Evaluation of anticonvulsant activity of Bellyache Bush using experimental animals

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Abstract

The present study evaluated the antiepileptic activity of *Jatropha gossypiifolia* Linn (Bellyache Bush) leaves using experimentally induced seizure models. Mice were subjected to maximum electroshock (MES), N-methyl-D-aspartate (NMDA) (75 mg/kg, s.c), Pentylenetetrazole (PTZ) (70 mg/kg, i.p) for anticonvulsant activity evaluation. Ethanolic extract of *Jatropha gossypiifolia* leaves were used at doses 100 mg/kg and 200 mg/kg.. Duration of flexion, extension, convulsion and stupor were measured. The mortality was evaluated by measuring % of protection. Both the doses of *Jatropha gossypiifolia* leaves and standard Phenytoin sodium protected 33.33%, 66.66% and 83.33% respectively and significantly ($P<0.001$) reduced the number of animals convulsing and significantly delayed the all phases of seizures in MES-induced seizures. Similar protection was observed in PTZ-induced seizures and NMDA induced seizures in mice. Therefore the present study offered a scientific proof to the traditional use of *Jatropha gossypiifolia* Linn in epilepsy. The anti-epileptic activity of leaf extract may be due to enhancement of GABAergic neurotransmission and/or inhibition of calcium current. The significant anti-epileptic activity may be attributed to complex active constituents of the leaf extract.

KEYWORDS: Antiepileptic activity, N-methyl-D-aspartate, Pentylenetetrazole, Phenytoin sodium, *Jatropha gossypiifolia*.

1. Introduction

The word epilepsy is acquired from the Greek word signifying "to seize" or "grab hold of", demonstrating that the individual having a seizure is "possessed"/out of control[1]. Epilepsy is an ongoing neurological problem that influences individuals of all ages. Around 50 million individuals worldwide have epilepsy. Almost 90% individuals with epilepsy are found in evolving regions[2]. An optimal antiepileptic medication ought to stifle all seizures without bringing about any undesirable effects. Shockingly, the medications accessible in the advanced medication not just neglect to control the seizure action in certain patients, however as often as possible reason undesirable effects that reach in seriousness from minimal impairment of the CNS to death from aplastic anemia or hepatic failure[3]. Traditional medication involves a significant spot in the medical services frameworks of developing nations. Individuals in developing nations rely upon traditional medication, since it is less expensive and more available than orthodox medication[4]. Herbal medication is currently enjoying a renewal in popularity in the west and in fact it is the principal form of medicine in many parts of the globe[5]. *Jatropha gossypiifolia* having a place with the family Euphorbiaceae[6] is a bush spice, stature 1.8 meter, gregarious with palmately 3-5 lobed leaves and dull red, ruby or purplish blossoms. Leaf edges, petioles and stipules are covered with glandular hairs[7]. *Jatropha gossypiifolia* originates normally practically the whole tropical region on the planet[8]. This plant is a local of Brazil, naturalized in many parts of India. It becomes on essentially a wide range of soils inside its reach. It is entirely expected in squander lands, side of the road, ineffectively tended rural fields and stream flood regions[9]. It has been recorded as a weed in India, Brazil, Jamaica and Trinidad [10]. The plant is accounted for to be advantageous to dyscrasia, anemia, dizziness and dysphonia. It is an anti-microbial, insecticidal and utilized in toothache and act as blood purifier[11]. The leaves are employed to carbuncles, dermatitis and itches and act as purgative. A decoction of the leaves is valuable for epilepsy, stomach ache, venereal sickness and as blood purifier[12]. Therefore the current study was designed to evaluate the antiepileptic activity of Bellyache bush leaves by employing various experimentally induced epilepsy models.

