IJBPAS, July, 2015, 4(7): 5021-5033

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

ANTICONVULSANT ACTIVITY OF THE AQUEOUS SEED FRACTION OF *CASSIA FISTULA* LINN. (FABACEAE) IN ICR SWISS ALBINO MICE

ANNE CATHERINE V. BARRO[1], MICHELLE Y. MORCO[1], NOLIN MERIBEL R. REALINGO[1], CATHRINA JANE M. SORE[1], MINE C. TAGRA[1], CEYSHELLE ANGELICA D.C. TAN[1]AGNES L. CASTILLO[1, 2, 3]

[1]Faculty of Pharmacy, University of Santo Tomas, Manila, Philippines

[2]Research Cluster for the Natural and Applied Sciences, University of Santo Tomas, Manila, Philippines

[3]Graduate School, University of Santo Tomas, Manila, Philippines

ABSTRACT

Epilepsy is the most widespread neurological problem and is manifested in erratic and periodic disturbance of neurologic activity called seizures. A preclinical study was performed to investigate the anticonvulsant activity of the aqueous seed fraction of *Cassia fistula* Linn. in ICR Swiss albino mice using Maximal Electroshock-induced (MES) and Strychnine-induced (STN) seizure models. The crude ethanolic extract, which was obtained by percolation, was fractionated using distilled water. The acute toxicity of the aqueous fraction was assessed. Then, three different doses (200 mg/kg, 400 mg/kg, and 800 mg/kg) of the non-toxic experimental fraction were administered intraperitoneally (i.p.). Duration of tonic-clonic seizures, percent protection from Tonic Hind Limb Extension (THLE) up to 10 seconds, and mortality were observed in the MES (0.2 seconds, 50 mA) model while latency, duration of tonic-clonic seizures, percent protection from tonic-extensor jerks, and mortality were monitored in the STN-induced (2 mg/kg; i.p.) model up to 30 minutes after induction of convulsion.

All doses of *C. fistula* aqueous seed fraction exhibited a protective effect against seizures by significantly decreasing the mortality caused by MES-induced convulsions ($p=0.021$). Also, the 400 mg/kg dose significantly decreased the occurrence of convulsions induced by STN ($p=0.009$) and showed potential to delay the latency of STN-induced seizures and decrease

the duration of convulsions induced by both seizure models. The results were expressed as median [IQR], mean ± SEM and count data (%) and were statistically analyzed by Kruskal-Wallis test, one-way ANOVA, and Generalized Fisher's Exact Test, respectively. Finally, phytochemical screening revealed the presence of flavonoids and saponins. The results acquired suggest that the aqueous seed fraction of *C. fistula* is a potential source for anticonvulsant agents and possesses anticonvulsant activity against MES-induced and STN-induced seizures in mice.

Keywords: Anticonvulsant, *Cassia fistula,* Maximal Electroshock, Strychnine

INTRODUCTION

Cassia fistula Linn. (Fabaceae) is a well-known tree for its medicinal properties. In folk medicine, it is used to treat tumors, and to relieve burns, convulsions, dysuria and gastrointestinal irritation. *C. fistula* is recognized as a carminative and laxative in Ayurvedic medicine [1].

C. fistula is one of the most preferred ethnomedicinal plants of the Bhoxa community used internally as a remedy for epilepsy in India [2]. The synergistic action of the metabolite production of *C. fistula* Linn. is primarily responsible for the plant's medicinal effects, which causes it to be a treatment for various conditions by people all over the world [3]. It is further emphasized that these biologically active chemical substances are responsible for the therapeutic potentials and form the foundations of modern prescription drugs [4]. *C. fistula* aqueous seed extract affects the central nervous system (CNS) by promoting sedation and reducing the level of anxiety in rats [5]. However, no pharmacological studies were performed to prove the anticonvulsant (AC) and antiepileptic (AE) properties of *C. fistula* [2].

