CHEMICAL COMPOSITION AND EVALUATION OF ANTIEPILEPTIC ACTIVITY OF LEAF EXTRACT OF *CUCURBITA MOSCHATA* IN EXPERIMENTAL ANIMALS

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Abstract:

Aim of the present investigation is designed to identify the phytoconstituents in ethanolic extract *Cucurbita moschata* and to assess the anti-epileptic potential of *Cucurbita moschata* leaf extract. One of the most frequent neurological illnesses is epilepsy. *Cucurbita moschata* has long been used to cure a variety of ailments. The present investigation was designed to identify the phytoconstituents in ethanolic extract *Cucurbita moschata* and to assess the anti-epileptic potential of *Cucurbita moschata* leaf extract. The National Institute of Standards and Technology database was used to interpret the mass spectrum of the GC-MS. The ethanolic extract was examined orally at different on experimentally induced animal. Phenytoin (40mg/kg), Valproic acid (200mg/kg) and Diazepam(10mg/kg) were used as the standard drugs for comparison. The parameters considered to assess the anti-epileptic activity were decrease in extensor phase, but there is no significance increase in onset of convulsion and decrease in the severity of status epilepticus as compared to the standard group. Significant anti-epileptic activity was showed by ethanolic suspension in MES model. Conclusively, the *Cucurbita moschata* leaf extract has potential to treat the grandmal epilepsy but there is no significant action against petitmal epilepsy and status epilepticus. GCMS analysis revealed presence of Pentacuclic terpenoid, Steroid acids and fatty acid esters. Antiepileptic activity may be attributed to pentacyclic triterpenoid, steroid and fatty acid esters.

Keywords: Pentacyclic terpenoid, Steroid, Fatty acid esters, Anti-epileptic activity, *Cucurbita moschata*, Phenytoin, Valproic acid

1. Introduction

Epilepsy is a seizure condition characterized by irregular electrical discharges from the brain, most commonly in the cerebral cortex. Epilepsy affects people of all ages, from newborns to the elderly, and has wide verities of causes and symptoms, including several common seizure forms and syndromes, as well as a lot that is not well classified. Fixed neurological deficits, psychiatric issues, learning disabilities, progressive disorders, and particularly in the old age group, are only a few of the comorbidities that complicate the assessment and care planning (Duncan et al. 2006). With the help of standard medications, approximately 75-80% of the epileptic patient may have tolerable seizure control. The current treatments are mainly prophylactic and are aimed at preventing seizures in those that are affected. In current therapy, verities of medications are available for the treatment of epilepsy, however, the biggest drawback is that they have unknown side effects, requiring the use of herbal extracts (Reddy et al. 2005). Pharmacological agents such as phenytoin, sodium valproate, and carbamazepine, which regulate the excessive abnormal electrical activity of brain neurons, are currently used to treat epilepsy. These drugs work by blocking the Sodium/Calcium channel and balancing the inhibitory and excitatory neurotransmitter system in the brain (Eerike et al. 2016). The plant kingdom is a good source of herbal and synthetic drugs. There has been a rising awareness of the value of medicinal plants in recent years. Plant-based drugs are widely available, inexpensive, safe, reliable, and have minimum side effects (Suresh et al. 2018).

Cucurbita moschata belongs to the Cucurbitaceae family. *Cucurbita .moschata* is primarily found in tropical America, where it has been used as a popular cooking vegetable for thousands of years. The seeds can also be consumed and have medicinal properties. Many countries, including China, Brazil, India, Yugoslavia, Mexico, Argentina and America, have historically used *Cucurbita moschata* as medicine. *Cucurbita moschata* is an annual dicotyledonous vegetable with a creeping or climbing stem that bears tendrils (up to 5m). The stem cylindrical, strong and the petioles are 12 to 30cm long. Hairiness can also be observed on the stem and leaves. The leaves are oval, triangular, or kidney-shaped, up to 20cm long and 30cm wide, deeply indented at the base, wavy, weakly lobed and toothed, and more or less white-spotted (Marie-Magdeleine et al. 2011).

