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Antimicrobial, cytotoxicity, mutagenicity and anti-epileptic potential of ethanol extracts of a multipurpose medicinal plant *Dalbergia sissoo*

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ABSTRACT

Dalbergia sissoo (Shisham) is a medicinal plant with great medicinal impact on the nervous system. The present study was aimed to evaluate the phytochemical screening, antimicrobial, cytotoxicity, mutagenicity and anti-epileptic potential of ethanol extracts of *Dalbergia sissoo* (bark and leaf ethanolic extracts) in epilepsy-induced rats. The bark and leaves extract of *D. sissoo* were found to be rich in phytochemicals tannins, flavonoids, saponins, reducing sugars, terpenoids, glycosides, and proteins, and possessed both antibacterial and antifungal properties against various pathogenic bacterial and fungal species. *In-vitro* cytotoxicity and mutagenicity profile evidenced that bark and leaf extracts were non-toxic and non-mutagenic, respectively. Finally, the anticonvulsant effect of these extracts was tested on Pilocarpine-induced seizures in albino Wistar rats. *In-vivo* studies revealed that both bark and leaf extracts of *D. sissoo* significantly reduced the intensity of the seizures, and duration of convulsions induced by Pilocarpine in albino Wistar rat models. The ethanolic extracts showed marked protective activities against Pilocarpine-induced seizures in rat models. A dose of 500 mg/kg of *D. sissoo* leaf extract delayed the onset of seizures by 144 s versus the control Pilocarpine. In conclusion, this work supports the folkloric use of *D. sissoo* in Pakistan for the treatment of epilepsy in a more rational and patient-friendly manner.

1. Introduction

Herbal medicine always remained an important part of the history of all Nations (Jaradat et al., 2017). According to the World Health Organization (WHO), 80% of the worldwide population prefer the intake of herbal medicines as a remedy against numerous infectious diseases. Herbal medicines are used in a variety of forms like as fruits, vegetables, drugs, injections or extracts, etc. For the cure of diseases and maintenance of health (Ahmad et al., 2017). Low and middle-income developing countries have always been suffered from the attack of almost every disease due to poor living standards and affording the cure of the disease remain problematic for them. Among the 391,000-plant species identified, 28,187 (7.2%) have been used in traditional medicine worldwide (Royal Botanic Gardens Royal Botanical Gardens Kew, 2017).

In general, natural products from medicinal plants are important sources of effective dynamic compounds for new therapeutic preparations (Edziri et al., 2018). A medicinal plant's therapeutic value depends on the association between the pharmacological activities and chemical structure of active phytocompounds (Alexa et al., 2018). Antimicrobial properties of plant-derived compounds are associated with functional and structural damages of target cells. During the last few decades, antimicrobial activities of both the plant extracts and essential oils were extensively studied (Anastasaki et al., 2017). Several studies have specified that protective effects of medicinal species against specific pathogens are due to their chemical constituents such as flavonoids, polyphenols and antioxidant compounds (Mohajer et al., 2016; Bilal et al., 2017).

According to the global burden of neurological diseases, epilepsy is considered as the third major cause of global burden after stroke and headache. Approximately, the worldwide incidence of epilepsy is 0.5–2%. Epilepsy is a neurological chronic disorder characterized by repeated seizures, at least two unprovoked seizures occurring more than 24 h apart (Emilie et al., 2018). In addition, it is also characterized by abnormal signaling of the autonomous nervous system leading to seizures and lack of consciousness (Ebrahimzadeh et al., 2017). Abnormal signaling of brain results in involuntary changes in body movements followed by unconsciousness and muscle shaking for a short period.

Different chemicals were used to cure epilepsy. Bromides have been

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used as chemicals in the first rational treatment of epileptic patients. Later on, various chemical and synthetic drugs were prepared and used as anti-epileptic drugs. Drugs used against epilepsy should have a broad activity spectrum, least side effects, low cost, rapid onset action and good oral bioavailability (Raju et al., 2017; Russo, 2017; Manchishi, 2018). Herbal treatments for epilepsy have been widely practiced in the diversified cultures of China, Europe, Iran, and America. Chinese herbal medicines for the treatment of epilepsy are the most popular and dominant. In developing countries, herbal medication for epilepsy was considered as a complementary medication for controlling seizures and maintaining health. Thousands of studies revealed the herbal treatments of epilepsy, and most of which are tested in the experimental laboratory animals (Liu et al., 2017).

