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Research Article

PHYTOCHEMICAL AND PHARMACOLOGICAL INVESTIGATIONS OF *ECLIPTA ALBA (LINN.) HASSAK* LEAVES FOR ANTIEPILEPTIC ACTIVITY

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ABSTRACT

Eclipta alba (Linn.) Hassak., is a herb commonly found throughout India. This plant is known to have various pharmacological activities and is traditionally used in treatment of epilepsy but it lacks adequate scientific proof of this activity and constituents responsible for it. The present paper describes the phytochemical and pharmacological investigations of *E.alba* leaves for antiepileptic activity. Different methods of extraction showed that Soxhlet method gave the best yield of 14.88 % with fresh dried leaves. The HPTLC method showed presence of Wedelolactone, Luteolin and β-amyrin. The residual solvent analysis method showed that the extract is free of residual methanol and hence safe for further pharmacological evaluation on experimental animals. The extract showed significant decrease in locomotor activity at the dose of 50mg/kg and dose dependent protection from seizures in MES model. The study confirmed that *E.alba* has good antiepileptic potential possibly due to presence of constituents like Wedelolactone, Luteolin and β-amyrin.

Keywords: Wedelolactone, Luteolin, β-amyrin, Maximal electroshock.

INTRODUCTION

Eclipta alba (Linn.) Hassk., is commonly known as False Daisy or Bhringaraj. It is a creeping and moisture loving herb commonly found on roadsides and waste lands throughout India. The plant has been reported to contain phytosterol, β-amyrin, triterpenes such as ecalbatin, echinocystic acid, ursolic acid, flavones such as Luteolin and coumarin such as wedelolactone [1]. The plant is known to have some important pharmacological activities such as hepatoprotective, antimicrobial, antinociceptive, analgesic, antiinflammatory, antiviral, immunomodulatory and nootropic activity[1].

E.alba has been used in traditional systems of medicine and also by traditional healers especially in South region of India for the treatment of epilepsy since ancient times [2]. Reports suggest that Wedelolactone and Luteolin, important constituents of *E.alba* have selectivity and affinity towards Benzodiazepine binding site on GABA receptor [3, 4]. Luteolin also inhibit the release of glutamate at cerebrocortical nerve terminals [5]. β-amyrin; a constituent in *E.alba* increases taurine level which is indirectly associated with reduced frequency of epileptic seizure, balance in glutamate levels and neuronal membrane stabilization [6]. All these claims suggest that *E.alba* may have anticonvulsant potential. Hence the objective of the study was to scientifically validate the different claims suggesting probable anticonvulsant activity of *E.alba*.

MATERIALS AND METHODS

Chemicals

All solvents used for analysis and experiments such as Petroleum Ether, Methanol, Butanol, acetic acid, toluene and ethyl acetate were purchased from Hi-Media Laboratories, Mumbai, India. Wedelolactone and Luteolin were purchased from Natural Remedies, Banglore. β-amyrin was purchased from Total Herb solution, Mumbai, India.

Plant Material

The whole plant of *E.alba* was collected in the month of June-August from the farms of Palghar, located near Mumbai where it grows like a weed. The marketed leaves of *E.alba* were collected from local market of Mumbai, India. The whole plant and leaves of *E.alba* were authenticated from Botany Department of Khalsa College, King's Circle, Mumbai and the voucher specimen was submitted to Institute of Chemical Technology, Matunga, Mumbai. Leaves were separated and subjected to shade drying at room temperature. The dried leaves were subjected to size reduction to a coarse powder with the help of grinder.

Extraction Methods

Soxhlet extraction

The weighed quantity of collected and marketed leaves were packed individually in two Soxhlet apparatus and defatted with Pet. Ether (1: 10 w/v) and then extracted with methanol (1: 10 w/v) for 18 hrs. The marc was finally concentrated under reduced pressure at 40°C. The percentage yield was calculated.