Cucurbita moschata has a wide variety of bioactivities, including hepatoprotection, antidiabetes, antiobesity, and anticancer. Freshly crushed seeds are used as an anthelmintic and for skin infection and inflammations. Pumpkin has anti-diabetic, anti-hypertensive, anti-tumor, immunomodulatory, antifungal, antibacterial, and anti-inflammatory properties, as well as antioxidant properties (Wang et

al. 2012). In the present study GCMS analysis and antiepileptic of ethanolic extract of *Cucurbita moschata* has been evaluated

2. Material and method

2.1.Collection and identification of plant material

The leaves of *Cucurbita moschata* were procured from Sullia and Mangalore Karnataka. India, during July 2020. The herbal drug was confirmed by Chairman, Department of applied Botany, Mangalore University (Mangalagangothri), Mangalore

2.2.Preparation of plant extract

Leaves were cleaned from adhering soil, dust, and other materials, and then it was dried under the shade for 20 days. After confirming the dryness, the leaves were pulverized in an electric grinder and coarse powdered. Coarse powder of *Cucurbita moschata* was subjected to maceration separation. For seven days, the powdered content was soaked in 90% ethanol. The mixture was filtered and the marc was pressed after the seventh day. This procedure was carried out three times. Both of the ethanolic fractions were mixed, and the ethanol was evaporated at low pressure. The thick syrupy consistency was obtained, and the contents were evaporated until dry extract was obtained. The extracted material was labelled and kept in desiccators for further use.

2.3.GCMS analysis

Ethanolic extract of *Cucurbita moschata* leaves were subjected to GCMS analysis. The instrument used was GC/MS Clarus 500 (Perkin Elmer). The Column used was Restek RtxR – 5, (30-meter X 0.25 mm) (5% diphenyl / 95% dimethyl polysiloxane). Injection temperature was 280 0C, Injection volume was 1.0 µl and run time was 60 min (Yasser et al. 2021)

2.4.Data Interpretation:

Figure 1 depicts the sample treated to GCMS and the total isolated peaks. All of the prominent peaks have extracted ion chromatograms. The mass of the compounds and fragments measured were compared with NIST for identification of probable compounds contained in the sample (Fig.-1).

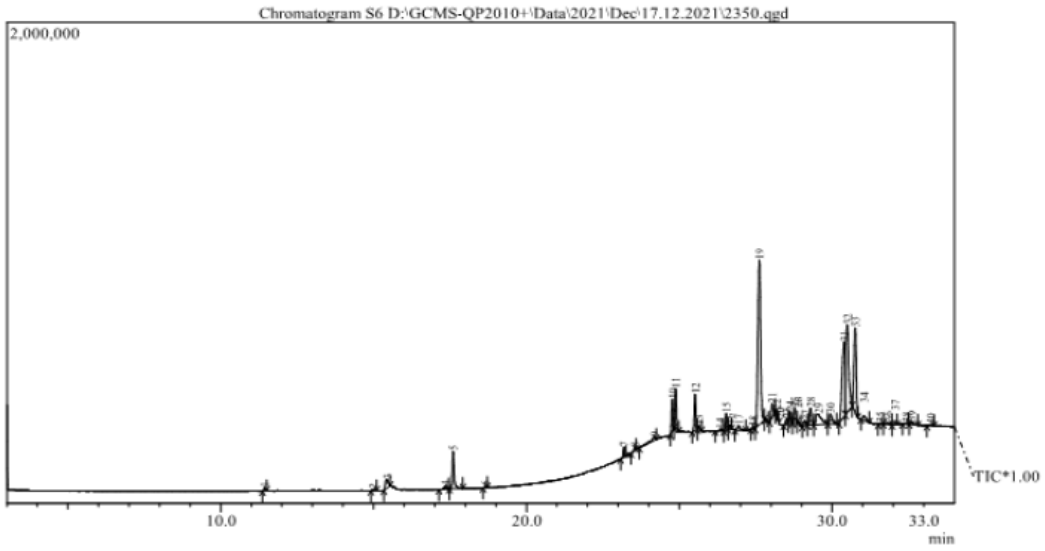

Fig.-1: GCMS Total Ion Chromatogram of ethanolic extract of *Cucurbita moschata* leaves

