vacuum evaporator at 40ºC.

Extraction by soxhlation process

Determination of percentage yield

The percentage yield of each extract was calculated by using following formula.

$$Percentage\ yield\ = \frac{Weight\ of\ Extract}{Weight\ of\ powdered\ drug}\ x\ 100$$

Phytochemical Screening

Phytochemical examinations were carried out for all the extracts as per the standard methods.

Detection of alkaloids: Extract were dissolved individually in dilute Hydrochloric acid and filtered.

Mayer's Test: Filtrates was treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

Wagner's Test: Filtrates was treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Dragendroff's Test: Filtrates was treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Hager's Test: Filtrates was treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

Detection of carbohydrates: Extract was dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Fehling's Test: Filtrates was hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

Detection of glycosides: Extract was hydrolysed with dil. HCl, and then subjected to test for glycosides.

Legal's Test: Extract was treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

Detection of saponins

Froth Test: Extract was diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

Detection of phenols

Ferric Chloride Test: Extract was treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Detection of tannins

Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

Detection of flavonoids

Alkaline Reagent Test: Extract was treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Lead acetate Test: Extract was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

Detection of proteins Xanthoproteic Test: The extract was treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

Detection of diterpenes Copper acetate Test: Extract was dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.[6-8]

Qualitative chromatographic analysis

Thin layer chromatography Thin layer chromatography (TLC) is based on the adsorption phenomenon. In this type of chromatography mobile phase containing the dissolved solutes passes over the surface of stationary phase.

Detection and Calculation of Rf. Value

Once the chromatogram was developed the Rf Value of the spot was calculated using the formula and results was depicted in Table 1.3.

$$R\text{-}f = \frac{Distance\ traveled\ by\ solute}{Distance\ traveled\ by\ solvent}$$

In vivo Antiepilepsy screening of hydroalcoholic leaves extract of Pueraria tuberosa

Drugs and chemicals

Pentylenetetrazole (PTZ), Diazepam were used in present study. Fresh solution was prepared before each experiment. All other reagents used were standard laboratory reagents of analytical grade and were purchased locally.

Toxicity study

Preliminary experiments were carried out on mice (n=6). Hydroalcoholic extract of Pueraria tuberosa were administered orally in different doses to find out the range of doses which cause zero and 100 % mortality of animals. Acute oral toxicity was conducted according to the method of Organisation for Economic Co-operation and Development (OECD). Animals were kept fasting providing only water, extract were given p.o. in doses of 500, 1000 and 2000 mg/kg/p.o. administered orally for 4 days of different groups of mice (n=6) and the animals were kept under observation for mortality as well as any behavioral changes for evaluation of a possible antiepileptic effect.

Pentylenetetrazole-induced seizures test

Mice were divided into three groups each containing six animals, and received hydroalcoholic extract of Pueraria tuberosa (100 and 200 mg/kg) and diazepam (3 mg/kg). Thirty minutes later seizures were induced by the pentylenetetrazole (80 mg/kg, i.p.). The animals were observed during the first 30 min for number of animals with convulsions i.e. latency and duration of myoclonic jerks, number of deaths and percent protection against convulsion and mortality.[9]

Maximal electroshock-induced seizures test

Mice were divided into three groups each containing six animals and treated with either hydroalcoholic extract of Pueraria tuberosa (100 and 200 mg/kg) and diazepam (3 mg/kg). Thirty minutes later seizures were induced by a current stimulus (18 mA, 50 Hz for 0.2 s) delivered by using corneal electrodes by a shock generator (Inco, India). The percent protection and duration of tonic hind limb extension (i.e., the hind limbs of animals outstretched at 180° to the plane of the body axis) was observed. Protection was defined as complete absence of tonic hind limb extension.[10]

RESULTS AND DISCUSSION

Determination of Percentage Yield

To obtain the percentage yield of extraction is very important phenomenon in phytochemical extraction to evaluate the standard extraction efficiency for a particular plant, different parts of same plant or different solvents used. The yield of extract obtained from samples using hydroacoholic solvent is depicted in the table 1.1.

Table 1.1: % Yield of Pueraria tuberosa.

S.No	Extracts	% Yiled (W/W)
1.	Pet .ether	2.47 %
2.	Hydroacoholic	7.85 %

Phytochemical screening of extract

Small portion of the dried extracts was subjected to the phytochemical tests using standard methods to test for alkaloids, glycosides, saponins, flavonoids and phenol separately for extracts of all samples. Small amount of each extract was suitably resuspended into the distilled water to make the concentration of 1 mg per ml. The outcomes of the results are discussed in the table 1.2.