Herbal drugs could better replace synthetic medicines for epilepsy, but with sufficient and effective data-based study (Ashrafzadeh et al., 2017; Shaheen and Kamran, 2017). Plant-based medicines are considered very crucial and effective in the struggle to manage seizures in epilepsy (Ahirrao et al., 2017). Different parts of the plants contain many ingredients that produce the required medicinal effects (Bilal et al., 2018; Meher et al., 2018). Therefore, plant-based medicines might be future anti-epileptic drugs due to their efficacy and effectivity. Moreover, plant-based epilepsy treatments would be more patient-friendly (Ahirrao et al., 2017). The therapies for epilepsy consider the use of anticonvulsant drugs that control repeated neuronal signals and prevention of reoccurring convulsions. Although a number of useful drugs are available to treat epilepsy but toxicity and side effects limit their application (Raju et al., 2017). Alternative therapies that lead towards the discovery of medically important compounds of medicinal plants has expanded throughout the world. Medicinal plants have potential therapeutic effects as well as fewer side effects (Rasheed et al., 2017a,b). Considering their safe therapeutic therapy for many diseases, scientists came to the concept of using medicinal plant extracts against epilepsy. Different plants such as *Paeonia officinalis, Bryonia alba, Acanthus montanus, Ficus platyphylla, Heliotropium strigosum, Pinus roxburghii,* etc. Are being used to cure epilepsy (Bum et al., 2011; Sahranavard et al., 2014; Rabiei, 2017).

Dalbergia sissoo is a multipurpose medicinal tree and is the best known for its timber wood. (Sau and Handral, 2014). *D. sissoo* also called as Indian Rosewood and belongs to family legume (Asif and Kumar, 2009). It is a large deciduous perennial tree that is found in the lower land region throughout India and is also indigenous to Bangladesh, Pakistan, Nepal, and Afghanistan (Asif and Kumar, 2009; Amrutkar et al., 2017). *D. sissoo* is widely used in folk medicine for several diseases. Many biologically active compounds have been isolated from this plant such as isoflavones, coumarins, flavones, and quinines. It also comprises of caviunin-7-*O*-glucoside, tectoridin, tectorigenin, iso-caviunin, 7-hydroxy- 4-methyl coumarin, bio-chanin-A and dalbergin (Amrutkar et al., 2017).

Different studies have been carried out by using different organic extracts of *D. sissoo* to explore its medicinal impact on the nervous system. It was found to be effective in improving memory by increasing the level neurotransmitters by repeated administrations of *D. sissoo* ethanolic extracts. The increase in memory was considered as a consequence of tannins present in *D. sissoo* (Sau and Handral, 2014; Sau and Handral, 2015). It was reported to be a brain tonic, has an antinociceptive effect and useful in enhancing neurological health conditions due to the presence of antioxidants. Moreover, *D. sissoo* has many phytoconstituents that are effective against mental disorders and related central nervous diseases (Thonda et al., 2014; Sau and Handral, 2015; Mannan et al., 2017). As *D. sissoo* shows an effective response against neurological diseases, it might be expected to have efficacy in protection against epilepsy. Therefore, in the present research, an attempt has been made to investigate the effect of ethanolic extracts of bark and leaves of *D. sissoo* in epilepsy-induced rats. The plant extracts were also tested for phytochemical screening, cytotoxicity, mutagenicity, and anti-microbial efficiency.

2. Methodology

2.1. Sample collection

The leaves and bark samples of *D. sissoo* were collected in bulk from the local vegetation of Jhelum, identified and verified by Dr. Ghazala, Associate Professor; Department of Botany; Government College for Woman, Jhelum. After verification, the samples were washed under running tap water to remove dust. The leaf and bark samples were dried in the shade for a week. Completely dried samples were finely ground into powdered form, stored in airtight bottles and labeled for subsequent use (Yasmeen and Gupta, 2016).