*2.5.*Acute oral toxicity study

A preliminary pharmacological investigation was carried out to evaluate the drug's acute pharmacological effects and safety. To determine the lethal dosage (LD50) of *Cucurbita moschata*, an acute toxicity study was done. These studies were done in female Albino Rats (150 to 200 g body weight), using "Up and Down Method" as per OECD 425 guidelines. Drug suspension (2000 mg/kg) was orally administered, to the overnight fasted rats. Then the animals were watched continually, once in 30 minutes for the next 4 hrs for any changes in their neurological and general behavioral and finally for death after 24hrs.The extract was found to be safe up to a dose of 2000mg/kg body weight (New OCED 425 guidelines 2001)

2.6. Selection of animals

Rats and Mice of either sex, 4-6 weeks, weighing (250-300g and 15-20g) were obtained from animal house, NUCARE, Paneer, Mangalore. The rats were appropriately grouped and then sheltered in distinct cages. The cages were kept under accepted lab conditions of temperature 25 ± 2° with an appropriate dark and light cycle of 12 hours. The animals were given complete access to standard food and water. The investigation was done following the guidelines of the CPCSEA, New Delhi, India. And the research work was permitted by the IAEC (NGSMIPS/IAEC/MARCH – 2019/135).

2.7.Experimental design

Maximal electroshock convulsion test (MES)

The maximal electroshock test is a measure of the ability of an anticonvulsant drug to abolish the tonic extensor component of the hind limb. The test extract was administered to groups of rats in doses of 100-400mg/kg body weight, p.o once in a day for 14 days. On the 14^{th} day after 30min of administration of the test drug, an electric shock (150mA, 0.2 sec) was applied using earclip electrodes. The hind limb flexion, hind limb

extension, and clonic phase were all recorded. The elimination or shortening of the extensor phase is used as an indicator of anticonvulsant activity. The ability of a compound to prevent MES seizure is believed to have the ability to prevent the spread of seizure through neural tissue. Activity against MES seizure is thought to indicate potential against grand mal epilepsy. The studies were carried out using three dose levels of plant extracts (Vogel 2002)

Animal grouping and dosing

Group I: Normal control: 0.6% w/v CMC 10ml/ kg p.o for 14 days

Group II: Standard drug group: Phenytoin 40mg/kg, i.p on 14 th day

Group III: Test extract: Low dose (100mg/kg) p.o once in a day for 14 days

Group IV: Test extract: Medium dose (200mg/ kg) p.o once in a day for 14 days

Group V: Test extract: High dose (400mg/kg) p.o once in a day for 14 days

Pentylenetetrazole-induced seizures

Pentylenetetrazole (PTZ) was administered by subcutaneous route in a dose of 60 mg/kg to all the groups of albino mice and up to 30 minutes following PTZ treatment, the animals were monitored for the commencement of clonic convulsion. The investigations were carried out using three dose levels of plant extracts. The animals were divided into five groups (n=6). The test extract was given for 14 days, followed by PTZ 30 minutes after the final dosage of the extract was given on the 14th day. The duration of the convulsion and the incidence of hind limb tonic extension (HLTE) were also recorded. If no Hind Limb Tonic Extension occurred during the time limit, the animals were considered protected. The onset of convulsion relative to controls was calculated.

Animal grouping and dosing

Group I: Normal control: 0.6% w/v CMC 10ml/kg p.o for 14 days

Group II: Standard drug group: Valproic acid 200mg/kg, i.p on 14th day

Group III: Test extract: Low dose (100mg/ kg) p.o once in a day for 14 days

Group IV: Test extract: Medium dose (200mg/ kg) p.o once in a day for 14 days

Group V: Test extract: High dose (400mg/ kg)p.o once in a day for 14 days

Lithium-Pilocarpine-induced Status epilepticus

The experiment was carried out by administering pilocarpine at a dose of 30mg/kg i.p, 24 hr after lithium sulfate (3mEq/kg, i.p.) to all groups of the experimental animals. 30 minutes before administration extracts in test groups, standard group, and the severity of status epilepticus was studied (Shirish et al. 2002).