Epilepsy is the most widespread serious neurological problem affecting 50 million people worldwide in 2004 [6]. In fact, a study confirmed that there are 230 in every 100,000 people suffering from epilepsy in the Philippines in 2003 [7].

Epilepsy is a disorder manifested in erratic and periodic disturbance of neurologic activity, called epileptic seizures. Epilepsy has diverse causes that may reveal underlying brain disease [8]. Seizures or convulsive episodes are an important feature of epilepsy, brain tumor, choking, brain infection, and fever (notably in children) [9].

Despite the availability of antiepileptic drugs (AEDs), there exists a resistance of over 30% patients [10]. Moreover, is it noted that most of these drugs have severe and dose-limiting adverse effects such as

chronic toxicity and teratogenicity [11]. Thus, there is a need to search for alternative medicines that suggest natural effects, low toxicity, and minimal or no adverse/side effects.

In this study, the researchers opted to evaluate the anticonvulsant activity of the aqueous seed fraction of *C. fistula* based on study findings that it has the potential to affect the CNS and is used as a remedy for epilepsy in ethnic communities [12]. The aqueous seed fraction was tested to inhibit physically and chemically induced seizures using Maximal Electroshock (MES) and Strychnine (STN), respectively, in animal models based parameters of latency time, duration, percent protection, and mortality.

MATERIALS AND METHODS

Equipment

An Electroconvulsiometer (ECT) was purchased at Orchid Scientifics and Innovatives India PVT. LTD. and was used for inducing convulsion to the test animals in the MES model. The activities of the test animals were recorded using a video camera.

Acquisition and Preparation of Plant for Extraction

The fruit of *C. fistula* was collected in Barangay Calaylayan, Abucay, Bataan. The plant material was authenticated at the UST Herbarium of the Research Center for the Natural and Applied Sciences (UST-RCNAS), University of Santo Tomas, España, Manila.

Extraction of Plant

Ground seed (336 g) was defatted using 1 liter of petroleum ether. The petroleum ether extract was collected after 24 hours. The defatted plant material was then extracted to exhaustion using 80% ethanol through percolation. A rotary evaporator apparatus was used to concentrate the ethanolic crude extract at a temperature not exceeding 50°C [13].

Fractionation

The dried ethanolic crude extract (40 g) was subjected to fractionation. The crude extract of *C. fistula* was suspended in 200 mL distilled water and DCM (150 mL x 3) using a separatory funnel. The aqueous fraction was collected and dried using a rotary evaporator and water bath at 40°C and was stored at 4° [13].

Test Animals

A total of 55 male ICR Swiss albino mice, weighing 23 to 38 g, were used for acute toxicity and anticonvulsant tests. The test animals were purchased at the Food and Drug Administration (FDA) and were housed at the UST-RCNAS Animal House in UST, España, Manila with IACUC approval code number of RC2014-1100725.

They were acclimatized for a duration of seven days prior to experimentation. The

animal house temperature was maintained at 25°C (± 3°C), with artificial lighting having the sequence of 12-hr light/12-hr dark cycle and humidity not exceeding 70% [14]. Diet of the test animals was based on the laboratory diet for rodents with an unlimited supply of water.

After acclimatization, five test animals were used for the limit test of the acute toxicity test. The remaining 50 test animals were divided into three groups, namely, the positive group, negative group, and experimental group. The group was further subdivided into 10 subgroups based on their treatment and seizure model. Each subgroup comprised of five mice. The experimental group received different doses (200 mg/kg, 400 mg/kg, and 800 mg/kg) of *C. fistula* aqueous seed fraction as drug treatment. NSS (10 mL/kg) was used for the negative control group. Diazepam (4 mg/kg) and Phenytoin (20 mg/kg) were used for the positive control group of the STN and MES models, respectively. The volume of administration was based on 0.1 mL/10 g body weight (BW).