Table 1.2: Phytochemical screening of extracts of Pueraria tuberosa.

S. No	Constituents	Hydroacoholic extract	Observation
1.	Alkaloids Dragendroff's test Hager's test	 -ve -ve	 Green coloured Not yellow coloured
2.	Glycosides Legal's test	 -ve	 Green coloured
3.	Flavonoids Lead acetate Alkaline test	 +ve +ve	 Yellow colour precipitate Yellow colour
4.	Phenol Ferric chloride test	 +ve	 Black coloured
5.	Proteins Xanthoproteic test	 -ve	 Green coloured
6.	Carbohydrates Fehling's test	 +ve	 Red colour precipitate
7.	Saponins Foam test	 +ve	 Layer of foam
8.	Diterpenes Copper acetate test	 +ve	 Green coloured
9.	Tannins Gelatin Test	 -ve	 Green coloured

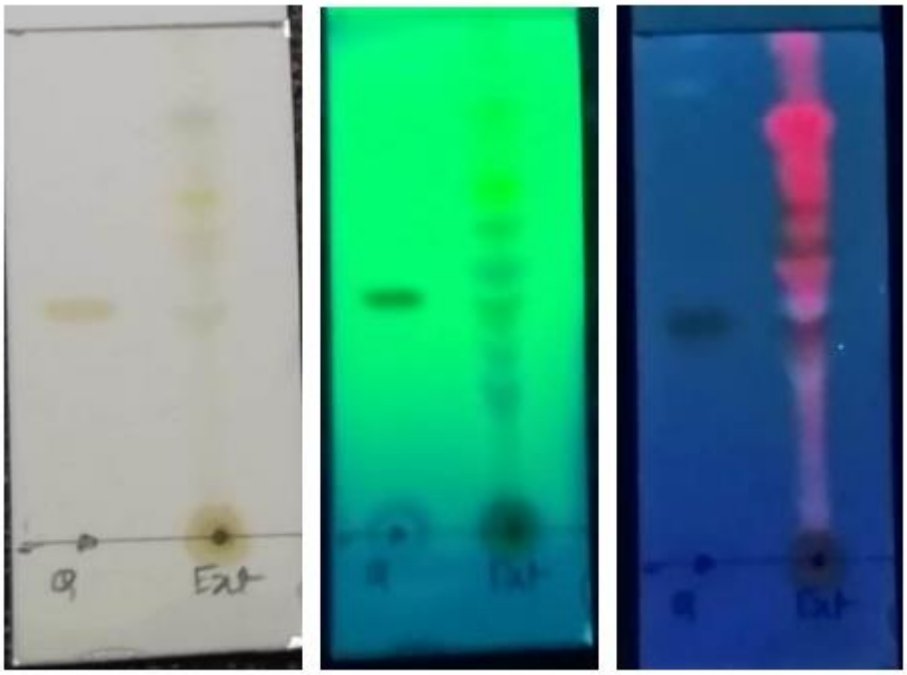
+ve= positive, -ve=negative

Results of phytochemical screening were detected flavonoids, phenol, carbohydrates, Diterpenes and saponins in hydroacoholic extracted of Pueraria tuberosa.

Results of thin layer chromatography of hydroalcoholic extract of Pueraria tuberosa.

Table 1.3: TLC chromatogram details of hydroalcoholic extract of Pueraria tuberose.

TLC chromatogram (Quercetin)		
S. No.	Mobile phase Toluene: Ethyl acetate: Formic acid (5:4:1)	Rf value
1.	(Quercetin) Dis. travel by mobile phase= 5cm No. of spot at long UV= 1 No. of spot at short UV = 1 No. of spot at normal light= 1	 Long UV - 0.66 Short UV - 0.66 Normal light - 0.66
2.	(Hydroalcoholic extract) Dis. travel by mobile phase= 5cm No. of spot at long UV = 8 No. of spot at short UV = 8 No. of spot at normal light= 6	0.32, 0.42, 0.56, 0.62, 0.68, 0.74, 0.82, 0.96 0.24, 0.34, 0.44, 0.52, 0.6, 0.68, 0.74, 0.86 0.4,0.56,0.6,0.7,0.76,0.86



1 st spot Quercetin standard
2 nd spot hydroalcoholic extract of Pueraria tuberosa
Figure 1.3: TLC of Quercetin

