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# Evaluation of Anticonvulsant Activity of *Prunus amygdalus* Batsch Kernels in Experimental Animal Model

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## Abstract
*Prunus amygdalus* batsch kernels are traditionally used as a remedy for various diseases. The aim of the present study is to evaluate the anticonvulsant activity of Prunus amygdalus batsch kernels in mice. The fine suspension of *Prunus amygdalus* kernels was used for assessing the anticonvulsant activity using various method by oral administration of two different doses of the extract (200mg/kg p.o and 400 mg/kg p.o). Preliminary phytochemical screening was carried out for determining the chemical constituents present in it. The anticonvulsant activity was evaluated by Maximal electroshock (MES) induced convulsion and Pentylenetetrazole (PTZ) induced convulsion. Biochemical parameters such as malondialdehyde (MDA) and reduced glutathione (GSH) were estimated in the isolated brain after completion of the anticonvulsant studies. The datas obtained from the results showed that the suspension possesses anticonvulsant activity at different doses of the extract (200mg/kg p.o and 400 mg/kg p.o) in a dose dependent manner at *P<0.05, **P<0.01, ***P<0.001 when compared with the positive control. Maximum activity was observed in 400mg/kg p.o. Flavanoids present in the kernels which possess antioxidant and anticonvulsant effect may be responsible for the anticonvulsant activity shown by the plant extract.

**Keywords**
anticonvulsant activity, Glutathione, Malondialdehyde, Maximum electroshock model, Pentylenetetrazole, *Prunus amygdalus* batsch.

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## INTRODUCTION
Epilepsy is a neurological disorder characterised by the occurrence of periodic seizures. Abnormal activity in brain results in the occurrence of behavioural alterations called seizures. As a result of an epidemiological studies, there are over 60 million people are affected by epilepsy and approximately 0.25 million new cases are added to this figure on yearly basis. About 28-30% of patients are unaffected to the available anticonvulsant drugs. Apart from that majority of patients suffer from a lot of problems like hepatotoxicity, gingival hyperplasia, depression, neurotoxicity and related CNS diseases as side effects. [1-2]

*Prunus amygdalus* batsch belonging to family Rosaceae is a deciduous tree widely seen in

Mediterranean climates with dry, warm summers and wet winters. The kernels of the plant possess wide variety of pharmacological activities like antistress, immunostimulant, lipid lowering, laxative activity and is highly useful in preserving vitality of the brain. Chemical constituents like Flavanoids and terpenoids are extracted from this plant. The literature survey reveals that *Prunus amygdalus* batsch kernels are conventionally used as nervine tonic in various ailments however not yet validated by scientific methods using animal experimental models. Hence, the current study is undertaken to evaluate the Anticonvulsant activity of the plant kernels in experimental animal models. [3-4].

## MATERIALS AND METHODS

### Plant materials and preparation of suspension

*Prunus amygdalus* batsch kernels were collected from local market of Iraq in July 2019. Identification and authentication was done by Dr. Sandhya P, Head of the Department of Botany, NSS College, Pandalam, Pathanamthitta district, Kerala. The herbarium specimen with voucher number BOHDOS-3/27/11/2020 was deposited at the same college for reference.

The husk of the plant kernels was removed initially, and dust particles were removed by thorough cleaning. Fine paste of *Prunus amygdalus batsch* kernel (PAS) suspension was prepared in distilled water by sonication for 20 minutes. The fine suspension was stored in airtight container in 2-8 degree Celsius and was left to room temperature before administration. [5]

### Preliminary phytochemical screening

The phytochemical screening of the suspension was performed to observe the presence and absence of different phytoconstituents like carbohydrates ( Molish's test, Benedicts test, Fehling's test), alkaloids (Mayer's test, Wagner's test, Dragondroff's test, Hager's test), glycosides (Modified Borntrager's test, Legal's test, Baljet's test, Keller killiani test), saponins (foam test), Flavanoids (Shinoda test, alkaline reagent test), phenolic compounds and tannins (ferric chloride test), phytosterols and terpenoids (Liberman Burchard's test), proteins (biuret test) according to standard methods. [6-7]

### Chemicals and reagents

Pentylenetetrazole (PTZ), phenytoin, diazepam and DTNB were purchased from SRL Chemicals (Mumbai, India). Thiobarbituric acid was purchased from LOBA CHEMIE (Mumbai, India). Trichloroacetic acid purchased from ISOCHEM laboratories (Coimbatore, India).