Acute Toxicity Test

Test animals used for acute toxicity test were fasted (*i.e.*, food was withheld, but not water) overnight prior to the administration of drug. Fasted animals were weighed and the observed BW was used for the computation of the dose administered. Test animals were weighed daily and changes in weight were recorded. The amount of drug to be administered to all the animals depended on its individual BW. Single dose was administered through gavage using a suitable intubation cannula. Individual records were made for each test animal for their weight, dose administration, response, and time of death [14].

Limit Test

Five Swiss albino mice were used in this experiment, all of which were given a 2000 mg/kg dose of the aqueous seed fraction of *C. fistula* orally. If the first test animal dies followed by three or more test animals, the main test must be done to determine the LD_{50} of the aqueous seed fraction). All test animals used for the acute toxicity test were humanely sacrificed using cervical dislocation [14].

Determination of Dose for Experimental Group

The doses for the experimental group were determined using an arbitrary logarithmic interval of 0.301. The doses were computed using the formula: dose= $10^{[(\log \text{dose } 1)+0.301]}$

Using this formula, the three different doses of the aqueous seed fraction were 200mg/kg, 400mg/kg, and 800mg/kg, respectively.

Convulsion Induction Methods

MES-Induced Model

Convulsion was physically induced 30 minutes after the administration of assigned drug treatment to each group using ECT. A drop of Proparacaine HCl Eyedrops 5 mg/mL 0.5% (Alcaine) was administered first on the eyes of the mice before stimulation for better electrode contact and to reduce the incidence of fatality. The mice were stimulated for 0.2 seconds with 50 mA current [15]. Tonic Hind Leg Extension (THLE) was observed from all the groups and results from each were compared. The duration of convulsion and the percentage of animals protected from THLE were determined for each dose [16].

STN-Induced Model

Drug treatments were first administered IP. After 30 minutes, 2 mg/kg of STN was administered IP to each group to chemically induce convulsions. The mice were observed for 30 minutes for the development of convulsion [17]. Parameters assessed were the latency time and duration of tonic-clonic convulsions, percent protection, and mortality up to 24 hours of the test animals.

Statistical Analysis of Data

The results were expressed as median [interquartile range (IQR)] for the latency, mean ± standard error mean (SEM) for the duration and count data (%) for percent protection and mortality. The data were then analyzed by Kruskal-Wallis Test, one-way analysis of variance (ANOVA), and Generalized Fisher's exact test, respectively. *P* values of <0.05 was the criterion used for statistical significance. All computations were performed using IBM SPSS Statistics Version 22.

RESULTS

Acute Toxicity Test

The mice were observed for any signs of toxicity for 14 days after administration of a 2000 mg/kg dose of *C. fistula* aqueous fraction. There were no mortalities, signs of adverse effects, and toxicity observed during the observation period, implying that the aqueous seed fraction of *C. fistula* is nontoxic. This was used as a baseline in determining the doses to be administered in the experimental group.

Induction of Convulsion

MES-Induced Model and STN-Induced Model Result

Figure 1 shows the mean duration of tonic-clonic convulsion for each model. Statistical analysis using one-way ANOVA showed that there is no significant difference in the mean duration of tonic-clonic convulsion [$F_{4,17}$=0.394, p=0.810] among the five groups in the MES-induced model. For the STN-induced model, all the doses decreased the duration of convulsion compared to the negative control. One-way ANOVA showed that there is no significant difference in the mean duration using the fractions and

negative control (Fig. 1) (p=0.86, p=0.244). This reveals that the fraction has the same effect as the negative control in terms of duration.

Using the one-way ANOVA, the results showed no significant difference in the mean duration of tonic-clonic convulsion in the MES-induced model. A significant difference was found in the presence of convulsion with THLE in the same model using the Generalized Fisher's Exact Test. Another parameter measured for the MES-induced model was percent protection. The results with Generalized Fisher's Test revealed a significant difference in the mortality using the three doses and the positive and negative controls. For the STN-induced model, Kruskal-Wallis test showed no significant difference in the mean latency using the extracts and negative control. A significant difference was seen in the tonic extensor jerks in the five groups, while no significant difference in the mortality was seen using the generalized Fisher's Test in the STN-induced model.