2.2. Extract preparation

The concentrated solution of each sample (bark, leaves) was prepared in 95% ethanol (5 g/50 mL). Sample mixture was kept overnight to yield ethanolic extracts. A mixture of both samples was then filtered using Whatman filter paper No.1. The filtrates were air dried for the evaporation of ethanol. The samples were re-dissolved in ethanol and labeled as *D. sissoo* bark (D.B.E), and leaf extract (D.L.E) (Yasmeen and Gupta, 2016).

2.3. Qualitative phytochemical screening

Qualitative analysis of extracts of *D. sissoo* bark and leaf was performed to determine the presence/absence of phytochemicals. Tests for tannins, flavonoids, saponins, terpenoids, glycosides, and anthraquinones were performed for the phytochemical screening. Proteins and reducing sugars were also determined. The standard protocols of qualitative phytochemical testing were followed (Bhattacharya et al., 2016).

2.4. Antimicrobial activity by a disc diffusion method

Antimicrobial screening of bark and leaf extracts of *D. sissoo* was performed against the selected bacterial and fungal strains. Three bacterial strains (i.e. *Staphylococcus aureus, Micrococcus leteus,* and *Enterobacter aerogenes*) and three fungal strains (i.e. *Aspergillus niger, Alternaria alternata,* and *Schizophyllum commune*) were used for antibacterial and antifungal screening of ethanolic extracts of *D. sissoo,* respectively. These strains were obtained from the microbial collection of Department of Biochemistry and Biotechnology, University of Gujrat. A disc diffusion method was used to detect the antimicrobial sensitivity of ethanolic extracts of *D. sissoo.* Briefly, three sterile discs were dipped in A (ampicillin solution), S_1 (D.B.E.) and S_2 (D.L.E.) and placed on nutrient agar (medium) containing *M. leteus, S. aureus* and *E. aerogenes* in separate Petri plates. Petri plates were then incubated at 37 °C for 24 h. After the designated time, the antibacterial activity was observed by visualizing the zone of inhibitions around discs in each plate against the three strains (Mostafa et al., 2018). For the antifungal activity, terbisil was used as a positive control (Ozcan et al., 2009). Three sterile discs were dipped in C (terbisil control solution), S_1 (D.B.E.) and S_2 (D.L.E.) and placed on medium (Potato Dextrose Agar) containing three fungal strains in separate Petri plates, which were then incubated at 28 °C for two days. The antifungal activity was then observed by visualizing the incubated Petri plates (Gul et al., 2017; Mostafa et al., 2018).

2.5. Hemolytic test

An earlier method described by Munir et al. (2016) was used to determine the hemolytic potential of *D. sissoo*. Different concentrations of ethanolic extracts of *D. sissoo* were prepared (2%, 4%, 6%, 8%, and 10%), and the percentage RBCs lysis was calculated using the following formula:

Lysis of RBCs (%) = (100 - Absorbance of sample/Absorbance of Triton X-100 × 100).

2.6. Mutagenicity test

Ames fluctuation method was performed to investigate the toxicity and mutagenicity of ethanolic extracts of *D. sissoo*. The inoculum of two mutant strains, (*S. typhimurium*; TA-98 and TA-100) were used for this activity (Razak and Aidoo, 2011).

2.7. In-vivo analysis of different parts of D. sissoo against epilepsy

Albino Wistar rats were selected for the *in-vivo* analysis of different parts of *D. sissoo* against epilepsy. *The in-vivo* study was carried out at the Department of Physiology, Government College University, Faisalabad. Rats (15) were divided into 5 groups with 3 rats in each group as follows;

Group 1: Normal group; dH_2O (2 mL/kg).
Group 2: Negative control Pilocarpine (100 mg/kg; i.p.).
Group 3: Positive control diazepam (5 mg/kg; p.o.) + Pilocarpine (100 mg/kg; i.p.).
Group 4: *Dalbergia sissoo* bark extract (500 mg/kg; p.o.) + Pilocarpine (100 mg/kg; i.p.).
Group 5: *Dalbergia sissoo* leaf extract (500 mg/kg; p.o.) + Pilocarpine (100 mg/kg i.p.).(i.p: Intraperitoneal and p.o: provide orally)

All rats were weighted and doses of each drug to be given to the rats were adjusted accordingly (Asif and Kumar, 2011). All the rats were then individually pretreated with *D. sissoo* bark and leaf ethanolic extracts and diazepam for about 30 min before injecting Pilocarpine (PC). After Pilocarpine injections (Mante et al., 2017), the rats were placed in the cage and visualized one by one for different physical parameters; onset of seizures (min), seizures duration (min), delay in seizures (min) and other epileptic symptoms (Amoateng et al., 2012; Mante et al., 2017).