### Animals

Healthy Swiss albino mice (15-20g) were procured from Government veterinary college, Mannuthy, approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). All animals were kept in propylene cages and reared at the animal house of St. Joseph's College of Pharmacy, Cherthala. The animals were maintained in a well-ventilated room with conductive environmental condition i.e; temperature (22 ±2 °C), humidity (45 ± 5 °C), and 12-hour dark and light cycle and fed *ad libitum* with normal laboratory chow standard pellet diet. All the studies were conducted in accordance with the guidelines approved by Institutional Animal Ethics Committee.

### Acute toxicity study

The acute toxicity study of *Prunus amygdalus* kernels was determined as per the OECD guideline and it is observed that there is no toxicity upto 2000mg/kg dose. Hence, 200mg/kg (approx. $1/10^{th}$ of 2000mg/kg body weight) and a high dose (twice that of $1/10^{th}$ dose) 400mg/kg body weight were selected for studies. [5]

### Evaluation of anticonvulsant activity

### Maximal electroshock (MES) induced convulsions

Twenty-four Swiss albino mice were selected and divided into four groups containing six animals each. Animals in group I received vehicle (water, 10 ml/kg p.o.) and served as disease control whereas standard drug phenytoin (25mg/kg i.p) was administered to animals in group II. Mice in group III and IV received test drug at the doses 200 mg/kg p.o. and 400mg/kg p.o. respectively. The test and standard drugs administered 30 minutes prior to MES induction. Electric current of 150 Ma for 0.2 s was administered to all the experimental animals through ear electrodes with the aid of electroconvulsiometer (ORCHID SCIENTIFIC). The animals were subjected to observation for 2 minutes. The time duration in various phases of convulsion were noted and compared to the control. Abolition of hind limb tonic extension was recorded as a measure of protection against MES induced seizure. [8]

### Pentylenetetrazole (PTZ) induced convulsion

Thirty mice were selected and allotted into four groups containing six animals each. Animals in group I received vehicle (water, 10 ml/kg p.o.) and served as normal control whereas in group II, animals were treated with vehicle (water,10 ml/kg p.o.) and served as disease control. Standard drug diazepam (5mg/kg i.p) was administered to animals in group III. Mice in group IV and V received test drug at the doses 200 mg/kg p.o. and 400mg/kg p.o. respectively. The duration of the study is 14 days. On the $14^{th}$ day after 60 minutes of water, diazepam, and test drug

administration, PTZ administer to all groups except the normal control group. The onset and duration of convulsion were noted. The anticonvulsant activity was assessed by the ability to decrease the onset and duration of convulsion. [9-10]

**Estimation of biochemical parameters**

**Collection of brain tissue and preparation of homogenates**

After the study period, animals were exposed to deep ether anaesthesia and sacrificed. For the preparation of homogenate, whole brain was dissected out and washed with ice-cold sodium phosphate buffer. Using a homogenizer brain tissue was homogenized with 10 times sodium phosphate buffer and it was centrifuged in a high speed refrigerated centrifuge (KEMI) at 3000rpm for 15 minutes. The resulted supernatant was used for the estimation of malondialdehyde (MDA) and reduced glutathione (GSH). [1]