2. Materials and Methods

2.1 Collection and authentication of Plant

The leaves of Bellyache bush was collected from the fields of Gandimaisamma, Dundigal, Hyderabad. The plant *Jatropha gossypiifolia* Linn. was authenticated by Dr.K.S.Murali Krishna, Department of Pharmacognosy, Marri Laxman Reddy Institute of Pharmacy. A sample specimen of plant material was deposited in the Herbarium of the institution. (Voucher number: MLRIP-64)

2.2 Preparation of Plant Extract

Fresh leaves of the plant were collected and washed with distilled water so as to remove dust and foreign particles. Leaves were then left on the clean surface to air dry, until well-dried and grinded to fine powder using a blender and sieved with a 40# sieve. After drying the powder, it was extracted in Soxhlet apparatus for 48 hours with 95% ethanol. The extract was filtered and concentrated in vacuum under reduced pressure to yield a syrupy mass using a rotary flash evaporator (yield obtained was found to be 7.6 % w/w). The extract was stored in an airtight container in a cool place and further used for the research.

2.3 Phytochemical analysis

Phytochemical screening of *Jatropha* gossypiifolia leaf extract was performed to detect the presence of steroids (Salkowski test), saponins (Foam test), tannins (Ferric chloride test), cardiac glycosides (Keller-killiani test), reducing sugars (Fehlings test), proteins (Xanthoprotein test), Starch (Iodine test), Triterpenoids (Liebermann Burchard test).

2.4. Procurement of Drugs

Pentlylenetetrazole (PTZ) was obtained from Chemdye Corporation, India, Phenytoin sodium was procured from Zydus Pharmaceuticals, India, Memantine was obtained from Intas Pharmaceuticals, India, Clonazepam was received from Torrent Pharmaceuticals, India & NMDA was obtained as gift sample from Sigma Aldrich Chemicals, India.

2.5. Selection of animals

Healthy albino mice weighing 25-30 g were selected for evaluation of antiepileptic activity. All animals were housed at ambient temperature (22 ± 1°C), relative humidity (55 ± 5%) and 12/12 h light/dark cycle. Mice had access to a standard pellet diet and water given *ad libitum*. The protocol of the study was approved by the Institutional Animal Ethics Committee (IAEC) as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (1567/PO/Re/S/11/CPCSEA) of our institution before commencement of experiment.

2.6 Acute toxicity study

Single dose acute toxicity investigation of Ethanolic extract of *Jatropha gossypiifolia* leaves (EEJG) was done in accordance to OECD guidelines 423.

2.7 Antiepileptic activity

2.7.1 Maximum electroshock induced seizure (MES) induced convulsions in mice

The electrical shock applied (150 mA for 0.2 s) through corneal electrodes to Swiss albino mice produced convulsion and those showing responses were divided into five groups of six animals in each.

Group I (Normal control): 1% Normal saline (1ml/100gm,p.o.)

Group II (Pathological control): (Electroconvulsiometer).

Group III (Positive control): Phenytoin sodium (25 mg/kg, i.p.)

Group IV: EEJG-1 (200 mg/kg, p.o.).

Group V: EEJG-2 (100 mg/kg, p.o.).

Drug pretreatment was given 30 min prior to the electric shock and animals were monitored for the duration of tonic flexion, tonic extension, convulsion, stupor and death/recovery.

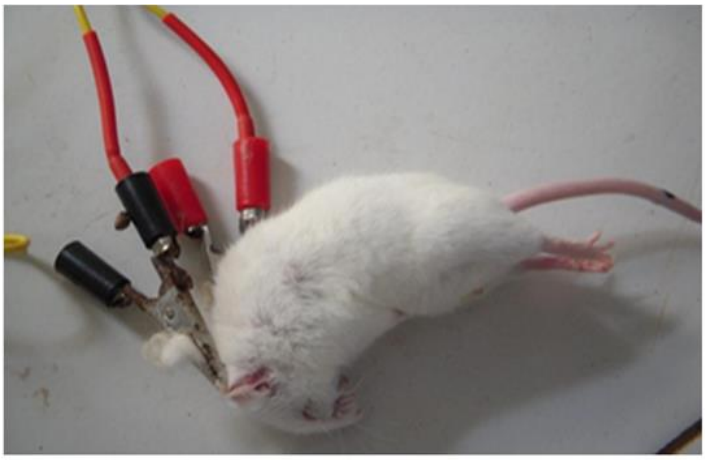

Figure 1. Mouse exhibiting Tonic extension phase in MES induced convulsions

2.7.2 N-Methyl-D-Aspartate (NMDA) induced convulsion in mice

Five groups of six mice received different treatments.