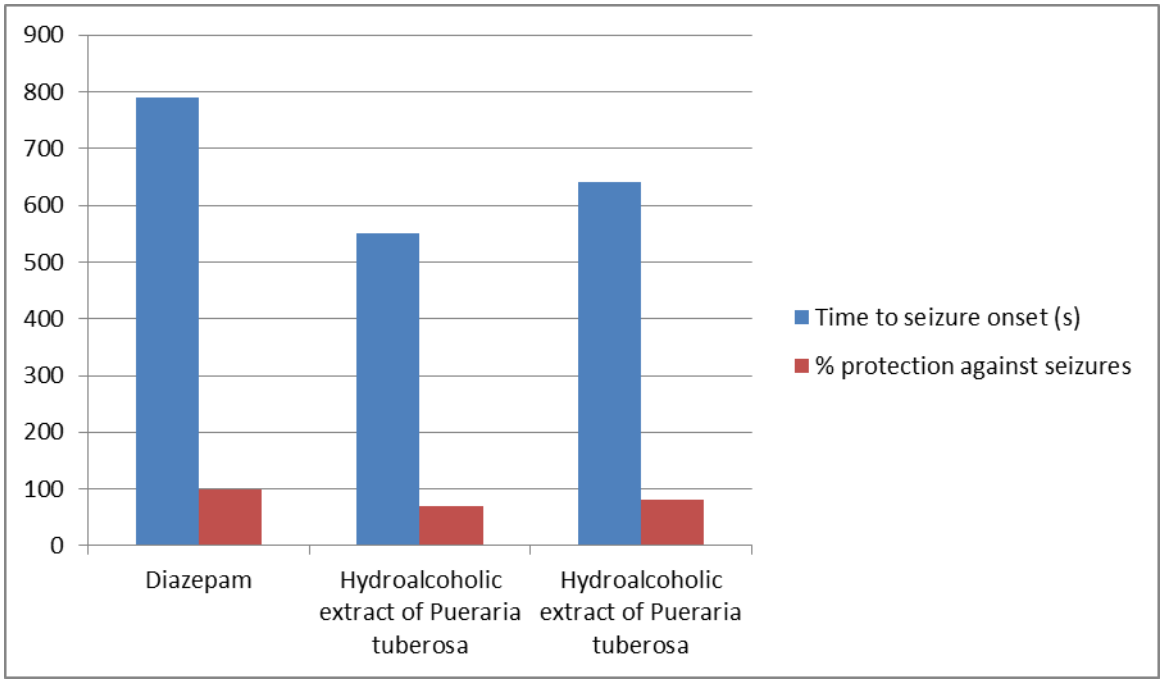
Results of antiepileptic activity of hydroalcoholic extract of P. tuberosa

Hydroalcoholic extract of Pueraria tuberosa shows antiepileptic property against epilepsy induced by Maximal electroshock (MES), and Pentylenetetrazole (PTZ)

Table 1.4: Effects of hydroalcoholic extract of Pueraria tuberosa on pentylenetetrazole-induced seizures

Treatment	Dose (mg/kg)	Time to seizure onset (s)	% protection against seizures
Diazepam	3	790.0 ± 12.00*	100
Hydroalcoholic extract of Pueraria tuberosa	100	550.20 ± 11.00	69.62
Hydroalcoholic extract of Pueraria tuberosa	200	640.10 ± 11.00*	81.01

Values are expressed as the mean ± SEM of six observations. *P<0.01 vs. saline treatment (One-way ANOVA followed by Dunnett's post hoc test).

