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EVALUATION OF ANTICONVULSANT ACTIVITY OF METHANOLIC EXTRACT OF *GISEKIA PHARNACEOIDES* LINN ON EXPERIMENTAL MICE

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ABSTRACT

The study was performed to evaluate the Anti convulsant activity of methanolic extract of *Gisekia pharnaceoides* (MEGP) in Albino mice. Anti convulsant activity of MEGP was investigated by using two methods maximal electro shock induced seizures [MES] and Pentylenetetrazole [PTZ] induced seizures. Evaluation was done on both models comparing with reference standard drug phenytoin (25mg/kg) and phenobarbitone sodium [40mg/kg]. Preliminary phytochemical investigation of methanolic extract of *Gisekia pharnaceoides* reveal the presence of carbohydrates, flavonoids, glycosides, proteins, steroids, alkaloids and results were found that MEGP dose of 100 mg/kg and 200mg/kg decreased in the extension phase when compared with control in MES induced convulsion and in PTZ method a dose of 100mg/kg and 200 mg/kg increased onset of convulsion and decreased duration of convulsion.

KEYWORDS: *Gisekia pharnaceoides,* Convulsions, MES, PTZ.

INTRODUCTION

Epilepsy, which has been described as a chronic disorder of the central nervous system of various etiologies, characterized by recurrent seizures due to excessive discharge of cerebral neurons is a major medical and social problem.[1] The incidence of the disease in developing countries is higher than that in developed countries and is reported to be 190 per one lakh people. There are many classes of anticonvulsants that are of clinical usefulness with good prognosis for controlling seizures in most patients. The currently available anticonvulsant drugs suffer from drawbacks like neurotoxicity, teratogenic and other dose related side effects.[2] In order to reduce the risk of side effects, overcome resistance and achieve safety and effectiveness, the use of anticonvulsant drugs combination is a fundamental strategy in assessment of epilepsy. There is a continuous and urgent need to discover new compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases. Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs.[3]

Gisekia Pharnaceoides belong to the family Molluginaceae and has been used in herbal medicine. It contains mostly citric acid, oxalic acid, Tartaric acid, Triacontane, Myristone, Tetracosanyl acetate Dotriacontane, The plant is used for the treatment on swellings and asthma, scabies. leucoderma, leprosy, loss of appetite and rhinitis in folklore medicine. In India the fresh plant is used as an anti-helminthic in case of taenia. In addition recent studies revealed inflammatory,[4] anti-helmintic,[5] anti-microbial[6] and antioxidant properties.[7] Therefore we made an attempt to evaluate anticonvulsant activity of *Gisekia Pharnaceoides*.

MATERIAL AND METHODS

PLANT MATERIAL

The fresh leaves of *Gisekia pharnaceoides* L were collected from local areas of Ongole Andhra Pradesh India. The leaves were Shade dried and ground to get a coarse powder.

PREPARATION OF EXTRACT

Extraction of the *Gisekia pharnaceoides* leafs by methanol as solvent. After the pulverization the fine powder was extracted by using the methanol solvent, first a thimble is prepared with blotting paper and about 150-200gms of *Gisekia pharnaceoides* powder is filled in it. After filling the thimble it is placed in the soxhlet apparatus. About 750 ml of methanol is filled in the round bottom flask [RBF] and the remaining is poured into the thimble which is placed in apparatus. This is done to saturate the powder and it is done till the siphon gets filled and acquires free flow of the solvent. In the next step the reflex condenser is placed over the soxhlet and which is made connected to the inlet and outlet. And

some porcelain chips are added into the RBF to avoid the vigorous boiling and the temperature is adjusted to 50°C this temperature is adjusted based on the methanol boiling point. Once the methanol boils in RBF the vapours enter the reflex condenser and gets condensed and falls onto the thimble in soxhlet apparatus this continues until the methanol vapours passes through the siphon and falls into the RBF. This cycle continued until the solvent gets colorless this is checked by the observing the siphon. The extraction is carried out for continuous 16 hours and the extraction mixture in RBF is taken and distilled to collect the extract.[8]