Group I (Normal control): 1% normal saline (1ml/100gm, p.o.)

Group II (Pathological control): NMDA (75 mg/kg, s.c)

Group III (positive control): Memantine (15mg/kg, s.c)

Group IV: EEJG-1 (200 mg/kg, p.o.)

Group V: EEJG-2 (100 mg/kg, p.o.)

Mice were injected subcutaneously with NMDA 75 mg/kg, 1 h after administration of the different treatments. They were observed for 30 min. Animals that did not exhibit turning behavior within the 30 min of observation were declared protected. Turning behaviour was characterized by two consecutive 360° cycles fulfilled by the same animal.

2.7.3 Pentylenetetrazole induced epileptic seizures in mice

Five groups of six mice received different treatments.

Group I (Normal control): 1% Normal saline (1ml/100gm, p.o.)

Group II (Pathological control): PTZ (70 mg/kg, i.p.)

Group III (Positive control): Clonazepam (0.1 mg/kg, i.p.)

Group IV EEJG -1 (200 mg/kg, p.o.)

Group V EEJG-2 (100 mg/kg, p.o.)

The animals that were not convulsed within the 10 min from the injection of PTZ were qualified as protected.

2.8 Statistical analysis

Results were represented as Mean ± S.E.M and the significance of the variation in the responses of treatment groups in comparison to the control was determined ANOVA (analysis of variance), one way subsequently using Dunnett's multiple comparison tests. $^{**}P<0.01$ & $^{***}P<0.001$ were treated as statistically significant by using graph pad prism 8.0.

3. Results and Discussion

3.1 Phytochemical analysis

Preliminary phytochemical investigation of *Jatropha gossypiifolia* leaves indicates the presence of steroids, saponins, tannins, cardiac glycosides, reducing sugars, proteins, Starch and Triterpenoids.

3.2 Acute toxicity study

Ethanolic extract of *Jatropha gossypiifolia* leaves (EEJG) was found to be non-toxic at 2000 mg/kg (OECD 423) after 14 days observation in single dose acute toxicity study. Therefore 100 mg/kg and 200 mg/kg doses were selected for Pharmacological studies.

3.3 Effect of EEJG on MES induced convulsions in Mice

The results indicates that EEJG at both the doses has significantly reduced that duration of flexion phase i.e 4.31±0.09 seconds (100 mg/kg) & 2.45±0.04 seconds (200 mg/kg) when equated with the pathological positive control (7.26± 0.05 seconds). The duration of extension has been significantly reduced by the leaf extract at higher dose (4.36±0.31 seconds) when compared to positive control (10.45±0.48 seconds). EEJG at 200 mg/kg displayed a mild reduction in convulsions (13.58±0.47 seconds) when compared to the positive control (17.38±0.49 seconds). A percentage protection of 33.33% with lower dose and 66.66% with higher dose of EEJG against MES induced epilepsy indicating antiepileptic action of *Jatropha gossypiifolia* leaves. The result of EEJG has been comparable with standard anti-epileptic drug Phenytoin 25 mg/kg, i.p. The results have been depicted in table 1 and figure 2.