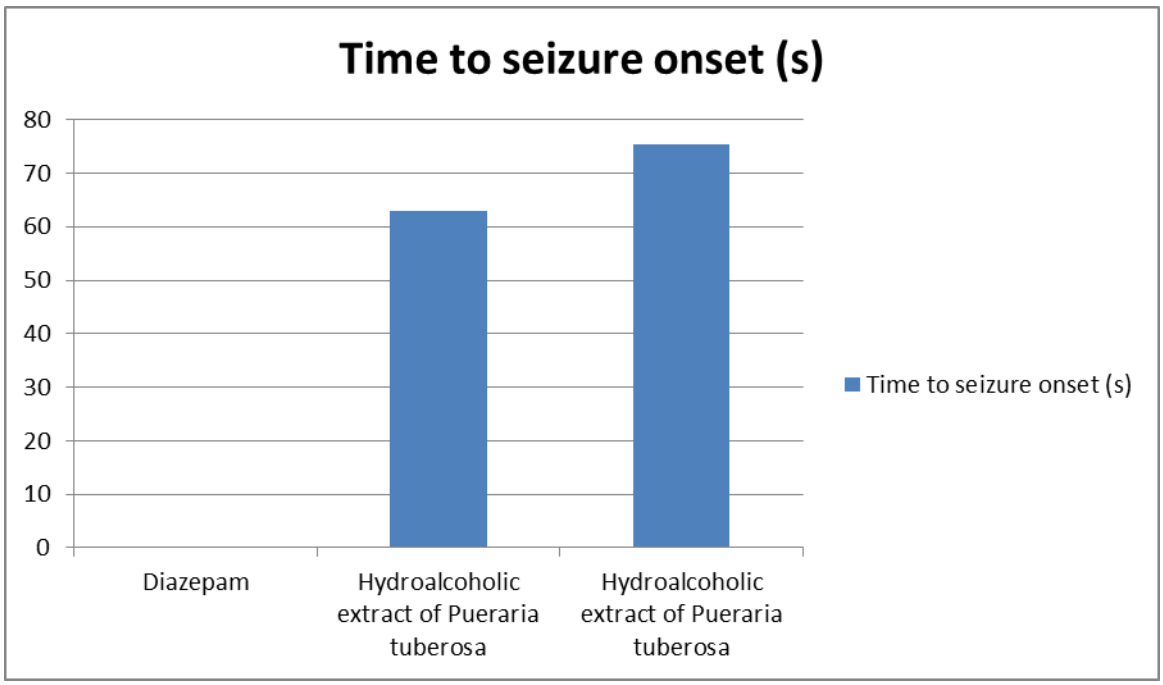


Maximal electroshock-induced (MES) seizure test

Table 1.5: Effects of hydroalcoholic extract of Pueraria tuberosa on MES induced seizures.

Treatment	Dose (mg/kg)	Time to seizure onset (s)
Diazepam	3	0.00 ± 0.00*
Hydroalcoholic extract of Pueraria tuberosa	100	62.85±2.45
Hydroalcoholic extract of Pueraria tuberosa	200	75.42±2.35*

Values are expressed as the mean ± SEM of six observations. *P



Epilepsy is a group of chronic neurological disorders characterized by sporadic episodes of convulsive seizures, sensory disturbance, abnormal behavior and loss of consciousness or all of these symptoms resulting from a brain dysfunction or an abnormal discharge of cerebral neurons. Drug therapy of epilepsy with currently available antiepileptic drugs is associated with side-effects, dose-related and chronic toxicity that involves virtually every organ system. Hazards of anticonvulsant therapy in pregnancy and teratogenic effects are well-

known. Moreover, all the currently available antiepileptic drugs have potential for adverse effects on cognition and behavior. This made man to search for alternative medicine from natural source.[11-12]

The yield of hydroacoholic extract was found to be 7.85% w/w. The phytochemical screening revealed the presence of flavonoids, phenol, carbohydrates, Diterpenes and saponins in hydroacoholic extracted of Pueraria tuberosa.[13]

Medicinal plants used for the therapy of epilepsy in traditional medicine have been shown to possess promising anticonvulsant activities. The folk or traditional medicinal uses of plants represent "leads" that could short cut the discovery of modern medicines with novel structures, which can be much cheaper and less timeconsuming. Several useful medicines derived from plants have been discovered from scientific investigation of traditional and folklore claims.[14]

We report that a hydroalcoholic extract of Pueraria tuberosa anticonvulsant effects against both PTZ- and MES-induced seizures. Higher prevalence, lack of awareness, cultural and social stigma and non-availability of proper diagnostic and treatment facilities are among the major problems in the developing countries. There is a pressing need for further research especially in the field of pharmacotherapy ofepilepsy to find drugs. Search for anti-epileptic agents has made man turn to alternative sources, indigenous system of medicine.