Extraction by continuous stirring method

The weighed quantities of collected and marketed leaves were taken individually in two double mouthed round bottom flasks and Pet. Ether was poured (1:10 w/v), temperature was maintained at 50ºC and placed on Magnetic stirrer for 24 hours. Condenser and thermometer were placed correctly. After 24 hours, Pet. Ether extract was collected and powder was then dried and packed with methanol (1: 10 w/v) for 24 hours. After 24 hours, extract was collected and solvent was replaced by fresh methanol (1:10 w/v) and again kept for 24 hours. Both the extracts were combined and sufficient quantity of solvent was recovered by distillation and remaining was evaporated under reduced pressure at 40°C. The percentage yield was calculated.

Extraction by Sonication

The weighed quantities of collected and marketed leaves were taken individually in two conical flasks and Pet. Ether (1: 10 w/v) was poured and placed on sonicator for 24 hours. After 24 hours, Pet. Ether extract was collected and powder was dried and soaked with methanol (1:10 w/v) for 24 hours. After 24 hours, extract was collected and solvent was replaced by fresh methanol (1:10 w/v) and again kept for 24 hours. Both the extracts were combined and sufficient quantity of solvent was recovered by distillation and remaining extract was finally concentrated under reduced pressure at 40°C. The percentage yield was calculated.

The Methanolic extract of freshly collected *E.alba* leaves (MEEA) prepared by soxhlet extraction was used for further analytical and pharmacological activities.

Preliminary qualitative phytochemical investigation

The preliminary phytochemical screening of methanol extract was carried out for the detection of alkaloids, saponins, coumarines, sterol, terpene, flavanoids and tannins using standard chemical tests as elaborated by Khandelwal (2002) and Kokate (2001)[7,8].

Residual Solvent Analysis by Gas Chromatography

Determination of the amount of residual solvent i.e. methanol in the dried test extract was performed using gas chromatography [9]. The extract was dissolved in toluene and injected in to the column. GC analysis was carried out by using a Chemito Gas Chromatograph (Ceres 800 plus) gas chromatograph equipped with a FID detector and a packed column OV 17 (chromatopak). The injector and detector temperatures were kept at 280°C and 320°C, respectively. Nitrogen was used as carrier gas, a flow rate of 0.6 mL/min; oven temperature programmed was 45°C held for 2 min and 45°C – 320°C at a rate of 10°C/min. respectively. The final temperature was 320°C held for 15min.

Identification of active constituents by chromatography (HPTLC)

The methanolic extract of *E.alba*, was subjected to HPTLC profile to determine the solvent system giving the best resolution [10]. The extract was applied as bands on HPTLC plates precoated with silica gel 60 GF_{254} plates and evaluated with CAMAG 3 scanner. Different solvent systems were modified for evaluation of various active constituents such as Toluene:ethylacetate:formic acid (11:6:1:0.1v/v/v) for wedelolactone, Toluene: ethyl acetate: formic acid methanol (3:3:0.8:0.2 v/v/v/v) for luteolin and hexane: ethyl acetate (80:20 v/v) for β-amyrin. The identification of the spots was confirmed by co-chromatography with the respective standards when observed at 254nm and 365nm and in white light

Acute oral toxicity

Acute Oral Toxicity study was performed according to OECD 425 (OECD, 2001)[11]. Animals were administered with single dose of extract and observed for mortality during 48 hours study period (short term) toxicity. Based on short-term profile of drug, the lethal and safe doses of the extract for animals was determined as per as OECD guideline [11]. The LD_{50} of the test extract was calculated using AOT 425 software provided by Environmental protection agency, USA.

Locomotor activity

As most of the drugs acting on Central Nervous System (CNS) influence the locomotor activities, the effect of extract on locomotor activity of mice was evaluated by method as elaborated in Mahendran *et al*, 2011 and Farooq *et al,* 2007[12,13]. Twenty four Male Swiss Albino mice (SAM) were selected and randomized into 4 groups (vehicle control, 50, 100 and 200 mg/kg respectively) of 6 animals each and dosed for 7 days. On the first day mice were placed individually in Actophotometer and locomotor scores were noted for 10 min. Locomotor activity was again recorded 60 min after last administration of the dose of the plant extract. Difference in the locomotor scores before and after drug treatment was recorded and inflexion ratio was calculated using following formula. Inflexion ratio (IR) = $(L^1 - L^0)/ L^0$, where L^0 is the initial score and L^1 is the final score).