3.4 Effect of EEJG on NMDA induced convulsions in Mice

The findings of NMDA induced convulsions in mice shows that the duration of tonic extension has been reduced significantly with higher dose of the leaf extract (5.80±0.30 seconds) when compared to the tonic extension duration of positive control mice (9.54±0.60 seconds). The convulsive turning behavior of the mice has been reduced with higher dose of the leaf extract indicating significant antiepileptic activity of *Jatropha gossypiifolia* leaves. However the antiepileptic activity was found to be less when compared to standard Memantine 15 mg/kg s.c treated mice. The results are shown in Figure 2 and Table 2.

3.5 Effect of EEJG on PTZ induced convulsions in Mice

The results obtained from PTZ induced convulsions model indicates that *Jatropha gossypiifolia* ethanolic leaves extract displayed a significant antiepileptic action by reduction in the duration of tonic extension phase of epilepsy when equated to the positive control. The number of animals exhibiting convulsions after 10 minutes of PTZ treatment has been reduced to 2 when compared to control mice (6 control mice was found convulsing). This data demonstrates the antiepileptic activity of *Jatropha gossypiifolia* leaves. Clonazepam 0.1 mg/kg has displayed greater antiepileptic effect against PTZ convulsions than the leaf extract. The results are shown in Figure 3 and Table 3.

Table 1: **Antiepileptic activity of *Jatropha gossypiifolia* Linn leaves extract on Maximal Electroshock induced convulsion in mice.**

Groups	Flexion (sec)	Extension (sec)	Convulsion (sec)	Stupor (sec)	Protection (%)
Pathological Control	7.26±0.05	10.45±0.48	17.38±0.49	110.42± 0.49	00
Phenytoin sodium 25mg/kg, i.p	2.25±0.09***	0.00±0.00	7.30 ±0.50***	92.96±0.51***	83.33
EEJG 100mg/kg, p.o	4.31±0.09**	7.35±0.13***	15.24±0.50	101.28±0.5	33.33
EEJG 200mg/kg, p.o	2.45±0.04***	4.36±0.31***	13.58±0.47**	105.25±0.47	66.66

EEJG - Ethanolic Extract of *Jatropha gossypiifolia* Linn. All values are expressed as mean ± SEM (n=6), Data analysed by two way ANOVA using Graph-Pad prism 8. ***P<0.001 & **P<0.01when compared to group I (Control).

Table 2: Anticonvulsant activity of *Jatropha gossypiifolia* Linn leaves extract on NMDA induced convulsion in mice

Groups	Duration of tonic extension	No of animals shows turning behavior after 30 minutes	No of animals shows protection	% Protection
Pathological Control (NMDA, 75 mg/kg, s.c)	9.54±0.60	6	0	00
Memantine 15mg/kg, s.c	0.58±0.18***	0	6	100
EEJG-100mg/kg, p.o	7.72±0.32	2	4	66.66
EEJG 200mg/kg, p.o	5.80±0.30**	1	5	83.33

EEJG - Ethanolic Extract of *Jatropha gossypiifolia* Linn. All values are expressed as mean ± SEM (n=6), Data analysed by two way ANOVA using Graph-Pad prism 8. ***P<0.001 & **P<0.01when compared to group I (Control).

Table 3: Antiepileptic activity of ethanolic leaf extract of *Jatropha gossypiifolia* Linn on PTZ induced convulsion in mice

Groups	Duration of tonic extension	No of animals shows convulsion after 10 min	No of animals shows protection	% of Protection
Pathological Control	12.1±2.57	6	0	00
Clonazepam 0.1mg/kg	1.2±1.63***	1	5	83.33
EEJG-100mg/kg	5.2±1.25*	3	3	50.00
EEJG-200mg/kg	3.7±1.17**	2	4	66.66

EEJG - Ethanolic Extract of *Jatropha gossypiifolia* Linn. All values are expressed as mean ± SEM (n=6), Data analysed by two way ANOVA using Graph-Pad prism 8. ***P<0.001, **P<0.01 & *P < 0.05 when compared to group I (Control).