Percent protection from THLE and tonic extensor jerks for MES and STN-induced models respectively was measured and presented in Figure 2. Twenty-percent protection was observed in the 200 mg/kg dose and 40% protection from both 400 mg/kg and 800 mg/kg in the MES-induced model. Phenytoin protected all the mice but none were protected by NSS. There is a significant difference (p=0.023) in the presence of convulsion with THLE between the five groups after performing the General Fischer Exact Test. Moreover, the percentage of those who had THLE when treated with 800 mg/kg, 400 mg/kg, 200 mg/kg and negative control do not differ (p=0.678), but significantly higher than those who were treated with positive control. For the STN-induced model, a significant difference (p=0.009) in the presence of tonic extensor jerks was observed. Moreover, the percentage of those who had tonic extensor jerks when treated with 800 mg/kg, 200 mg/kg, and negative control do not differ (p=0.301), but significantly higher than those who were treated with 400 mg/Kg and diazepam (4mg/kg) or positive control (p=0.444).

The presence of tonic extensor jerks was used to compute for the percentage of test animals protected from tonic extensor jerks. For percent protection in the STN-induced model, *C. fistula* aqueous fraction showed 20% protection from both 200 mg/kg and 800 mg/kg doses, and 60% protection was observed from the 400 mg/kg dose as shown in Fig. 2.

Statistical analysis of the mortality of all treatments in the MES and STN-induced model is shown in Table 1. For the MES-

induced model, mortality of 60% was only observed from the negative control. It showed that there is a significant difference (p=0.021) in the mortality among the five treatments. Specifically, the mortality of those treated with the negative control is significantly higher than those treated with positive control, 800 mg/kg, 400 mg/kg, and 200 mg/kg (p=1.000). A mortality of 60%, 40%, 20%, and 80% was observed from the 200 mg/kg, 400 mg/kg, 800 mg/kg, and negative control groups, respectively in the STN-induced model. The positive control (Diazepam) protected all mice and showed no mortality.

Mean Latency was also measured for the STN-induced model. As shown in Table 2, there is no significant difference in the mean latency using the fractions and negative control. This shows that the fraction has the same effect as the negative control in terms of latency (p=0.051).

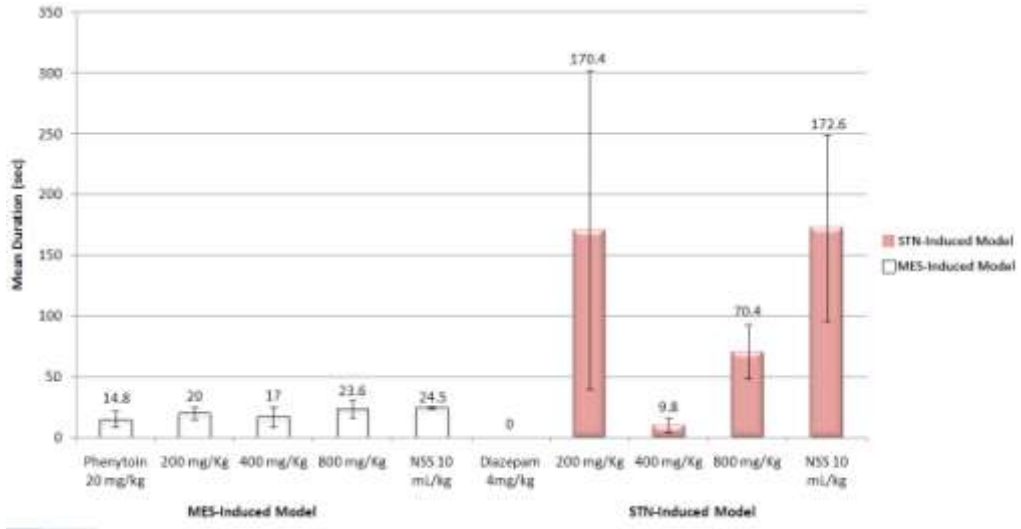

Figure 1: The Mean Duration of the MES-induced and STN-induced Tonic-Clonic Convulsions for the Five Treatments