2.8. Statistical analysis

Mean and standard deviation of the results (taken in triplets) were calculated.

3. Results and discussion

3.1. Qualitative phytochemical analysis

Phytochemicals are secondary metabolites that are present in plants. The phytochemical tests revealed the presence of biologically active compounds in *D. sissoo* bark and leaf extracts. The bark and leaves extracts of *D. sissoo* possessed tannins, flavonoids, saponins, reducing sugars, terpenoids, glycosides, and proteins. Glycosides play several important roles in living organisms (Saravanan et al., 2018), while saponins are being used as adjuvants during oral drug delivery (Shobha et al., 2014). However, anthraquinones were not detected in *D. sissoo* leaf extract (Table 1). The presence of phytochemicals in *D. sissoo* extracts make it effective in the treatment of different diseases. The present study has explored that most of the biologically active phytochemicals are present in this plant extracts. The medicinal properties of this plant are attributed to the presence of biologically active phytochemicals and might be used as a therapeutic to various diseases.

3.2. Antimicrobial screening of plant crude extracts

Fig. 1 portrays that the bark and leaf extracts of *D. sissoo* were found to be active against the bacterial strains. Notably, the bark and leaf extracts of *D. sissoo* had remarkable anti-bacterial activity against *E. aerogenes, M. leteus,* and *S. aureus.* The clear zone of inhibitions recorded around the discs dipped in leaf and bark extracts against these bacterial strains indicate effective anti-bacterial activities. Results of the antibacterial activity of *D. sissoo* extracts suggested that *E. aerogenes* was the most resistant bacterial strain as compared to the other two strains (Fig. 2). Moreover, *D. sissoo* bark extract showed a strong antibacterial activity as compared to the leaf extract. In various studies, the phytochemicals present in plant extracts, are found to be associated with the antimicrobial effects. These plant-based antibacterial compounds are therapeutically important because they can serve the purpose associated with the synthetic antimicrobials without any side effects (Mehta et al., 2014; Khalid et al., 2017). The bacterial *Enterobacter* strain causes various human infections including diabetes mellitus, hepatobiliary disease; chronic renal failure, ulcers of the upper gastrointestinal tract and immune-suppression (Jha et al., 2016). *Dalbergia sissoo* bark and leaf extracts showed high activity against this strain, thereby highlighting their efficiency in the treatment of these infections. The bark and leaf ethanolic extract of *D. sissoo* were also evaluated for antifungal activities. Results indicated that *D. sissoo* extracts show antifungal activities against *A. niger, A. alternate,* and *S. commune* (Fig. 3). Both extracts i.e. bark and leaf showed minimum antifungal activity against the fungal strains. Therefore, both bark and leaf extracts of *D. sissoo* are sufficiently effective in suppressing the fungal growth of all the three available strains and can be used against the infections caused by these fungal strains.

Table 1
Phytochemical screening of ethanolic extracts of *Dalbergia sissoo*.

Phytochemicals	Test	*D. sissoo* bark extract	*D. sissoo* leaf extract
Tannins	Lead acetate test	+ve	+ve
Flavonoids	Test by $FeCl_3$	+ve	+ve
Saponins	Froth test	+ve	+ve
Reducing Sugars	Fehling test	+ve	+ve
Terpenoids	Copper acetate test	+ve	+ve
Glycosides	Conc. H_2SO_4 test	+ve	+ve
Proteins	Millon's test	+ve	+ve
Anthraquinones	Born Trager's test	+ve	-ve