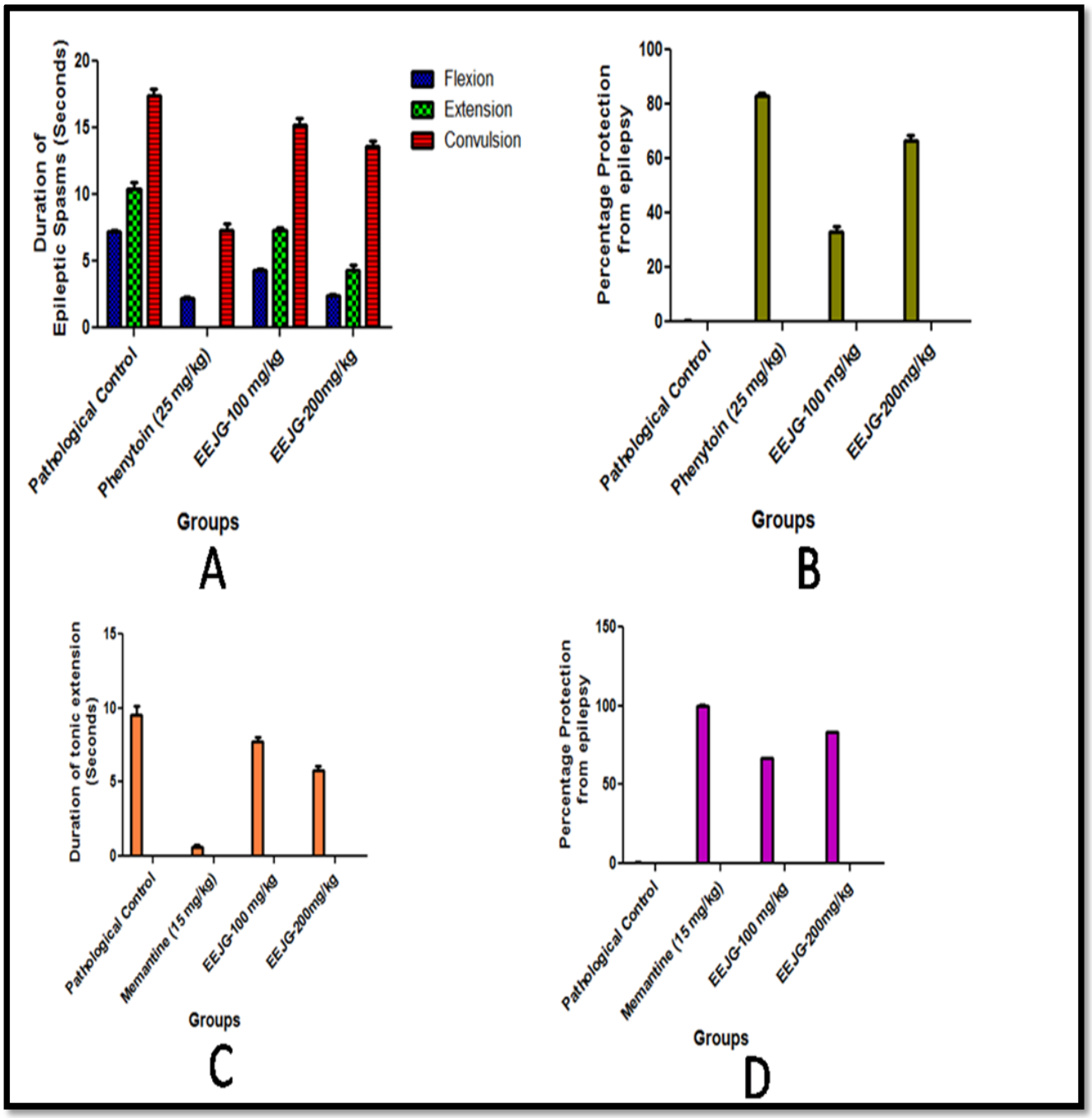

Figure 2: A. Effect of EEJG on duration of Epileptic spasms in MES induced convulsions in mice. B. Effect of EEJG on percentage protection from epilepsy in MES induced convulsions in mice. C. Effect of EEJG on duration of tonic extension in NMDA induced convulsions. D. Effect of EEJG on percentage protection from epilepsy in NMDA induced convulsions in mice.