The data obtained in the present study demonstrated hydroalcoholic extract of Pueraria tuberosa had significantly inhibited the convulsions induced by MES and PTZ. The MES test in mice is a suitable model for grand mal epilepsy. MES test in mice is used primarily as an indication for compounds, which are effective in grand mal epilepsy. PTZ-induced convulsions in mice are a suitable model for petit mal epilepsy. PTZ is GABA antagonist. This assay has been used primarily to evaluate AED. Drugs which antagonize PTZ-induced seizures are generally useful in petit mal epilepsy. It has been indicated that PTZ-induced seizures can be prevented by drugs that reduce T-type Ca2+ currents, such as ethosuximide and also by drugs that enhance GABAA receptor-mediated inhibitory neurotransmission, such as benzodiazepines and Phenobarbital88-89. The results of our study reveal that hydroalcoholic extract of Pueraria tuberosa significantly inhibited the convulsions induced by MES and PTZ. Previous phytochemical investigations revealed the presence of different chemical compounds90. It is also found that many flavonoids could act as benzodiazepine-like molecules in the central nervous system and modulate GABA-generated chloride currents in animal models of anxiety, sedation and convulsion.[15]

CONCLUSION

In conclusion, hydroalcoholic extract of Pueraria tuberosa was found to have marked anticonvulsant activity against MES and TZ-induced convulsions. Although forskolin is a promising candidate molecule for the anticonvulsant activity of hydroalcoholic extract of Pueraria tuberosa, it is possible that the bioactivity is mediated by a combination of two or more molecules. Further experiments will be required to identify the active molecules(s) and their mechanism(s) of action.

REFERENCES

1. Beaumont A, Whittle IR. The pathogenesis of tumour associated epilepsy. Acta Neurochir, 2000; 142(1): 1–15.
2. KleinM, Engelberts NHJ, van der Ploeg HM, Kasteleijn-Nolst Trenité DGA, Aaronson NK, Taphoorn MJB, et al. Epilepsy in low-grade gliomas: the impact on cognitive function and quality of life. Ann Neurol, 2003; 54(4): 514–20.
3. Taphoorn MJB, Sizoo EM, Bottomley A. Review on quality of life issues in patients with primary brain tumors. Oncologist, 2010; 15(6): 618–26.
4. Büttner A, Gall C, Mall G, Weis S. Unexpected death in persons with symptomatic epilepsy due to glial brain tumors: a report of two cases and review of the literature. Forensic Sci Int, 1999; 100(1–2): 127–36.
5. Kerkhof M, Dielemans JCM, van Breemen MS, Zwinkels H, Walchenbach R, Taphoorn MJ, et al. Effect of valproic acid on seizure control and on survival in patients with glioblastoma multiforme. Neuro Oncol, 2013; 15(7): 961–7.
6. Dr. Mamta maheshwari, Prabhat Jain, Prachi Sharma, Priyanka Sharma. Antimicrobial activity of hydroalcoholic extract of Juglans regia. Asian Journal of Pharmaceutical Education and Research, 2019; 8(3): 1-5.
7. Umme Atifa, Hemant Kumar Sharma, Prabhakar Budholiya, Prabhat Jain. Study of bioactive constituents and antidepressant potential of hydroalcoholic extract of Urtica urens L. in mice. Asian Journal of Pharmaceutical Education and Research, 2019; 8(3): 53-60.
8. Geeta Parkhe, Deepak Bharti. Phytochemical Investigation and Determination of Total Phenols and Flavonoid Concentration in Leaves Extract of Vitex trifolia Linn. Journal of Drug Delivery & Therapeutics, 2019; 9(4-A): 705-707.
9. Nisar, M., Khan, I., Simjee, S.,U., Gilani, A.,H., Obaidullah, Perveen, H., 2008. Anticonvulsant, analgesic and antipyretic activities of Taxus wallichiana Zucc. J Ethnopharmacol, 116: 490–4.
10. Swinyard, E., A., Brown, W.,C., Goodman, L.,S., 1952. Comparative assays of antiepileptic drugs in mice and rats. J Pharmacol Exp Ther, 106: 319–30.
11. Jerome E, Timothy AP. A Comprehensive Textbook. 2nd ed. Philadelphia: Lippincott Williams and Wilkins; 1997. What is Epilepsy? pp. 1–7.

12. Vogel GH. Pharmacological Assays. Germany: Springer; 1997. Drug Discovery and Evaluation, p. 487.
13. Evidence for involvement of GABA in the action of benzodiazepines: studies on rat cerebellum. Costa E, Guidotti A, Mao CC Adv Biochem Psychopharmacol, 1975; (14): 113-30.
14. Gopalakrishnan S, Saroja K, Elizabeth JD. Chemical investigations of aerial parts of Acalypha fruticosa Forssk. Scholars Research Library. Der Pharma Chem, 2010; 2: 383–9.
15. Effect of Gentiana olivieri on experimental epilepsy models. Aslan M, Orhan DD, Orhan N Pharmacogn Mag, 2011 Oct; 7(28): 344-9.