3.3. Hemolytic assay

The hemolytic assay was carried out by using different concentrations of *D. sissoo* bark and leaf extracts (2%, 4%, 6%, 8%, and 10%) prepared in ethanol. It was observed that the bark and leaf extracts of *D. sissoo* showed very less hemolysis of RBC's that indicated their non-toxic nature, which agrees well with the result of previous research of hemolytic potential of *D. sissoo* gum (Munir et al., 2016). PBS buffer used as a negative control shows zero hemolytic efficiencies whereas; Triton X-100 shows a hemolytic activity up to 97.7%. The results indicate that all the sample concentrations do not affect human blood RBCs and were non-toxic. Fig. 4 depicts that the leaf extract of *D. sissoo* was less toxic as compared to bark extract. Maximum hemolysis caused by leaf extract was only 5.09%, whereas, for the bark extract, the lysis was recorded up to 5.35%. Therefore, bark and leaf extracts of *D. sissoo* would be effective for the medication against different human diseases due to their non-toxicity.

3.4. Mutagenicity assay

The Ames fluctuation method was performed to investigate the mutagenicity of ethanolic extracts of *D. sissoo* by using two bacterial strains of *Salmonella typhimurium* TA98 and TA100 (Table 2). After experimentation, firstly, the blank plate was observed and then

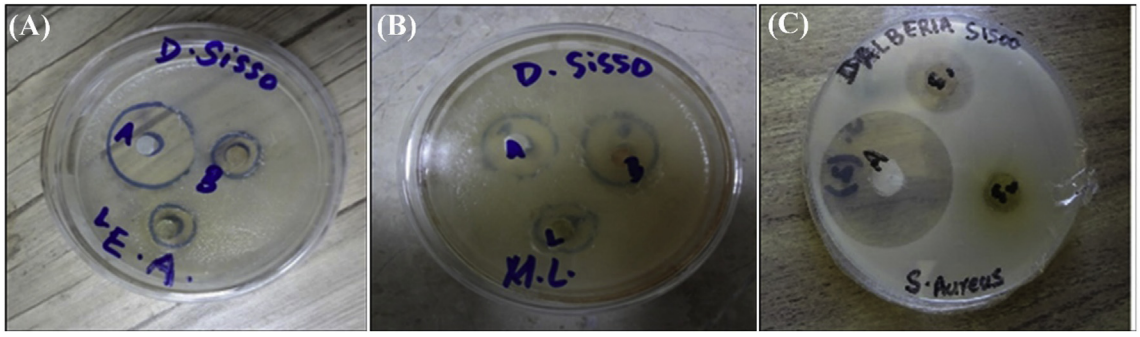

Fig. 1. Antibacterial activity of ethanolic extract (bark and leaf) of *Dalbergia sissoo* against A) *Enterobacter aerogenes*, B) *Micrococcus leteus*, and *Staphlococcus aureus*.

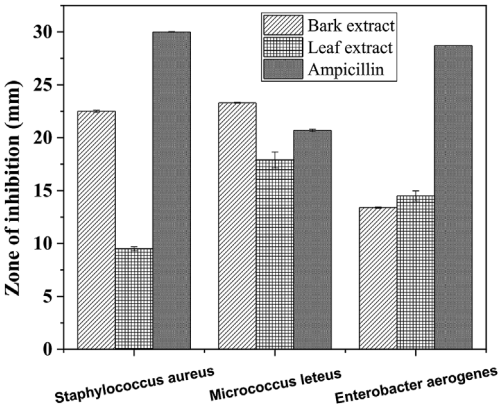

Fig. 2. Antibacterial activity of ethanolic extract (bark and leaf) of *Dalbergia sissoo* against A) *S. aureus*, B) *M. leteus* and C) *E. aerogenes*.