Maximal Electroshock Test [MES]

This method is probably the best validated preclinical test that predicts efficacy of a drug against generalized seizure of the tonic-clonic type and was performed as described by Toman *et al*, 1946 and Mahendran *et al*, 2011[12, 14, 15, 16]. Treatment [Vehicle/Phenytoin (25mg/kg)/MEEA (50, 100 and 200 mg/kg)] was administered orally to rats (6/group) for 7 days. One hour after the last treatment, seizures were induced in rats by delivering electroshock of 150mA for 0.2s by means of electroconvulsiometer (Dolphin, Mumbai) through a pair of ear clip electrodes to all groups. Each animal was placed into individual plexiglass transparent cage. A decrease in duration of hind leg extension (HLE) was noted as a parameter indicating anticonvulsant activity. All the experimental groups were compared with the respective control treated with vehicle.

Statistical analysis

The values were expressed as mean ± SEM. The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Dunnett's test.

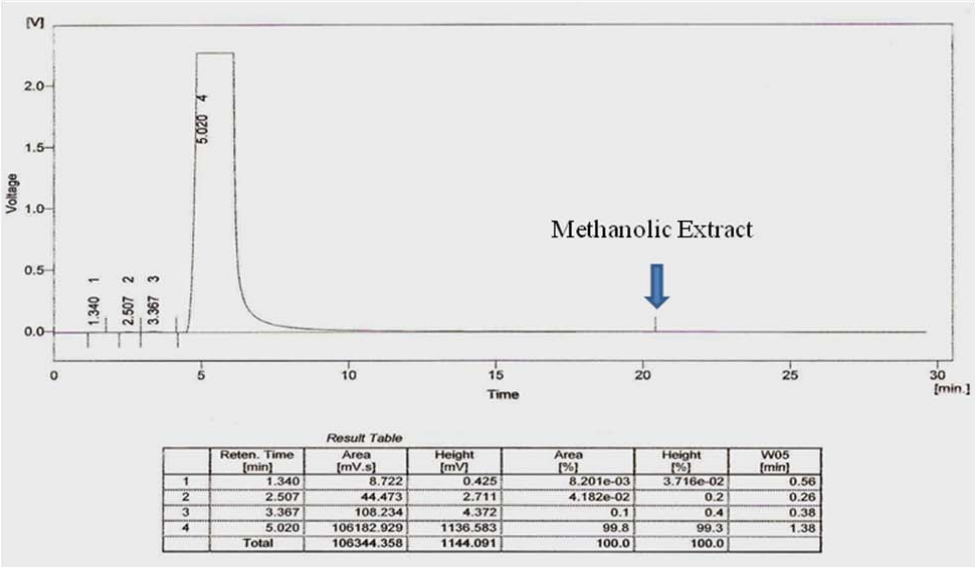
RESULTS AND DISCUSSION

Preliminary phytochemical screening and extraction

The extractive yield of extracts prepared by various extraction techniques is mentioned in Table no 1. The results indicated that methanol extract of dried leaves powder exhibited a maximum yield of 14.88% by Soxhlet extraction process and the marketed powder of leaves exhibited a maximum yield of 8.8% by sonication process. Thus, it was observed that the fresh dried leaves of *E.alba* showed highest extractive yield quantity compared to marketed powder of *E.alba* leaves subjected to Soxhlet extraction method. The prepared extract was subjected to qualitative chemical test. Methanol extract of dried leaves of *E.alba* indicated the presence of alkaloids, carbohydrates, phenolic compounds and coumarins.