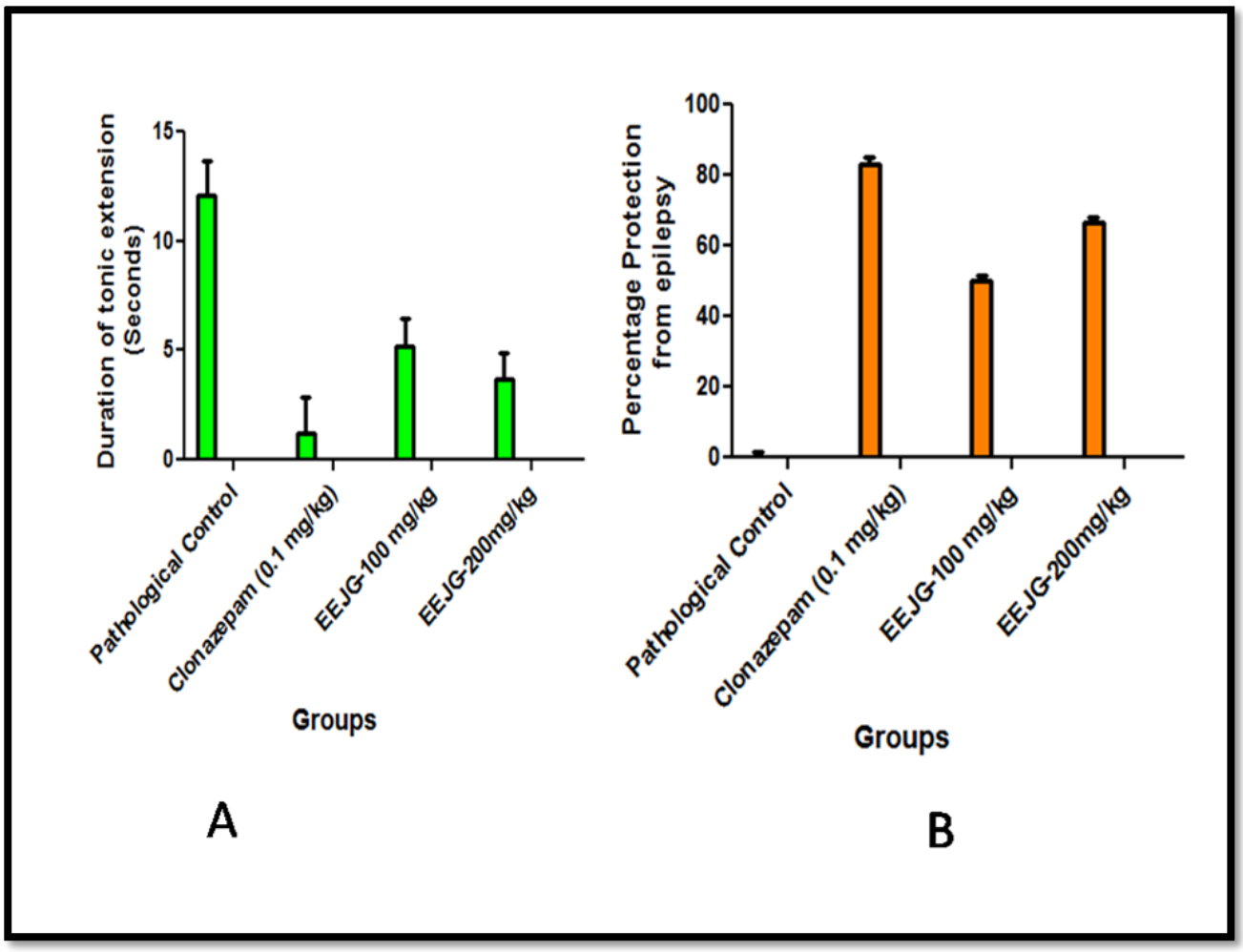

Figure 3: A. Effect of EEJG on duration of tonic extension in PTZ induced convulsions. B. Effect of EEJG on percentage protection from epilepsy in PTZ induced convulsions in mice.