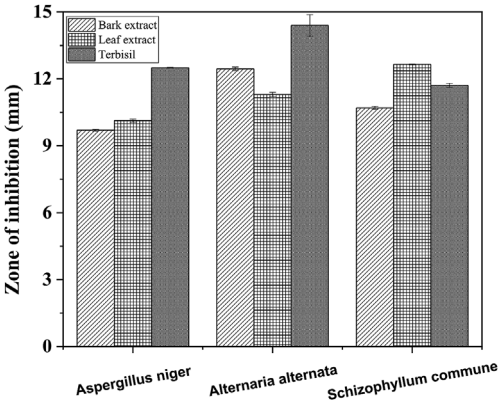

Fig. 3. Comparison of the anti-fungal activity of *D. sissoo* against A) *A. niger*, B) *A. alternate*, and C) *S. commune*.

accordingly the other plates were observed separately. Test sample 1 (*D. sissoo* bark) showed the yellowish coloration of 9 wells from 96 wells against TA98 and 5 wells from 96 wells against TA100, indicating a negligible mutagenic activity. Similarly, test sample 2 (*D. sissoo* leaf) showed the yellowish coloration of 7 wells from 96 wells against TA98 and 3 wells from 96 wells against TA100, showing its non-mutagenicity. Therefore, results indicate that none of the samples possess mutagenic activity. This same pattern was observed in a previous study of *D. sissoo* (gum extracts) reported by Munir et al. (2016).

3.5. In-vivo analysis of different parts of Dalbergia sissoo

The experimental study indicated that administration of ethanolic extracts of *D. sissoo* at a dose of 500 mg/kg, half an hour before injecting Pilocarpine, resulted in a significant delay in the onset, duration,

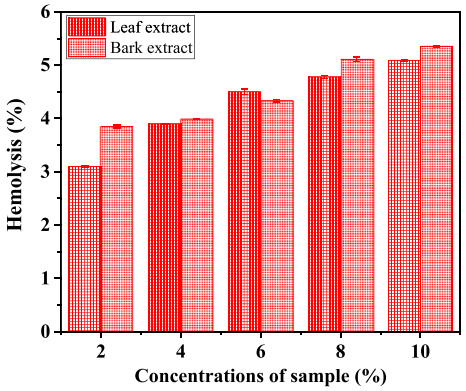

Fig. 4. Comparison of hemolytic activities of different concentrations of *D. sissoo* ethanolic extracts (bark and leaf).

Table 2
Mutagenic activity of ethanolic extracts of *Dalbergia sissoo* in Ames Fluctuation Assay.

Treatment	No. of Positive Wells/96 Wells	Results
Salmonella TA 98		
Background	20/96	–
Standard ($K_2Cr_2O_7$)	93/96	+ve
Test sample 1 (*D. sissoo* bark)	9/96	-ve
Test sample 2 (*D. sissoo* leaf)	7/96	-ve
Salmonella TA 100		
Background	25/96	–
Standard (NaN_3)	91/96	+ve
Test sample 1 (*D. sissoo* bark)	5/96	-ve
Test sample 2 (*D. sissoo* leaf)	3/96	-ve

and intensity of seizures. In negative control group 2, no cure treatment was given, therefore; after injecting PC, epileptic seizures started after about 2:19 min. Pretreatment half an hour before injecting PC, in groups 3, 4 and 5; resulted in the delay in starting of epileptic seizures. In positive control group 3, the rats were pretreated with diazepam half an hour before injecting PC, therefore, the epileptic seizures started after 5:26 min. In case of group 4 and 5, when pretreated with *D. sissoo* bark and leaf extract half an hour before injecting PC, the seizures begin after 3:28 and 4:43 min (Table 3). The bark extract of *D. sissoo* delayed the onset of seizures by 1:09 min whereas, the leaf extract delayed the onset of the seizures by 2:24 min as compared to the negative control. Moreover, *D. sissoo* leaf extract was more effective in delaying the seizures as compared to bark extract (Fig. 5). The ethanolic extracts of *D. sissoo* were not only effective in delaying the onset of seizures but were also reduced the duration of seizures as compared to Group 2. The leaf extract of *D. sissoo* was observed to be more effective in treating epileptic seizures and other symptoms as compared to the bark extract.

Fig. 5 compares the duration of seizures induced by Pilocarpine in

Table 3
Physical and Behavioral observations of the antiepileptic effect of *Dalbergia sissoo*.