Table 1: Percentage yield of different extraction method

Material	Percentage Yield		
	Soxhlet	Continuous Stirring	Sonication
Market Powder	1.16%	4.5%	8.8%
Dried Leaves Powder	14.88%	9.5%	10%



Result Table

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	1.340	8.722	0.425	8.201e-03	3.716e-02	0.56
2	2.507	44.473	2.711	4.182e-02	0.2	0.26
3	3.367	108.234	4.372	0.1	0.4	0.38
4	5.020	106182.929	1136.583	99.8	99.3	1.38
	Total	106344.358	1144.091	100.0	100.0	

Fig. 1: Residual Solvent Analysis by Gas Chromatography

Residual Solvent Analysis by Gas Chromatography

Residual solvent analysis is an important step to ensure that the dried extracts are completely free of the solvents or quantities of solvents present in the extracts are within permissible limits. In our study residual solvent analysis ensured that the dried methanolic extract of *E.alba* leaves was completely free of methanol. Extract was found to be free of residual methanol and was safe for the administration to the animals. (see Figure 1)

Identification of active constituents by chromatography

Methanolic extract of *E.alba* was found to contain principle constituents like Wedelolactone, Luteolin and β-amryin having R_f values of 0.56, 0.72 and 0.32 respectively. The 'photo-documentation of HPTLC fingerprints indicated presence of Wedelolactone, Luteolin and β-amryin in the extract are shown in Figure 2,3 and 4 respectively.