Animal grouping and dosing

Group I: Normal control: 0.6% w/v CMC 10ml/kg p.o for 14 days

Group II: Standard drug group: Valproic acid 200mg/kg, i.p on 14th day

Group III: Test extract: Low dose (100mg/ kg) p.o once in a day for 14 days

Group IV: Test extract: Medium dose (200mg/ kg) p.o once in a day for 14 days

Group V: Test extract: High dose (400mg/ kg)p.o once in a day for 14 days

2.8.Statistical analysis

The results were presented as a mean SEM. The ANOVA test was used for statistical analysis, followed by a post hoc multiple comparison test using SPSS software. A statistically significant p-value is one that is less than 0.05.

3. Results and discussion

3.1.GCMS analysis of ethanolic extract of *Cucurbita moschata* leaves

Sixteen Bioactive compounds were identified from GCMS analysis of ethanolic extract of *Cucurbita moschata* leaves. They are 1,1-Diethyl-1,2,3,4-tetrahydronaphthalene, hexanedioic acid bis (2-ethylhexy1) ester, 1-Docosanol methyl ether, Phthalic acid hexadecyl propyl ester, Isopropyl stearate, 10-Octadecenoic acid methyl ester, Caryophyllene, Trilinolein, p-coumaric acid, Hexadecanoic acid-methyl ester, Pentadecanoic acid-14-methyl- methyl ester, 3-methyl-Cyclooctene, 9-Octadecenoic acid (Z)- methyl ester and cis-13-Octadecenoic acid—methyl ester β-Sitosterol and Lupeol (Table-1)

Table-1: Phytoconstituents revealed from GC-MS analysis of ethanolic extract of *Cucurbita moschata* leaves

Peak	R.Time	Area	Area%	Compound name
1	11.644	199128	0.10	1,1-Diethyl-1,2,3,4-tetrahydronaphthalene
2	15.378	1934076	0.97	Hexanedioic acid bis (2-ethylhexy1) ester
3	15.758	331022	0.17	1-Docosanol methyl ether
4	17.556	25096962	12.61	Phthalic acid hexadecyl propyl ester
5	18.626	34461415	17.31	Isopropyl stearate
6	23.362	163650	0.08	Octadecenoic acid methyl ester
7	23.600	8270183	4.16	Caryophyllene
8	24.472	514947	0.26	Trilinolein
9	25.083	166811	0.08	p-coumaric acid
10	25.670	876926	0.44	Hexadecanoic acid-methyl ester
11	26.624	5906384	2.97	Pentadecanoic acid-14-methyl- methyl ester
12	26.771	153745	0.08	3-methyl- Cyclooctene

13	26.853	739038	3.71	9-Octadecenoic acid (Z)- methyl ester
14	27.672	62949757	31.63	B-Sitosterol
15	30.796	50089747	25.17	Lupeol
16	31.233	527281	0.26	cis-13-Octadecenoic acid—methyl ester

3.2.Maximal electroshock convulsion test (MES)

The ethanolic leaf extract of *Cucurbita moschata* suggested that the pre-determent with different doses (100mg/kg, 200mg/kg, and 400mg/kg) exhibited a dose-dependent significant decrease in the extensor phase when compared with the normal control group. The rats administered with the extract showed almost comparable results as that of the standard (phenytoin) treated group as seen in Table-2.

Table-2: Effect of ethanolic extract of *Cucurbita moschata* on the decrease in extensor phase in MES

Group	Treatment	Onset of convulsion
I	Control	11.8333 ± 0.60093^{b}
II	Standard (Phenytoin)	0 ± 0^{a}
III	Ethanolic extract-100mg/kg	7.5000 ± 0.4281
IV	Ethanolic extract-200mg/kg	7.0000 ± 0.3651
V	Ethanolic extract-400mg/kg	6.5000 ± 0.4281

a=p<0.05 When compared to groupI(control) b=p<0.05 When compared to group II (Standard). All the values are expressed as mean ±SEM(n=6)