4. Conclusion

The results obtained in the current study indicate that the ethanolic extract Bellyache Bush leaves was successful in suppression of epilepsy induced by various models. Enhancement of GABAergic neurotransmission or sodium channel blockade or inhibition of calcium ion channel may be one of the mechanisms involved in the anticonvulsant activity of the extract of *Jatropha gossypiifolia* Linn. As it has shown the significant effect on different models such as

MES, PTZ and NMDA, further studies are required to ascertain the exact mechanism of antiepileptic action. It is also believed that the presence of various phytoconstituents in the plant leaves i.e. steroids, saponins, tannins, glycosides, triterpenes, proteins may be responsible for anticonvulsant activity and justify its use as a traditional folk remedy for central nervous system related activities.

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6. References

1. Uppala PK, Krishna BM. Experimental evaluation of anticonvulsant activity of Hydrocotyle asiatica linn (Centella asiatica) in Albino mice. Pharm Biotika. 2014;1:59-73.

2. Roy SP, Kumar KS, Manharlal PN, Muralikrishna KS. EVALUATION OF MOOD ELEVATING ACTIVITY OF MADHUCA LONGIFOLIA VAR. LATIFOLIA BARK BY IN VIVO METHODS.

3. Tabuti JR, Dhillion SS, Lye KA. Traditional medicine in Bulamogi county, Uganda: its practitioners, users and viability. Journal of Ethnopharmacology. 2003 Mar 1;85(1):119-29.

4. Bhandari AM, Sathwara JA. Antiepileptic activity of Murraya koenigii leaf extracts. PharmaTutor. 2016 Nov 1;4(11):41-4.

5. Hasan M, Das N, Al Mahmud Z, Abdur Rahman SM. Pharmacological evaluation of naproxen metal complexes on antinociceptive, anxiolytic, CNS depressant, and hypoglycemic properties. Advances in pharmacological sciences. 2016 Jul 11;2016.

6. Kirtikar KR, Basu BD. Indian Medicinal Plants. 1 [sup] st ed, Vol. 3. Dehradun: International Book Distributor. 1980:676.

7. Ogundare AO. Antimicrobial effect of Tithonia diversifolia and Jatropha gossypifolia leaf extracts. Trends in applied sciences Research. 2007;2(2):145-50.

8. Burkill HM. The useful plants of west tropical Africa. Volume 2: Families EI. The useful plants of west tropical Africa. Volume 2: Families EI.. 1994(Edn 2).

9. Akshada K, Madhuri P, Kiran W, Sandip P, Magdum CS, Naikwade NS. A comprehensive review of Jatropha gossypifolia Linn. Pharmacognosy Reviews. 2008;2(4 suppl.):2-6.

10. Csurhes S, Edwards RH. Potential environmental weeds in Australia. Biodiversity Group, Environment Australia; 1998.

11. Balée WL. Footprints of the forest: Ka'apor ethnobotany-the historical ecology of plant utilization by an Amazonian people. Columbia University Press; 1994.

12. Bandyopadhyay U, Biswas K, Chatterjee R, Bandyopadhyay D, Chattopadhyay I, Ganguly CK, Chakraborty T, Bhattacharya K, Banerjee RK. Gastroprotective effect of Neem (Azadirachta indica) bark extract: Possible involvement of H+-K+-ATPase inhibition and scavenging of hydroxyl radical. Life sciences. 2002 Nov 1;71(24):2845-65.

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