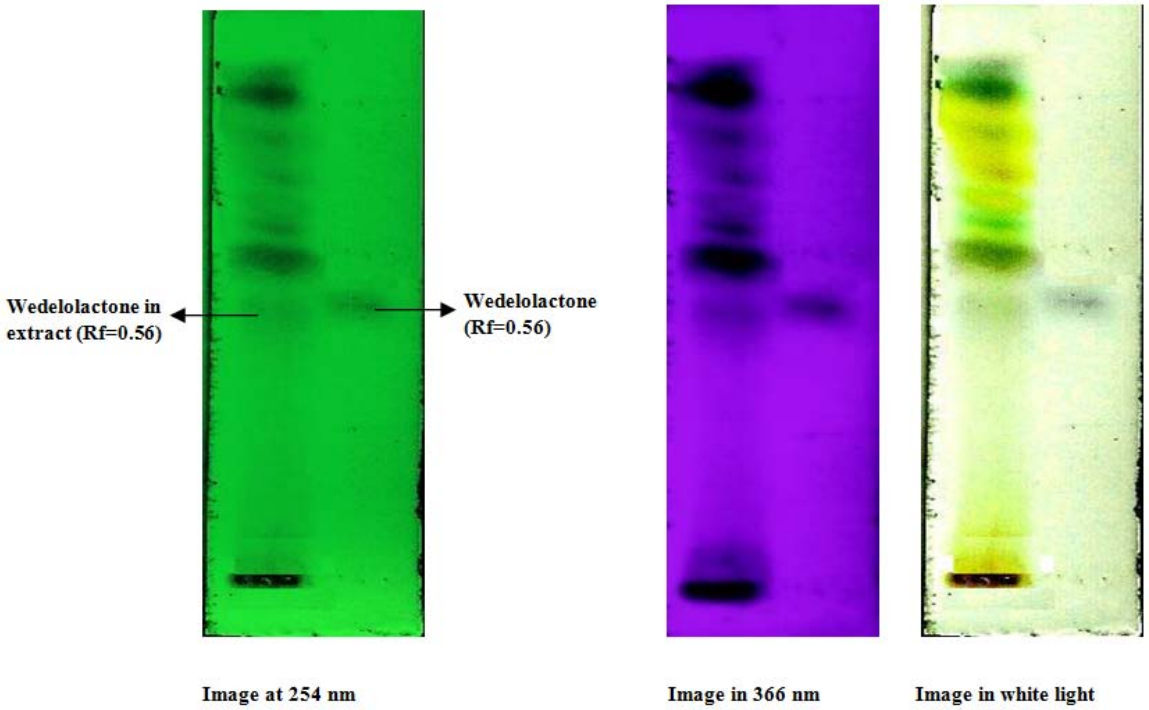


Fig. 2: HPTLC of MEEA for Wedelolactone

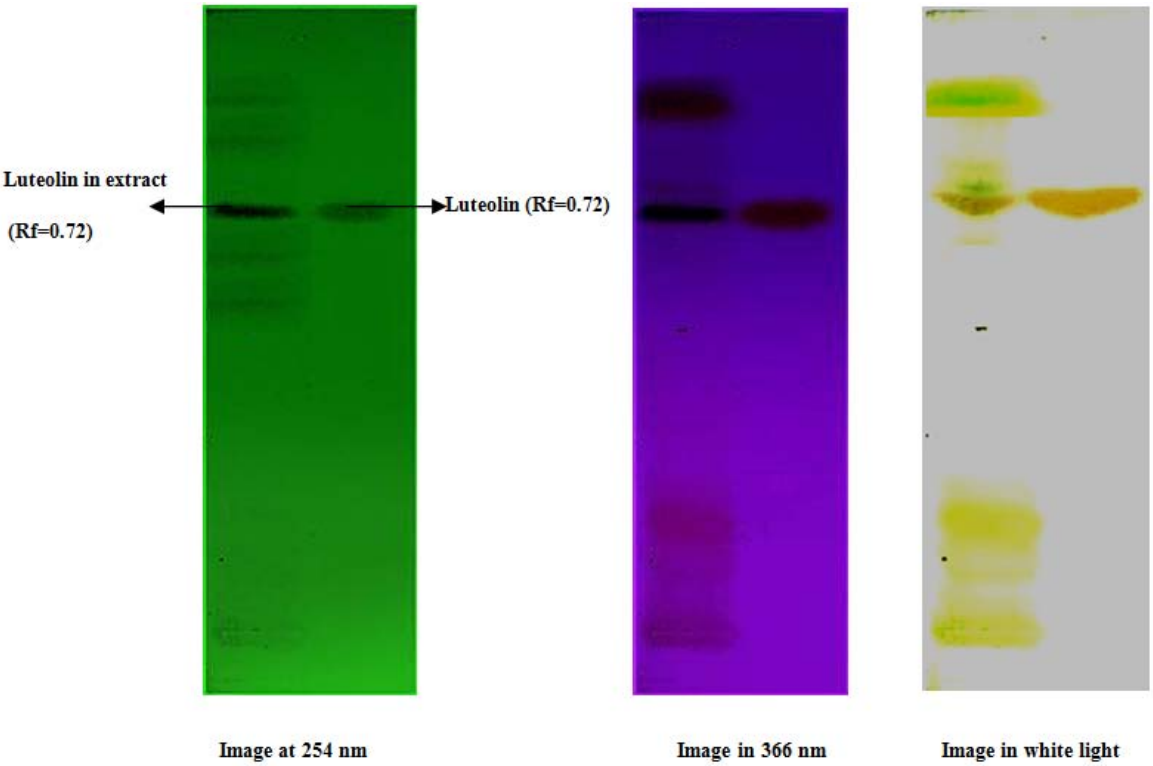


Fig. 3: HPTLC of MEEA for Luteolin

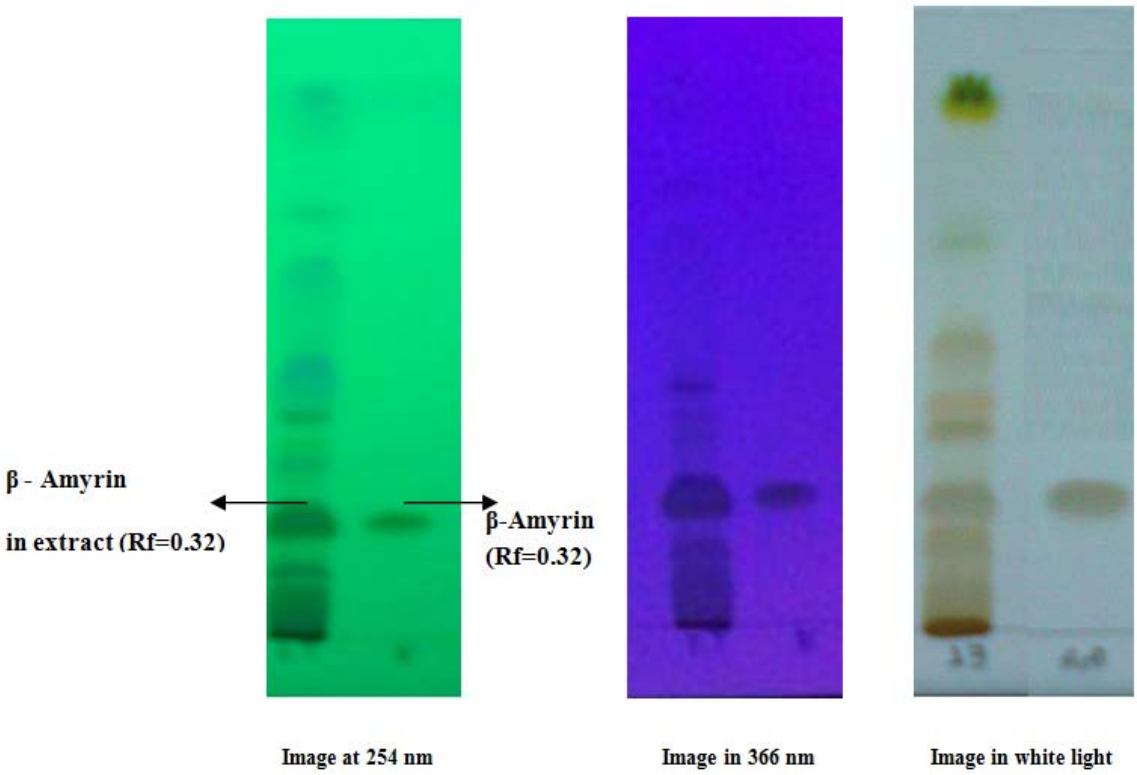


Fig. 4: HPTLC of MEEA for β-amyrin

Acute oral toxicity

Acute oral toxicity study in mice according to AOT 425 showed than MEEA has LD_{50} greater than 2000 mg/kg means the drug is safe upto 2000mg/kg.

Locomotor activity

Locomotor activity is considered as an index of alertness and decrease in the activity indicated CNS depression [17]. In the present study MEEA showed decrease in locomotor activity at all the three dose levels i.e. 50, 100 and 200mg/kg with a significant decrease at 50mg/kg dose. (see Figure 5).

Maximal Electroshock Model

Maximal electroshock (MES) model of epilepsy was used for evaluation which is arguably the best-studied and most useful animal model of seizures [18]. Animals treated with 50, 100 and 200 mg/kg of MEEA showed significant decrease in the duration of time spent in extensor phase in a dose dependent manner where as Phenytoin completely protected animals from extensor phase when compared with vehicle control. Protection against HLE in MES predicts the ability of a test drug to prevent the spread of seizure discharge from the epileptic focus in brain [18]. Generally the drugs which inhibit the voltage gated sodium channels are active in this model of epilepsy [19]. (see Figure 6).