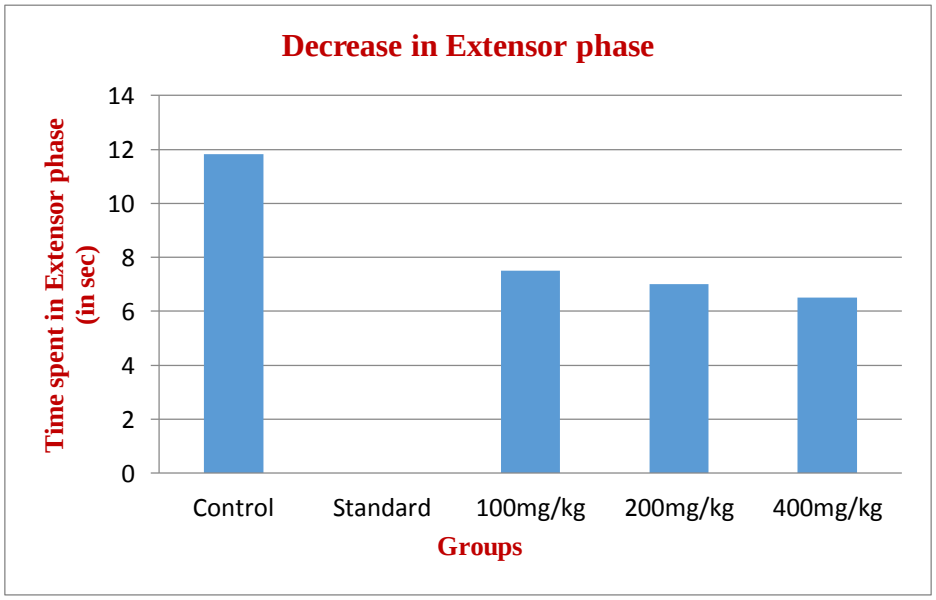

Fig 2: Effect of *Cucurbita moschata* on decrease in extensor phase in MES

3.3.Pentylene tetrazole induced seizure

The ethanolic extract of *Cucurbita moschata* leaf revealed that when compared to the control group, pretreatment with different dosages of the extract (100mg/kg, 200mg/kg, and 400 mg/kg) did not exhibit a significant delay in the onsets of convulsion. This indicates that the extract of *Cucurbita moschata* did not have any effect on the PTZ model. However, *Cucurbita moschata* test extract was statistically insignificance ($p>0.05$) when compare to a normal control group. The above result demonstrates that the ethanolic leaf extract fail to show antiepileptic activity against petit mal epilepsy as seen in table-3

Table-3: Effect of ethanolic extract of *Cucurbita moschata* on pentylenetetrazole induced seizures

Group	Treatment	Onset of convulsion in sec
I	Control	177.83 ± 11.730^{b}
II	Standard (valproic acid)	624.00 ± 1.0331^{a}
III	Ethanolic extract-100mg/kg	224.83 ± 7.9599
IV	Ethanolic extract-200mg/kg	161.50 ± 6.1734
V	Ethanolic extract-400mg/kg	171.67 ± 5.4873

a=p<0.05 When compared to groupI(control) b=p<0.05 When compared to group II (Standard). All the values are expressed as mean ±SEM(n=6)