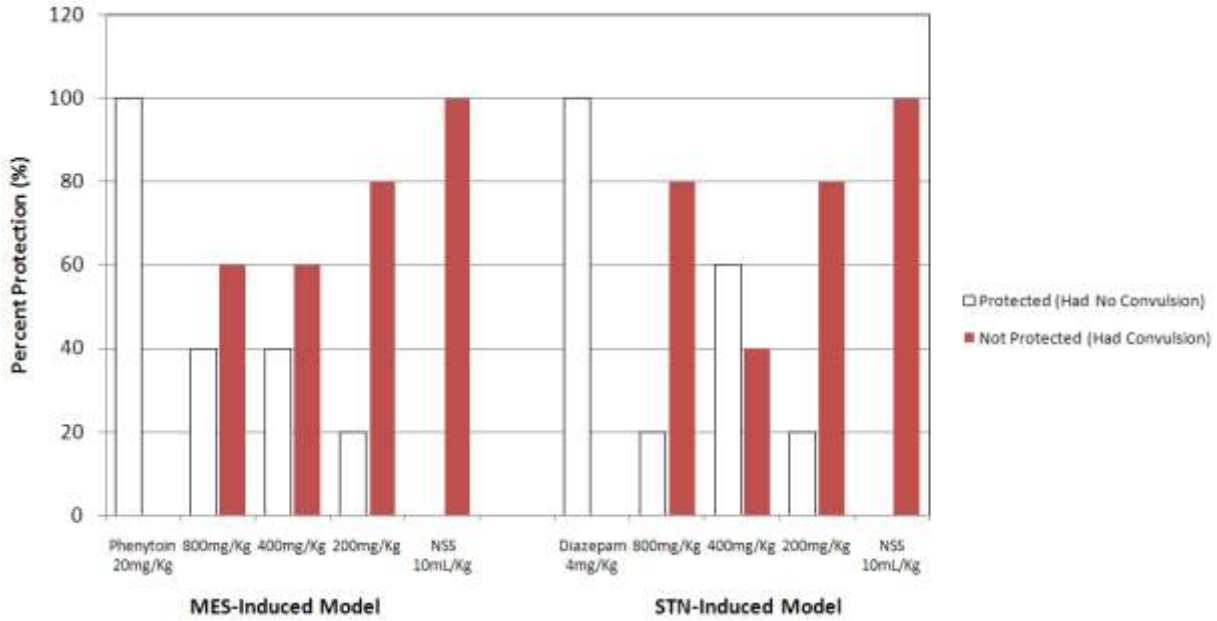

Figure 2: Percent Protection from THLE for MES and STN-Induction Models

Table 1. Mortality of the five groups in the MES- and STN-induced Models				
Model	**Group**	**Dead**	**Alive**	**p-value**
MES Model	**Phenytoin 40mg/Kg**	**0 (0%)**	**5 (100%)**	**0.021**
	800mg/kg	**0 (0%)**	**5 (100%)**	
	400mg/kg	**0 (0%)**	**5 (100%)**	
	200mg/kg	**0 (0%)**	**5 (100%)**	
	NSS 10mL/Kg	**3 (60%)**	**2 (40%)**	
STN Model	**Diazepam 4mg/Kg**	**0 (0%)**	**5 (100%)**	**0.128**
	800 mg/Kg	**1 (20%)**	**4 (80%)**	
	400 mg/Kg	**2 (40%)**	**3 (60%)**	
	200 mg/Kg	**3 (60%)**	**2 (40%)**	
	NSS 10mL/Kg	**4 (80%)**	**1 (20%)**	