Group	Group Name	Treatment Drug and Dose	Epileptic Seizures	Other Epileptic Symptoms
Group 1	Normal	dH_2O (2 mL/kg)	No seizures	–
Group 2	-ve control	Only Pilocarpine (100 mg/kg)	Intensive seizures	Head nodding, mouth frothing, forelimbs, and hind-limbs jerks.
Group 3	+ve control	Diazepam (5 mg/kg) + Pilocarpine (100 mg/kg)	Very mild seizures	Head nodding only.
Group 4	Treatment group	D.B.E. (500 mg/kg) + Pilocarpine (100 mg/kg)	Mild and less intensive seizures	Head nodding, mouth frothing, forelimb jerks and rubbing.
Group 5	Treatment group	D.L.E. (500 mg/kg) Pilocarpine (100 mg/kg)	Mild and less intensive seizures	Head nodding, mouth frothing.

different treatment groups. The height of the peaks shows the duration of seizures, whereas the color indicates the intensity of seizures. In Group 2 (Negative group): seizures lasted for about 53:25 min and were violent as shown by dark green color. Similarly, in Group 3 (Positive group): the seizures persisted only for about 13:06 min indicating its high efficiency in controlling seizures. In Group 4 (D.B.E. treated group); the seizures duration was significantly reduced to 37:03 min and a significant reduction in seizure's intensity was observed. Lastly, the seizures duration was reduced to approximately 47 min in Group 5 (D.L.E. treated group) with a great reduction in the intensity of seizures. In Pilocarpine-induced seizure models, the seizures were violent and lasted for a long time. While pre-treatment with ethanolic extracts of *D. sissoo,* there was a significant reduction in the intensity and duration of seizures. The results unveiled that the *D. sissoo* extracts treatment has a significant effect on the nervous system against the PC-induced seizures showing their anticonvulsant properties in rat models. In addition, the findings revealed that the pretreatment with the *D. sissoo* extracts delayed the onset of seizures as well as shortened the time duration of the seizures induced by the PC. In the present study, the anti-epileptic effect of *D. sissoo* extracts was compared with diazepam (antiepileptic drug). Diazepam prevents the development of electroencephalography and behavioural alterations induced by pilocarpine as well as the subsequent neuropathological alterations in pilocarpine-induced rats (Curia et al., 2008), while pilocarpine induces status epilepticus, via a primary peripheral effect on white blood cells, leading to elevated serum levels of IL-1β, which in turn alters BBB permeability (Vezzani, 2009).

Interestingly, the *D. sissoo* leaf extract had greater anticonvulsant effect as compared to *D. sissoo* bark extract. However, the anti-epileptic effect of *D. sissoo* extracts was less but significant as compared to diazepam. Mante et al. (2017) studied the anti-epileptic effect of *Antiaris toxicaria* aqueous extract in PC-induced seizures in male Wistar rats. They reported that *A. toxicaria* produced a significant anti-epileptic effect on the latency, onset, and duration of seizures. Similar results were also observed by *D. sissoo* in albino Wistar rats. Drugs from plant sources have been used for the treatment of various diseases since ancient times. Nowadays, the use of herbal drugs to cure inflammation and pain is gaining popularity due to their effectiveness, fewer side effects, low cost, and availability. Non-steroidal anti-inflammatory drugs act by inhibiting cyclooxygenase and the production of prostaglandins. The presence of flavonoids has been reported in Dalbergia species, and flavonoids are known to inhibit prostaglandin synthetase (Asif and Kumar, 2009).

3.6. Comparison of Dalbergia sissoo with first Marijuana approved drug against epilepsy

Epidiolex is the first plant-based epileptic drug derived from marijuana on 27th June 2018. Earlier research evaluated the anti-epileptic effect of Marijuana in PTZ-induced seizures rat models. Results showed that different doses of Marijuana delayed the onset of seizures in rats as compared to control PTZ (Fig. 6). At a dose of 400 mg/kg of Marijuana, the delay in the onset of seizures was 84.7 s Whereas, at doses of 600 and 800 mg/kg of Marijuana, the delay in onset of seizures was 77 and 129 s versus onset of seizures in the control PTZ treated rats (Namvar et al., 2016). Later on, Cannabidiols were extracted from Marijuana and first plat based epileptic drug Epidiolex was made and approved by the FDA. Comparing the above results of Marijuana with our results, *D. sissoo* leaf was found to be more effective in delaying the onset of seizures as compared to marijuana. A dose of 500 mg/kg of *D. sissoo* leaf extract delayed the onset of seizures by 144 s versus the control Pilocarpine. Overall, the results demonstrated that the *D. sissoo* plant has anti-epileptic properties. As the synthetic anti-epileptic drugs have side effects in the long term, in such cases it is safe to conclude that the use of *D. sissoo* plant can be effective in preparing an epileptic drug like Epidiolex in near future.