**Estimation of malondialdehyde**

The MDA level was determined by Esterbauer and Cheeseman method, which includes the reaction of Thiobarbituric acid (TBA) and MDA or MDA-like substance at Ph 2-3 at 90$^{o}$C for 15minutes. Here formation of a pink pigment having 532 nm absorption maxima is the product of the reaction. In this reaction, the protein was precipitate by mixing the sample with 2 volumes of cold 10% (w/v) trichloroacetic acid. After that the supernatant was obtained by centrifugation and it was reacted with an equal volume of 0.67% (w/v) TBA in a boiling water bath for 10 minutes. After cooling, the absorbance was taken at 532nm in UV spectrophotometer at room temperature against appropriate blank. The concentration of MDA was estimated using the molar extinction coefficient (ε =1.53×105 $M^{-1}cm^{-1}$). The results were expressed as nmol per g protein in brain tissues. The concentration of MDA was calculated using the following formula. [1]

The concentration of MDA = Absorbance at 532nm X **D /L x ε**

Where,

L: Light path (1cm).

ε: 1.53 x 105 $M^{-1}Cm^{-1}$.

D= Total volume/ volume of sample [11-12]

**Estimation of reduced glutathione**

The reduced GSH in brain was determined by Ellman method (Ellman 1959). A 1ml brain supernatant along with 1% trichloroacetic acid were subjected to precipitation for 5-10 minutes. Then the samples underwent centrifugation at 1200×*g* for 15 min at 4°C. After centrifugation, 1ml of supernatant was mixed with 2,7ml of phosphate buffer (0.1 M, Ph 8) and 0.2 ml of 5, 5'- dithio-bis-2-nitrobenzoic acid (DTNB). Formation of yellow colour was occurred, and it is measured immediately at 412 nm using UV spectrophotometer. The result was expressed in terms of nmol per mg protein. [1]

**Statistical evaluation**

Results are expressed as mean ± SEM. Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Dunnett's post hoc multiple comparison test using software graph pad prism version 8.

## RESULTS AND DISCUSSIONS

**Phytochemical screening**

The preliminary phytochemical screening of *Prunus amygdalus batsch* kernel suspension showed that the kernels possess flavanoids, glycosides, proteins and amino acid, tannins, carbohydrates, steroids, phenols and volatile oils.

**Anticonvulsant activity**

**Effect of PAS on MES induced seizures**

MES method is generally employed for the determination of the anticonvulsive effect in grand mal epilepsy which is related to intensity of current stimulus and the dose. There are four phases for convulsion i.e, flexion, extensor, clonus and stupor. The decrease in the duration of hind limb extension was indicated as a protective effect. A dose dependent decrease in duration of various phases of convulsion was observed in *Prunus amygdalus* suspension treated groups.

The data obtained from the results have shown that the groups treated with PA suspension 400mg/kg. p.o and 200mg/kg. p.o significantly decreased the duration of flexion ($P<0.01$, $P<0.05$) hind limb extension ($P<0.01$, $P<0.05$), clonus ($P<0.01$, $P<0.05$) and stupor ($P<0.01$, $P<0.05$) phases in a dose dependent manner compared to the control respectively. However, phenytoin treated animals showed 100% protection against MES induced hind limb tonic extensor phase. The results suggest that PA suspension possess anticonvulsant effect against grandmal epilepsy.

**Effect of PAS on PTZ induced seizures**

In PTZ model, the different parameters noted were the onset and duration of clonic convulsions, and the percentage protection of convulsions relative to control group. The ability to abolish clonic seizures is considered as an indicator for anticonvulsant activity. All groups treated with Pentylenetetrazole (60 mg/kg i.p) were compared with the diseased control group. The test suspension at the doses 400mg/kg and 200 mg/kg significantly ($P<0.01$, $P<0.05$) increased the time of onset of convulsions, decreased the duration of seizures, increased the % protection and decreased the % mortality in a dose

dependent manner. The groups treated with PA suspension 400mg/kg. p.o, delayed the onset of convulsion, significantly reduced the duration and reduced mortality to 83.33 % and group treated with 200mg/kg.p.o. delayed the onset of convulsion, decreased the duration of seizures and reduced mortality to 66.67 %. 100% protection was observed in groups treated with diazepam 5mg/kg. The result from the study reveals that PAS possess protective activity against petitmal epilepsy

**Effect of PAS on malondialdehyde (MDA) level**

The MDA level was dramatically increased in the brain of diseased control group in PTZ model. Diazepam significantly supressed this increase (P<0.001). PA suspension treated groups show reduced MDA levels in a dose dependent manner. The test suspension at doses of 200mg/kg.p.o. and 400mg/kg.p.o. significantly reduced the MDA level at P<0.01 and P<0.001 respectively. The groups treated with PAS 400mg/kg.p.o. showed more activity than the group treated with 200mg/kg.p.o.