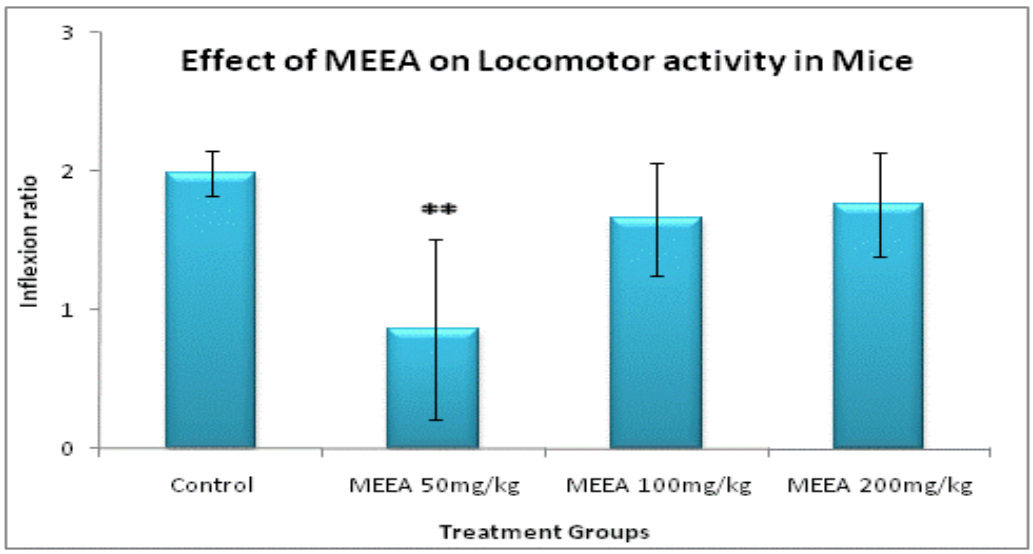


Fig. 5: Effect of MEEA on Locomotor activity

N=6, Data are expressed as mean +SEM, Statistical analysis by one-way ANOVA followed by Dunnett's test. Significance at *P<0.05, ** P<0.01 & ns-not significant vs. control.

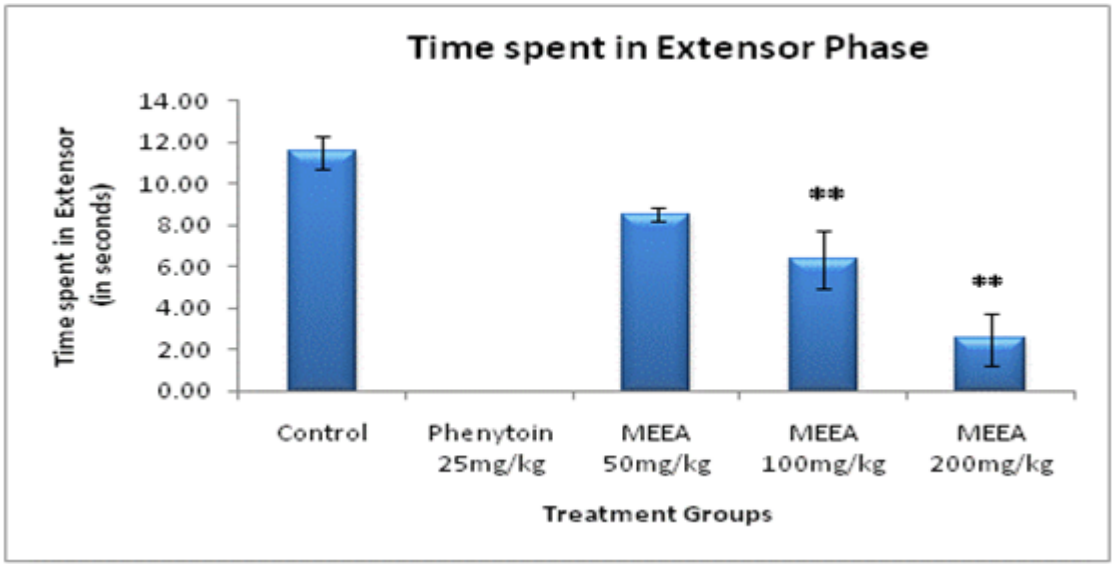


Fig. 6: Effect of MEEA in MES model

N=6, Data are expressed as mean +SEM, Statistical analysis by one-way ANOVA followed by Dunnett's test. Significance at *P<0.05, ** P<0.01 & ns-not significant vs. control.

CONCLUSION

The present study demonstrated a dose dependent increase in the seizure threshold (decrease in the extension), indicating anticonvulsant property of the extract against the MES model. This activity may be due to its inhibition of voltage gated sodium channels. The bio active constituents namely Wedelolactone, Luteolin and β-amyrin may be responsible for activity.

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