PRELIMINARY PHYTOCHEMICAL SCREENING

Extract was tested for Glycosides, Steroids, Alkaloids, and Flavanoids.[9]

EXPERIMENTAL ANIMALS

Albino mice of either sex weighing between 20-30g were procured from Animal house, QIS College of pharmacy, ongole, for experimental purpose. The animals were acclimatized to laboratory condition for 7 days. The animals were housed in clean, sterile polypropylene cages in a well-ventilated room under hygienic conditions and were exposed to 12 h day and night cycle. The animals were fed with commercial rat pellet feed and were given water and libitum. All animal studies were performed in accordance to guideline of CPCSEA and Institutional Animal Ethical committee [IAEC] of central Animal house, QIS College Pharmacy, ongole.(Reg No: 1921/PO/ReS/16/CPCSEA.

ACUTE TOXICITY STUDIES

Toxicity studies were performed for Methanolic extract as per OECD guidelines–420, fixed-dose procedure. Fixed-dose levels of extracts starting from 50, 100, 200, 500, 1,000, increasing upto 2,000 mg/kg body weight were given, and signs and symptoms of toxicity were observed for next 48 hr. No toxicity or death was observed in the experimental mice when they were subjected to toxicity study.[10]

EVALUTION OFANTICONVULSANT ACTIVITY

MAXIMAL ELECTRO SHOCK INDUCED SEIZURES [MES][11,12]

Protection against electroshock induced seizures in mice or rats is used as an indication for compounds which may prove effective in 'grand mal epilepsy'. Electric stimuli evoke tonic hind limb extensions, which are suppressed by anti-epileptic drugs. The animals were given maximal electroshocks of 150 mA for 0.2s to the cornea by using electro convulsometer. Twenty four healthy and convulsion free albino mice (20-30 g) were randomly divided into four groups (n=6). Group I received no treatment, served as control animals. Group II received standard drug Phenytoin (25 mg/kg body weight, per oral). Group III received low dose of MEGP (100mg/kg, per oral), Group IV received high dose of MEGP (200mg/kg, per oral). The plant extracts at the dose of 100 and 200 mg/kg, standard drug phenytoin (25mg/kg) and vehicle control were administered 30 min prior to MES. The vehicle treated animals exhibit the characteristic maximal electroshock convulsions which can be divided into 5 phases- (a) tonic flexion, (b) tonic extensor, (c) clonic convulsions, (d) stupor and (e) recovery or death. The animals were observed for 2 min after the shock. Disappearance of the hind limb extensor tonic convulsions was taken as the criterion of protection.

PENTYLENETETRAZOLE [PTZ] INDUCED SEIZURES[13,14]

This test is considered as indicative of anticonvulsant activity of drugs against 'petit mal seizures'. PTZ produces generalized asynchronized clonic movements which are super ceded by tonic convulsion characterized by flexion of limbs followed by extension. The excitatory effects of PTZ may be due to decrease in neuronal recovery time in the postsynaptic pathway of the spinal cord. Twenty four healthy and convulsion free albino mice (20-30 g) were randomly divided into four groups (n=6). Group I received no treatment, served as control animals. Group II received standard drug phenobarbitone sodium (40 mg/kg body weight, per oral). Group III received low dose of MEGP (100mg/kg, per oral), Group IV received high dose of MEGP (200mg/kg, per oral). The plant extract at the dose of 100 and 200mg/kg, standard drug phenobarbitone sodium and vehicle control were administered 30 min prior to PTZ (80 mg/kg per i.p).The onset and number of death after showing tonic hind limb extension were also recorded. Mice that did not convulse 30min after pentylenetetrazole administration were considered protected.

STATISTICAL ANALYSIS

Statistical analysis was carried out using graph pad prism software. All were expressed as Mean + Standard mean error. Groups of data were compared with one way analysis of variance followed by Dunnett test. Values were considered statistically significant at $p<0.01$.

RESULTS

PRELIMINARY PHYTOCHEMICAL INVESTIGATION

The preliminary phytochemical investigation of the methanolic extract of *Gisekia Pharnaceoides* showed that it may contain carbohydrates, flavonoids, glycosides, proteins, steroids and alkaloids.