**Effect of PAS on reduced glutathione (GSH) level**

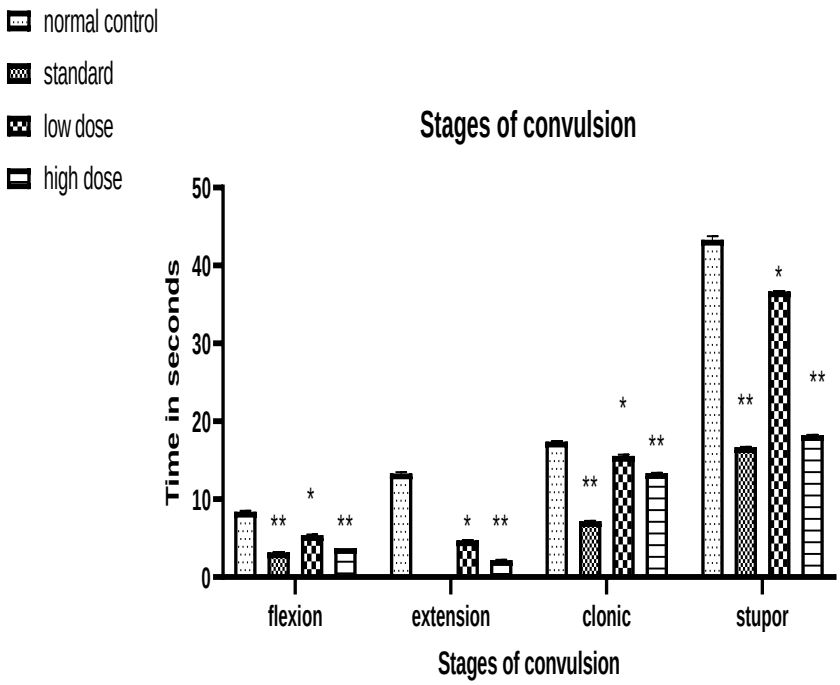
There was a significant decline in GSH level in the brain of diseased control group in PTZ model. Diazepam (P<0.001) and PA suspension treated groups significantly increased the GSH levels in a dose dependent manner. The test suspension at doses of 200mg/kg. p.o and 400mg/kg. p.o significantly increased the GSH level at**P<0.01 and ***P<0.001respectively. The groups treated with PAS 400mg/kg. p.o showed more activity than the group treated with 200mg/kg.p.o.

**Table 1: Effect of test drug (200 and 400 mg/kg) on the MES induced convulsion in mice.**

| Group | Flexion (sec) | Extension (sec) | Clonic (sec) | Stupor (sec) |
|---|---|---|---|---|
| Disease control (10ml/kg water, p.o) | 8.4 ± 0.13 | 13.3 ± 0.18 | 17.4 ± 0.07 | 43.3 ± 0.46 |
| Standard (25mg/kg phenytoin, i.p) | 3.2 ± 0.01** | _ | 7.2 ± 0.02** | 16.7 ± 0.04** |
| Low dose (200mg/kg test, p.o) | 5.4 ± 0.07* | 4.72 ± 0.02* | 15.57 ± 0.15* | 36.7 ± 0.01* |
| High dose (400mg/kg test, p.o) | 3.72 ± 0.01** | 2.18 ± 0.06** | 13.66 ± 0.03** | 18.23 ± 0.02** |

Experimental data in table 1 were expressed as Mean ± S.E.M. The difference between experimental groups was compared by One-way Analysis of Variance (ANOVA), followed by Dunnett's Multiple Comparison Test. The results were considered statistically significant when *P<0.05, **P<0.01, ***P<0.001 compared with disease control.