4. Conclusions

In this investigation, the antimicrobial, cytotoxicity, mutagenicity and anti-epileptic potential of ethanol extracts (bark and leaf extracts) of *Dalbergia sissoo* were evaluated in epilepsy-induced rats. The *D. sissoo* extracts showed potential antibacterial and antifungal properties against various pathogenic bacterial and fungal species owing to the presence of various phytochemical constituents. In addition, the ethanolic extracts showed marked protective activities against Pilocarpine-induced seizures in rat models without exhibiting any cytotoxicity and mutagenicity. However, extensive studies are required to evaluate the

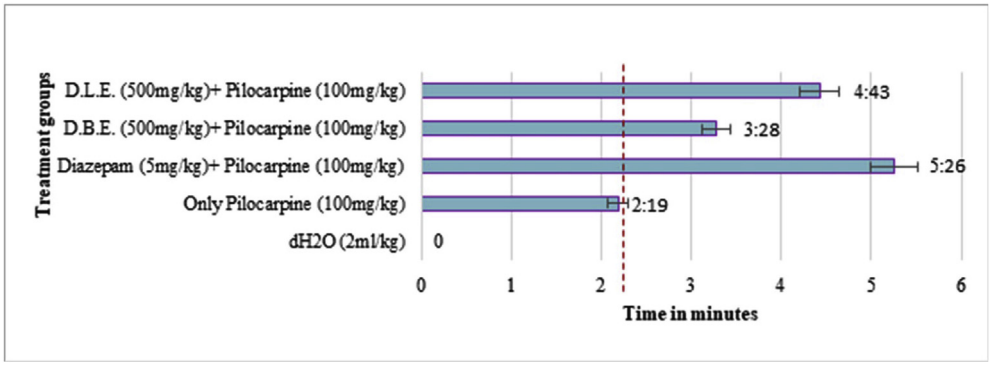

Fig. 5. Onset of seizures after injecting PC in different treatment groups.

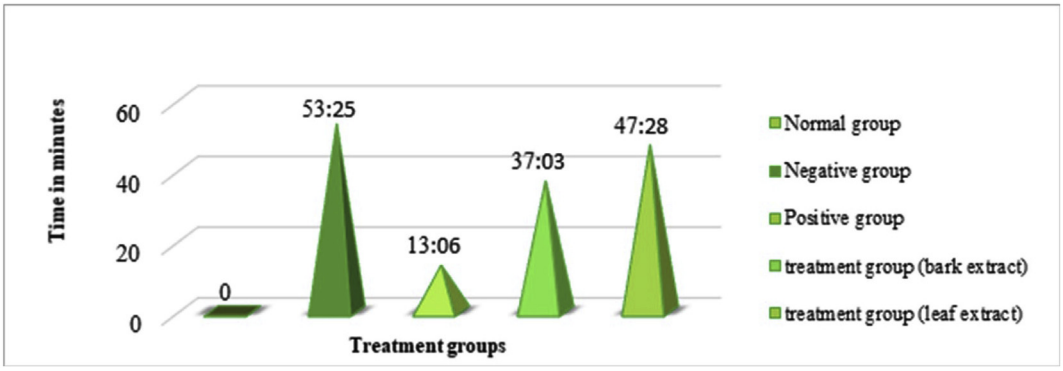

Fig. 6. Comparison of seizures duration in the treatment groups.

precise mechanism and the safety profile of *D. sissoo* as a therapeutic remedy for epilepsy disorders. In conclusion, this research work supports the folkloric use of this plant in Pakistan for the treatment of epilepsy in a safer and patient-friendly way.

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