Table 2. Latency (sec) of Convulsion in the STN-induced Model			
Group	**Latency (sec)**	**X^2 stat**	***p*-value**
800 mg/Kg	**469 [377.5 – 1202.5]**	**9.450**	**0.051**
400 mg/Kg	**1800 [256.5-1800]**		
200 mg/Kg	**249 [187.5 – 1072.50]**		
NSS 10 mL/Kg	**469 (341.5-548]**		
Diazepam 4 mg/Kg	**1800 [1800 – 1800]**		

Values expressed as median (IQR).

DISCUSSION

The results of the STN- and MES-induced seizure models suggest that the aqueous seed fraction of *C. fistula* possesses significant anticonvulsant activity. The MES Model is the best-validated and most widely used preclinical test in screening AEDs [15]. MES-induced seizure models are used for the evaluation and discovery of drugs for generalized tonic-clonic seizures and partial seizures. AEDs that inhibit MES-induced seizures act by inhibiting voltage-dependent sodium channels and impeding glutamatergic excitation through

the activation of N-methyl-D-aspartate (NMDA) receptors [18].

All doses of *C. fistula* aqueous seed fraction (200 mg/kg, 400 mg/kg, and 800 mg/kg) exhibited protective effect against seizures by decreasing the duration of tonic-clonic convulsions and significantly decreasing the mortality caused by MES-induced convulsions. Thus, the anticonvulsant activity exhibited by the aqueous seed fraction shows that it could have blocked the seizure spread by inhibiting sodium channels and glutamatergic excitation through NMDA receptor [18]. A previous study on *C. obtusifolius* aqueous fraction prevented MES-induced seizures where it deemed more effective than the DCM fraction [13].

Strychnine stimulates spinal reflexes by directly antagonizing glycine receptors [19]. The potential to inhibit strychnine-induced seizures by *C. fistula* aqueous fraction indicates its effect on glycine receptors in the spinal cord. The flavonoids present in *C. fistula* aqueous seed fraction could have exhibited anticonvulsant activity because the flavonoids possess anticonvulsant activity [20].

AED action in the CNS is to block the voltage-dependent sodium channels and T-type calcium channels and promote the neurotransmission of γ-aminobutyric acid (GABA) receptors [21].

It is confirmed that the plant constituents that affect the CNS through their anticonvulsant activity are alkaloids, lipids, terpenes, triterpenoids, flavonoids, and coumarins [2]. Saponins also participate in the anticonvulsant activity of plants that belong to the Fabaceae family [13]. The hydroalcoholic seed extract of *C. fistula* is rich in alkaloids, tannins, flavonoids, saponins, triterpenoids, steroids, glycosides, and anthraquinones [22] and in the aqueous fraction of *Astragalus obtusifolius* (Fabaceae), the flavonoids, saponins, and alkaloids were mostly extracted from the aqueous fraction and the results suggested that the aqueous fraction might be responsible for the inhibition of seizures [13]. With these, the aqueous seed fraction of *C. fistula* is a potential source of phytochemical constituents, specifically alkaloids, flavonoids, and saponins that exert anticonvulsant activity.

However, lipophilic substances readily pass the blood blood brain barrier (BBB) and produce effective CNS activity. In this study, the aqueous fraction was used which may have passed through the BBB through active transport and may have caused the reduced effectivity of the experimental fraction on some of the parameters of convulsion [23].Thus, the petroleum ether and DCM fractions of the *C. fistula* seed

may also exhibit anticonvulsant activity because of its lipophilicity [13] [24].

CONCLUSIONS AND RECOMMENDATIONS

Saponins and flavonoids were found to be present in the *C. fistula* aqueous seed fraction. The *C. fistula* aqueous fraction showed no toxicity at the dose of 2000 mg/kg and is considered nontoxic.