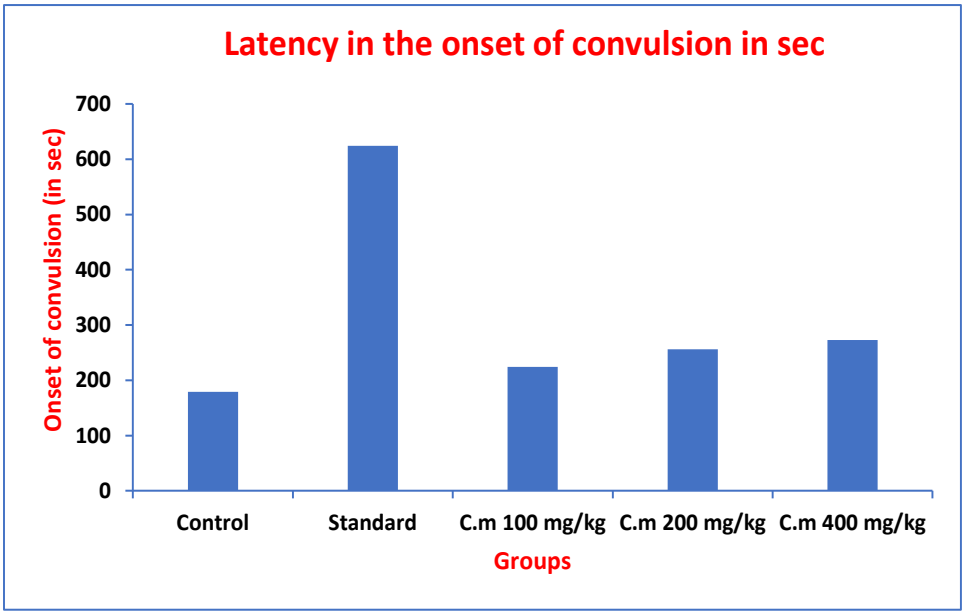

Fig 3: Effect of *Cucurbita moschata* on PTZ induced Seizure

3.4.Lithium pilocarpine induced status epilepticus

The antiepileptic activity of an ethanolic extract of the leaf of Cucurbita moschata was evaluated, and it was found that pre-treatment with different doses of the extract (100mg/kg, 200mg/kg, and 400mg/kg) did not result in significant reductions in the severity of status epilepsy when compared to

the control group. The group of animals treated with the test extracts showed almost comparable results as that of control group confirming that the extract of *Cucurbita moschata* did not have any effect on the Lithium-pilocarpine induced status epilepticus model. However, *Cucurbita moschata* test extract was statistically insignificance ($p>0.05$) when compare to a normal control group as seen in Table-4

Table-4: Effect of ethanolic extract of *Cucurbita moschata* in lithium pilocarpine induced status epilepticus

Group	Severity of Status Epilepticus mean±SEM (min)									
	0	15	30	45	60	75	90	120	150	180
Control	0	1.61±0.12[b]	3.01±0.0.14[b]	3.80±0.11[b]	4.01±0.15[b]	4.36±0.40[b]	2.01±0.12[b]	3.82±0.17[b]	2.04±0.18[b]	1.04±0.16[b]
Standard	0	0.47±0.07[a]	0.91±0.41[a]	0.68±0.005[a]	0.99±0.009[a]	1.00±0.12[a]	1.50±0.008[a]	1.02±0.16[a]	0.50±0.008[a]	0.46±0.16[a]
100mg/kg	0	0.49±0.02	2.8±0.03	3.0±0.23	3.8±0.03	3.7±0.49	1.91±0.02	3.6±0.26	1.9±0.21	0.94±0.01
200mg/kg	0	1.4±0.28	2.8±0.47	3.0±0.22	3.6±0.14	3.68±0.49	1.87±0.26	3.6±0.46	1.8±0.22	0.91±0.01
400mg/kg	0	1.46±0.30	2.82±0.04	2.82±0.22	3.44±0.19	2.97±0.41[b]	1.84±0.19	3.58±0.44	1.8±0.22	0.8±018

a=p<0.05 When compared to groupI(control) b=p<0.05 When compared to group II (Standard). All the values are expressed as mean ±SEM(n=6)