**Graph 1: Effect of PA suspension on MES induced seizure**



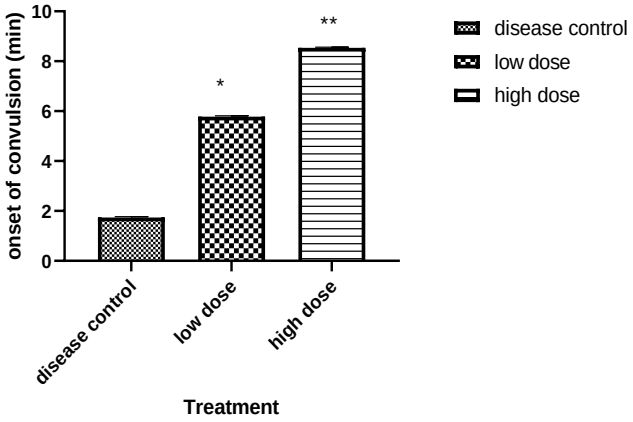

*P<0.05, **P<0.01

**Table 2: Effect of Prunus amygdalus batsch kernels suspension (200 & 400 mg/kg) on the PTZ-induced convulsion in mice**

| Group | Onset of convulsion (min) | Duration of convulsion (min) | Number of deaths | Number of recoveries | Percentage protection (%) |
|---|---|---|---|---|---|
| Normal control (10ml/kg water, p.o) | - | - | - | - | - |
| Disease control (10 ml/kg water, p.o) | 1.74 ± 0.03 | 4.19 ± 0.04 | 6 | 0 | 0 |
| Standard (5 mg/kg diazepam, i.p) | _ | _ | 0 | 6 | 100 |
| Low dose (200 mg/kg test, p.o) | 5.78 ± 0.03* | 2.26 ± 0.01* | 2 | 4 | 66.67 |
| High dose (400 mg/kg test, p.o) | 8.54 ± 0.02** | 1.16 ± 0.02** | 1 | 5 | 83.33 |

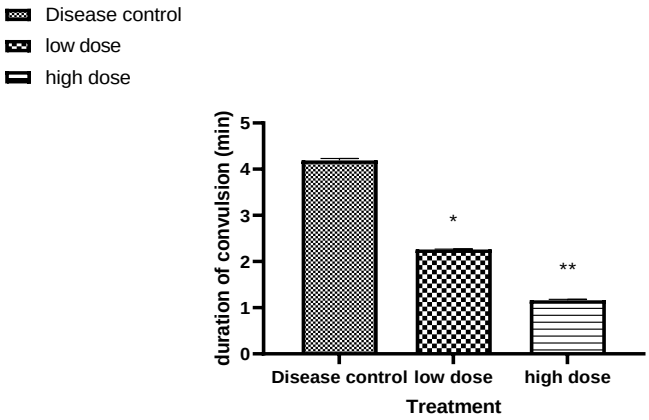
Experimental data in **table 2** were expressed as Mean ± S.E.M. The difference between experimental groups was compared by One-way Analysis of Variance (ANOVA), followed by Dunnett's Multiple Comparison Test. The results were considered statistically significant when *P<0.05, **P<0.01, **P<0.001 compared with diseased control.