The 400 mg/kg dose of the aqueous fraction of *C. fistula* showed a decrease in the presence of tonic extensor jerks in the STN-induced model and also a decrease in duration of tonic-clonic convulsion in the STN and MES model. Therefore, it can be concluded that the aqueous seed fraction of *C. fistula* possesses potential anticonvulsant activity and it could be a potential source for anticonvulsant agents.

Further studies are required for the identification of the specific constituents and the exact mechanism of action responsible for the anticonvulsant activity of *C. fistula*. According to these research findings, which were consistent to previous studies, *C. fistula* extracts exhibit minimal toxicity. Therefore, together with its significant anticonvulsant activity, it has the potential to be used in combination with standard antiepileptic drugs, thereby reducing the dose and, consequently, the associated side effects. Comprehensive pharmacological and clinical researches need to be conducted for the determination of the oral bioavailability and possible synergistic interactions with standard epileptic drugs.

The use PTZ as the chemical inducer of convulsion is also recommended since it has a profound effect on GABA activity. The DCM and petroleum ether fractions may be tested for its anticonvulsant activity. Also, a larger sample size is recommended to provide better results for statistical analyses.

ACKNOWLEDGEMENT

The researchers would like to express their gratitude to the Faculty of Pharmacy, University of Santo Tomas, Philippines.

REFERENCES

[1] Bhalerao, S.A., & Kelkar T.S. (2012). Traditional Medicinal Uses, Phytochemical Profile and Pharmacological Activities of *Cassia fistula* Linn. *International Research Journal of Biological Sciences, 1(5),* 79-84.

[2] Sharma, J., Gairola, S., Gaur, R. D., Painuli, R. M., & Siddiqi, T. O. (2013). Ethnomedicinal plants used for treating epilepsy by indigenous communities of sub-Himalayan region of Uttarakhand, India. *Journal of Ethnopharmacology , 150*, 353-370.

[3] Bahorun, T., Neergheen, V. S., & Aruoma, O. I. (2005).

Phytochemical constituents of *Cassia fistula*. *African Journal of Biotechnology , 4* (13), 1530-1540.

[4] Deshpande, S., Kewatkar, S. & Painthankar, V. (2013). Pharmacognostical Evaluation of Roots of *Cassia fistula* Linn. *Der Pharmacia Lettre, 5 (1),* 121-135.

[5] Vafaei, A. A., Miladi-Gorgi, H., Moghimi, H. R., & Ameri, M. (2011). The effect of fruit *Cassia fistula* aqueous extract on sleeping time and the level of anxiety in mice. *J Gorgan Uni Med Sci , 12* (4), 1-6.

[6] World Health Organization. (2004). *Epilepsy in the Western Pacific Region: A Call to Action: Global Campaign against Epilepsy*. Geneva, Switzerland: World Health Organization.

[7] Naval, G.M. (2013, September 5). Better treatment of epileptics urged. *Malaya*. Retrieved November 19, 2014 from http://www.doh.gov.ph/sites/default/files/090513-0001.pdf

[8] Fisher, R.S., Boas, W.E., Blume, W., Elger, C., Genton, P., Lee, P., et al. (2005).Epileptic Seizures and Epilepsy: Definitions Proposed by the International League Against Epilepsy (ILAE) and the International Bureau of Epilepsy (IBE). *Epilepsia, 46(4),* 470-472.

[9] Campellone, J.V (2014, February 10). Seizures. Retrieved November 18, 2014, from http://www.nlm.nih.gov/medlineplus/ency/article/003200.htm

[10] Suong, L. K., Lan, T. T., & Nguyen, V. P. (2009). Anti-convulsive Activity of NL197, a derivative from 4(3H)Quinazolinon on Chemical Induced Seizure Mice. *International Electronic Conference on Synthetic Organic Chemistry* , 1-7.