MAXIMAL ELECTRO SHOCK INDUCED SEIZURES [MES]

The results of anticonvulsant activity of *Gisekia Pharnaceoides* are shown in table-1.Oral administration of methanolic extract of *Gisekia Pharnaceoides* produced significant dose-dependent protection from convulsions than control. The methanolic extract of *Gisekia Pharnaceoides* 200mg/kg, p.o and 100mg/kg, p.o has shown a significant ($P<0.01$) protection from

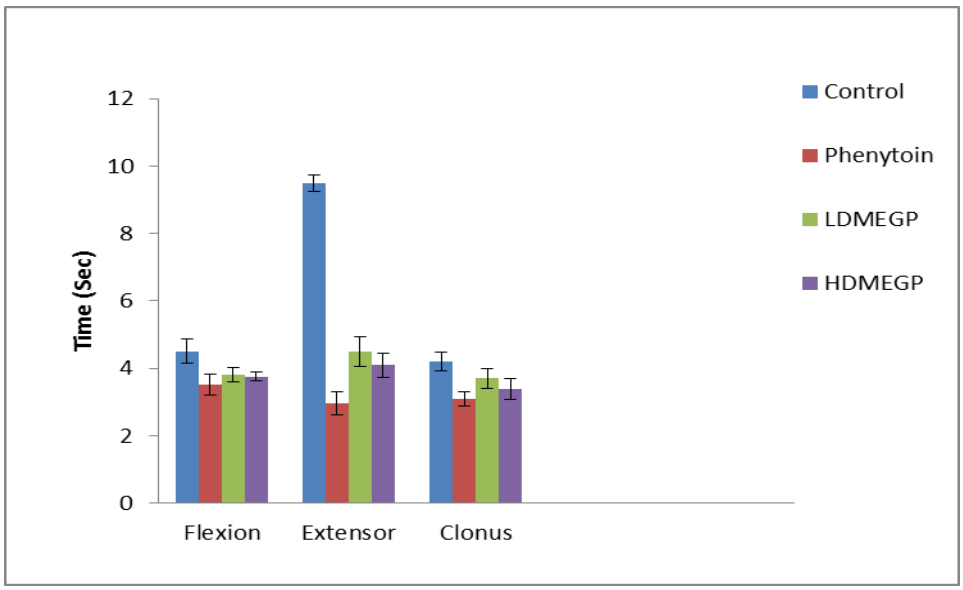
convulsions. Phenytoin treated mice shown a significant protection when compared to control.

Table No.1: Effect of Methanolic extract of *of Gisekia Pharnaceoides* on MES induced convulsions

Groups	Treatment	Dose mg/kg	Time in various phases of convulsion (s)				
			Flexion	Extensor	Clonus	Stupor	R/D
I	Control	10ml	4.51±0.35	9.5±0.25	4.2±0.28	178±1.5	R
II	Phenytoin	25	3.52±0.3	2.95±0.34*	3.1±0.22	81±1.2	R
III	LDMEGP	100	3.81±0.25	4.5±0.44*	3.7±0.30	115±1.1	R
IV	HDMEGP	200	3.76±0.12	4.1±0.36*	3.4±0.31	97±1.9	R

LDMEGP= Low dose of MEGP, HDMEGP= High dose of MEGP, R= Recovered, D= Death

*P<0.01 considered statistically significant as compared with control group.



Graph.1: Effect of Methanolic extract of *of Gisekia Pharnaceoides* on MES induced convulsions

PENTYLENETETRAZOLE [PTZ] INDUCED SEIZURES

The result of PTZ induced convulsion depicted in Table No-2, the test extracts and standard drug delayed the onset of time and decreased the duration of convulsion. The MEGP at the dose of 100 mg/kg and 200 mg/kg significantly (P<0.01) delayed the onset of convulsion and decreased the duration of convulsion in PTZ induced convulsion when compared to the solvent control group. This effect also comparable with those result produced by standard.