**Graph 2: Effect of PA suspension on onset of convulsion in PTZ induced seizures in mice.**




*P<0.05, **P<0.01

**Graph 3: Effect of PA suspension on duration of convulsion in PTZ induced seizures in mice.**



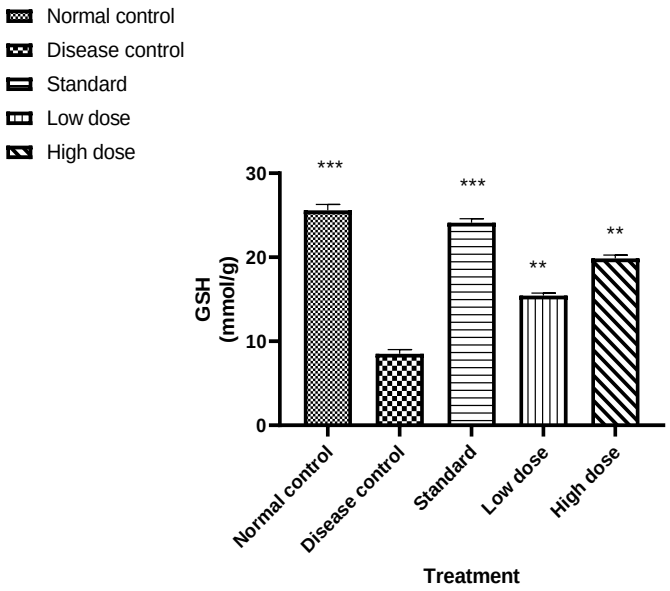

*P<0.05, **P<0.01

**Table 3: Effect of *Prunus amygdalus batsch* kernels suspension (200 & 400 mg/kg) on malondialdehyde (MDA) level and glutathione (GSH) level**

| Group | GSH (mmol/g) | MDA (nmol/mg) |
|---|---|---|
| Normal control | 25.59 ± 0.70*** | 22.54 ± 0.60*** |
| Disease control | 8.50 ± 0.52 | 36.70 ± 0.80 |
| Standard | 24.11 ± 0.47*** | 23.29 ± 0.31*** |
| Low dose (200 mg/kg) | 15.44 ± 0.30** | 31.80 ± 0.19** |
| High dose (400 mg/kg) | 19.85 ± 0.40*** | 28.18 ± 0.42*** |

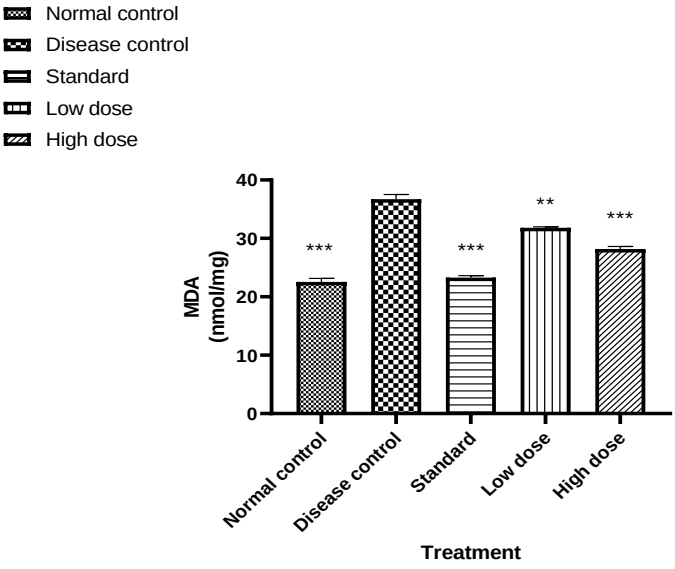
Experimental data in **table 3** were expressed as Mean ± S.E.M. The difference between experimental groups was compared by One-way Analysis of Variance (ANOVA), followed by Dunnett's Multiple Comparison Test. The results were considered statistically significant when *P<0.05, **P<0.01, ***P<0.001 compared with diseased control group.

**Graph 4: Effect of PA suspension on Glutathione (GSH) level**




*P<0.05, **P<0.01, ***P<0.001

**Graph 5: Effect of PA suspension on malondialdehyde (MDA) level**




*P<0.05, **P<0.01, ***P<0.001

## CONCLUSION

Since ancient times, *prunus amygdalus batsch* kernels belonging to family Rosaceae were traditionally employed as a nervine tonic in various ailments. The present study involves investigation on the anticonvulsant activity of *Prunus amygdalus batsch* kernels in Swiss albino mice. The fine suspension of the test drug was evaluated for its anticonvulsant activity using its low dose and high dose i.e; 200 mg/kg. p.o and 400 mg/kg.p.o. The methods opted for the evaluation of anticonvulsant activity are Maximum electroshock model and Pentylenetetrazole model. These two models represent the Generalised seizure and Absence seizure. Biochemical evaluation also performed to evaluate the oxidative stress after PTZ administration. Preliminary phytochemical screening of Prunus amygdalus batsch kernels revealed the presence of flavonoids, phenols, tannins, terpenoids and volatile oil.