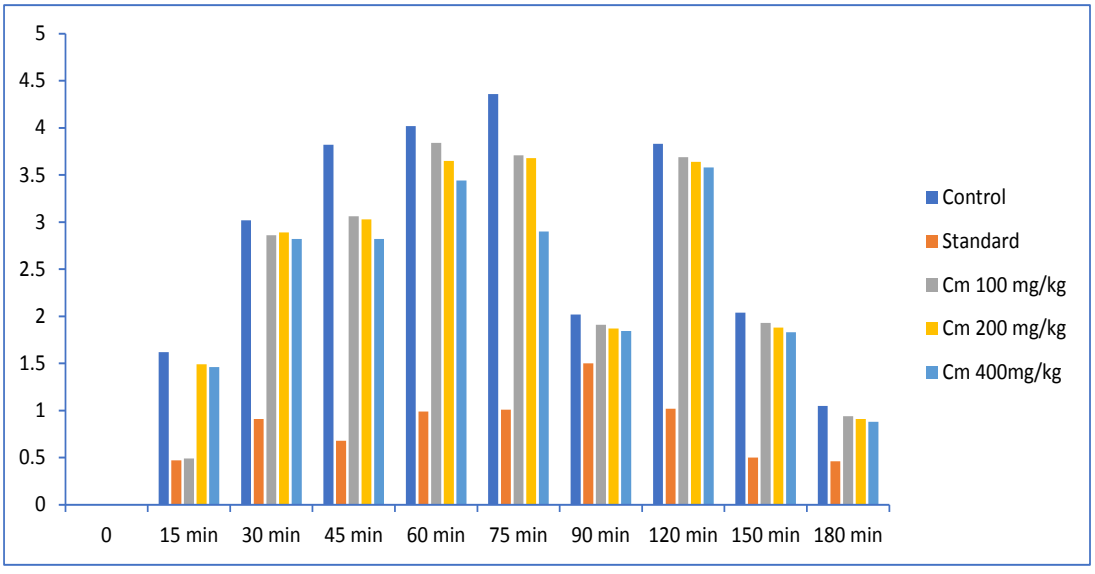

Fig 4: Effect of *Cucurbita moschata* in lithium pilocarpin induced Seizure

MES model is one of the well-accepted models for the evaluation of the anti-epileptic activity. When compared to the control, Cucurbita moshata extract dramatically decreased the duration of the extensor phase in this model in a dose-dependent manner. In dosages of 100mg/kg,

200mg/kg, and 400mg/kg, the ethanolic extract protected the mice against seizures and shortened the length of hind leg extension where as the control did not show any protection, it representing that the ethanolic extract is effective against Seizures induced by MES. 400mg/kg, 200mg/kg and 100mg/kg doses of ethanolic extract produced epilepsy of 6.5, 7.0 and 7.5 respectively; compared with the control (11.83).The extensor phase in phenytoin treated group was found to be 0. A sodium channel blocker, phenytoin, is utilised as a first-line therapy for partial and tonic clonic seizures.

PTZ model **i**s another model of screening anti-epileptic activity. Ethanolic extract at dosages of 100mg/kg, 200mg/kg, and 400mg/kg did not delay the onset of clonic convulsions in this model whereas in control treated animals convulsion appeared in a short duration. When comparing the test and control groups, $p>0.05$ indicates that the results are insignificant. Valproic acid is used to treat absence, tonic-clonic, and myoclonic seizures as a first-line therapy. It is also beneficial in some epileptic syndromes and can be administered for partial seizures.

When compared to the control group, the lithium-pilocarpine model at dosages of 100mg/kg, 200mg/kg, and 400mg/kg in this ethanolic extract did not reduce the severity of status epilepticus. When the test and control groups are compared, $p>0.05$ indicates that the results are not statistically significant. Diazepam is the standard drug used.

From this investigation, the results of the present study demonstrated that the rats and mice which are pre-treated with the *Cucurbita moschata* leaf extract significantly decreased the development of Epilepsy induced by MES model when compared to control group but not in the PTZ and Lithium pilocarpine indicating that the test extract is effective against grandmal epilepsy but does not shown action against petitmal and status epilepticus. Therefore it seems that the extract could be effective only against grandma epilepsy.

4. Conclusion

Cucurbita moschata exhibited significant activity in a dose dependent manner. The Extract suspension at 400 mg/kg exhibiting significant antiepileptic activity and comparable effect as that of the standard drugs in MES model used in the study. Based on these results we can conclude that *Cucurbita moschata* possess significant ant-epileptic activity in grandmal epilepsy. These observations provide pharmacological proof and support on the traditional use of *Cucurbita moschata* as an anti-epileptic agent. *Cucurbita moschata*. Antiepileptic activity may be attributed to steroid like β-sitosterol, pentacyclic terpenoid like Lupeol and fatty acid esters identified from GC-MS analysis of ethanolic extract of *Cucurbita moschata*.

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