[11] Gahlot, K., Abid, M., Lal, V. K., & Jha, S. (2013). Sedative and Anticonvulsant Activities of the Ethanol Root Extract of Flemingia chappar Benth. *Tropical Journal of Pharmaceutical Research , 12* (2), 203-208.

[12] Kumari, B.P., Sujatha, D., Gopi Chand, C., Divya, K., Malleswari, I., & Ranganayakulu, D. (2012). Evaluation of antiepileptic activity and probable mechanism of action of Coleus amboinicus in MES and PTZ models. *Journal of Pharmacy Research , 5* (3), 1587-1591.

[13] Sayyah, M., Khodaparast, A., Yazdi, A., & Sardari, S. (2011). Screening of the anticonvulsant

activity of some plants from Fabaceae family in experimental seizure models in mice. *DARU , 19* (4), 301-305.

[14] Organisation for Economic Co-operation and Development. (2008, October 16). Acute Oral Toxicity – Up-and-Down Procedure. *OECD Guideline for Testing of Chemicals*. Paris, Cedex, France.

[15] Castel-Branco, M., Alves, G., Figueiredo, I., Falcao, A., & Caramona, M. (2009). The Maximal Electroshock Seizure (Mes) Model in the Preclinical Assessment of Potential New Antiepileptic Drugs. *Methods Find Exp Clin Pharmacol , 31* (2), 101-106.

[16] Bum, E. N., Nkantchoua, G. N., Njikam, N., Taiwe, G. S., Ngoupaye, G. T., Pelanken, M. M., et al. (2010). Anticonvulsant and Sedative Activity of Leaves of Senna spectabilis in Mice. *International Journal of Pharmacology , 6* (2), 123-128.

[17] Azikiwe, C. A., Siminialayi, I. M., Brambaifa, N., Amazu, L. U., Enye, J. C., & Ezani, M. C. (2012, October 7). Anticonvulsant activity of the fractionated extract of Crinum jagus bulbs in experimental animals. *Asian Pacific Journal of Tropical Disease* , 446-452.

[18] Sayyah, M., Mandgary A., & Kamalinejad, M. (2002). Evaluation of the anticonvulsant activity of the seed acetone extract of *Ferula gummosa* Boiss. against seizures induced by pentylenetetrazole and electroconvulsive shock in mice. *Journal of Ethnopharmacology*, 82 (2–3), 105–109.

[19] Olatokunboh, A.O., Kayode, Y.O., & Adeola O.K. (2009). Anticonvulsant activity of *Rauvolfia vomitoria* (Afzel). *African Journal of Pharmacy and Pharmacology*, 3(6), 319-322.

[20] Duraisami, R., Srinivasan, D., & Ramaswamy, S. (2009). Anticonvulsant activity of bioflavonoid gossypin. *Bangladesh Journal of Pharmacology*, 4, 51-54.

[21] Praveen, C., Ayyanar, A., & Perumal, P. T. (2011). Practical synthesis, anticonvulsant, and antimicrobial activity of N-allyl and N-propargyl di(indolyl)indolin-2-ones. *Bioorganic & Medical Chemistry Letters , 21*, 4072-4077.

[22] Bhalodia, N. R., Acharya, R. N., & Shukla, V. J. (2011). Evaluation of in vitro Antioxidant Activity of hydroalcoholic seed extracts of

Cassia fistula linn. *Free Radicals and Antioxidants , 1* (1), 68-76.

[23] Deeken, J. & Löscher, W. (2007). The Blood-Brain Barrier and Cancer: Transporters, Treatment, and Trojan Horses. *Clinical Cancer Research, 13*(6), 1663-1674.

[24] Kasture, V. S., Kasture S. B., & Chopde, C. T. (2002). Anticonvulsive activity of *Butea monosperma* flowers in laboratory animals. *Pharmacology Biochemistry and Behavior,* 72 (4), 965–972.