Table No.2: Effect of Methanolic extract of *of Gisekia Pharnaceoides* on PTZ induced convulsions

Groups	Treatment	Dose mg/kg	Onset of convulsion in minute	Duration of convulsion in minute
I	Control	10ml	1.80±0.2	2.4±0.3
II	Phenobarbitone	40	5.5±0.25*	1.5±0.26
III	LDMEGP	100	3.6±0.15*	2.2±0.22
IV	HDMEGP	200	4.5±0.21*	1.8±0.19

LDMEGP= Low dose of MEGP, HDMEGP= High dose of MEGP

*P<0.01 considered statistically significant as compared with control group

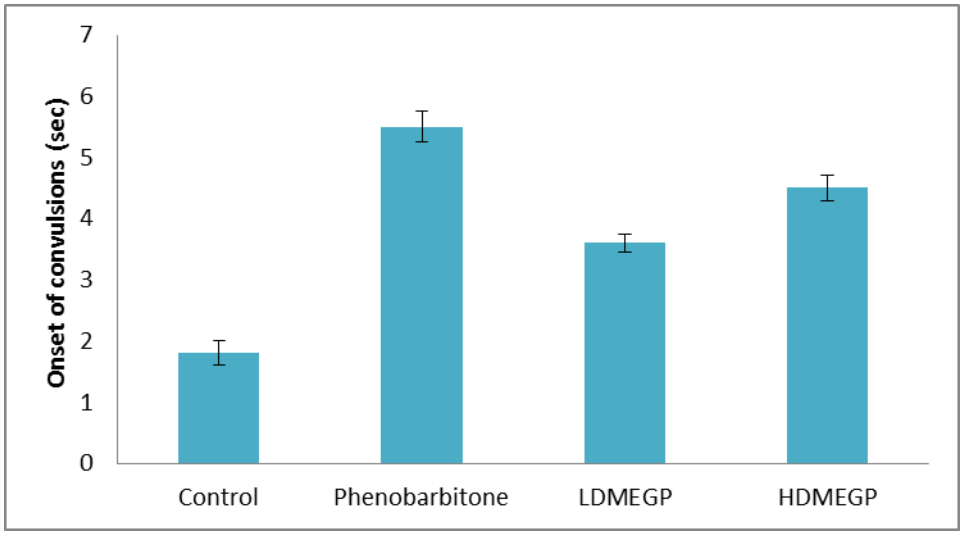


Table No.2: Effect of Methanolic extract of *of Gisekia Pharnaceoides* on PTZ induced convulsions

DISCUSSION

Preliminary phytochemical analysis performed showed that Carbohydrates, flavonoids, glycosides, tannins, phenols, reducing sugar, terpenoids and saponins were detected in the methanolic extract of plant. In MES induced convulsion, MEGP at the dose of 100 mg/kg and MEGP at the dose of 200mg/kg significantly ($P<0.01$) failure in extensor phase when compared with the control group and the result is comparable to that produced by phenytoin. Similarly in study of PTZ induced convulsion, the MEGP at the dose level of 100 &200 mg/kg significantly ($P<0.01$) increased the onset of convulsion while a significant ($P<0.01$) decrease in the duration of convulsion.

Pentylenetetrazole (PTZ) is convulsant drug used to induce convulsions, while ability of an agent to inhibit convulsion in comparison with the untreated mice. Pentyltetrazole (PTZ) destabilizes nervous cell membrane to produce convulsion. GABA is the predominant inhibitory neurotransmitter in the mammalian CNS and is widely implicated in epilepsy, mediating inhibition of neuronal responsiveness (excitability) and activity by increasing the chloride ion conductance through opening of the chloride ion channel.[15] The findings of the present study, therefore, tend to suggest that the Gisekia extract might have inhibited and/or attenuated PTZ-induced seizures by enhancing or in some ways interfering with GABAergic neurotransmission.

CONCLUSION

It was concluded that the methanolic extract of *Gisekia Pharnaceoides* in small to moderate doses (100-200 mg/kg) enhances the anticonvulsant effects in different animal models. This effect may be attributed to different mechanisms including the brain glutamate and increasing brain GABA levels, inhibition of free radical generation, scavenging of reactive oxygen species and reactivation of antioxidant defenses. Results of this study direct the light towards the possible use of *Gisekia Pharmaceoides* In the treatment of epilepsy.

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