In MES test, the decrease in the duration of hind limb extension produced by the suspension was considered as a protective effect. The high dose of suspension produces more protective effect than low dose.

In PTZ test, the groups treated with PA suspension 400mg/kg. p.o, delayed the onset of convulsion, significantly reduced the duration and reduced mortality than the group treated with 200mg/kg.p.o. The major causes responsible for producing the neuronal changes which mediate the behavioural deficits in neurodegenerative disorders are free radical and oxidative stress. Antioxidants are effective in rodent models of epilepsy, stroke and Alzheimer's disease. The suspension at doses of 200mg/kg.p.o. and 400mg/kg.p.o. significantly reduced the MDA level whereas the suspension at doses of 200mg/kg p.o. and 400mg/kg. p.o. significantly increased the GSH level. The groups treated with PA suspension 400mg/kg. p.o. showed more protective activity than the disease control group.

In conclusion, these findings suggest that the anticonvulsant effect of the kernels may be attributed to the recorded phytoconstituents of the plant especially the Flavanoids, terpenoids and their antioxidant actions.

Majority of currently available anticonvulsant drugs are not able to cure the underlying disease process as well as epileptic patients are become highly refractory to current therapies. This situation demands the emergence of various new drugs, that could solve the underlying disease process of epileptogenesis. Chemical constituent flavanoids has a main role in GABA-A receptors as it has the ability to bind with the receptor as a ligand, that shows its wide chance to be used as an anticonvulsant medication.

**Compliance with ethical standard**

All animal experiments were approved by Institutional Animal Ethical Committee (IAEC). Proposal number is SJCP/IEC/2020/12/16.

## REFERENCES

1. Talha Jawaid, Mehnaz Kamal, Richa Singh, Deepa S, Vidya D, Mukty S. Anticonvulsant, and neuroprotective effects of methanolic extract of *Cinnamomum camphora* leaves in rat brain. Oriental pharmacy and experimental medicine.2018;18(1):1-10.
2. Krishna M C, Durraivel S, Madhan M E. Antiepileptic activity of *Alstonia scholaris* LINN. On MES, PTZ and Strychnine induced convulsions in rats. International journal of pharmacy and biological sciences.2016;6(1):207-213.
3. Ali J E, Rashid J, Rana J E. The importance of almond (Prunus amygdalus L) and its by-products. Food chemistry.2010;120(2):349-360.
4. Abdullah. Mohd K, Mohd K H. Badam (*Prunus amygdalus* Bail.): A Fruit with Medicinal Properties. International journal of herbal medicine. 2017;5(5):114-117.
5. Kirti. S. Kulkarni, S B kasture, S A Mengi. Efficacy study of Prunus amygdalus (almond) nuts in scopolamine-induced amnesia in rats. Indian Journal of Pharmacology.2010; 42(3): 168-73.
6. Kokate CK. Practical Pharmacognosy. Vallabh Prakasham Delhi, 5th Edition 1991:107-121.
7. Jayaram J. Laboratory Manual in Biochemistry. New age International (P) Ltd, 1st Edition 1981:51.
8. Balakrishnan S, Pandhi P, Bhargava V K. Effects of Nimodipine on the efficacy of commonly used antiepileptic drugs in rats. Ind J Exp Biol. 1998; 36:51-54.
9. Swinyard EA. Laboratory evaluation of antiepileptic drugs: review of laboratory methods. Epilepsia. 1969; 10:107-19.
10. Kulkarni SK. Handbook of Experimental Pharmacology. Vallabh Prakashan, 3rd Edition 1999: 131-134.