Evaluation and Comparison of Anti-Epileptic Activity of Methanolic Extracts of Seeds of *Cuminum Cyminum L.* and *Centratherum Anthelminticum L.* in Seizure Model

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Abstract

Objective: Evaluation and comparison of anti-epileptic activity of methanolic extracts of seeds of *Cuminum cyminum L.* and *Centratherum anthelminticum L*. in strychnine-induced seizure model.
Methods: The study was conducted in the Department of Pharmacology, University of Karachi. After 15 days of dosing, experiment was performed. A total of 40 healthy mice were selected from animal house of the Pharmacology department, University of Karachi, and were equally divided into four groups. Group 01 was on DMSO (dimethyl sulphoxide) organic solvent as control; Group 02 was on methanolic extract of *Cuminum cyminum L*. (500 mg/kg); Group 03 was on methanolic extract of *Centratherum anthelminticum L*. (200 mg/kg) and Group 04 was on reference drug diazepam (3 mg/kg). On the day of experiment, after giving doses of DMSO, methanolic extract of *Cuminum cyminum L.*, methanolic extract of *Centratherum anthelminticum L*. and diazepam, respectively to all groups, strychnine was administered and animals were observed for latency period and duration of jerks. Mortality rate was also evaluated.
Results: Group 02 and 03, receiving methanolic extracts of *Cuminum cyminum L*. and *Centratherum anthelminticum L*. showed significant seizure protection as observed by delayed seizure onset ($p \leq 0.001$) and decreased total duration of convulsions in groups 02 and 03 ($p \leq 0.01$, $p \leq 0.05$) respectively. The mortality rate also decreased significantly ($p \leq 0.05$) in comparison with group 01.
Conclusion: Anticonvulsant activity of methanolic extracts of *Cuminum cyminum L*. and *Centratherum anthelminticum L.* could be due to their antioxidant activity and further enhanced by potentiation of GABAergic and glycinergic activity. Further studies are required to confirm exact mechanism of action.
Keywords: Cuminum cyminum, convulsions, seizures, dimethyl sulphoxide, strychnine.
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Introduction

Medicinal herbs are the potential sources of drugs for therapeutic aids. They have played a significant role in health systems all over the world and are used for a number of different diseases and also for maintaining proper health. The biological properties of plants are used in alternative medicine. Currently, several scientific investigations are being conducted to determine and isolate the active compounds of plants. Medicinal plants play a vital role in the development of new drugs. Today, 80% of the developing population depends on traditional medicine. Herbal drugs referred to as plant materials or herbs, involve the use of whole or parts of plant. The *Cuminum cyminum L*. and *Centratherum anthelminticum L.* are used as analgesic, antipyretic, anti-inflammatory and anti-oxidant drugs. It

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has been observed from many studies that phenolic contents of plant have antioxidant and disease-curing activity[1,2].

Seeds of *Cuminum cyminum L*. (black cumin) are widely used in ayurvedic medicine. It is also used as a spice in Indian and Pakistani foods because of its specific aroma and hot flavouring effect. It is locally called as 'Shahijeera' in India[3,4]. It is a flowering plant and belongs to Apiaeceae (Umbelliferae) family. Cumin plants are usually cultivated all over the world because of their culinary use[5].

The phytochemicals reported in ripe cumin seeds are: essential oils, rich monoterpene aldehydes, cumin aldehyde, terpene hydrocarbon, phenyl ethanol and fixed oil (p-cymene). In addition to fixed oils, proteins, cellulose, sugar and also some minerals such as calcium, potassium, sodium, iron, magnesium and phosphorus[6,7]. Ancient Greeks kept them as holy seeds because these seeds are mentioned in the Bible[8]. The flavonoids and phenolic compounds of cumin are responsible for its antioxidant activity by quenching free radicals[9,10]. The decoction of cumin seeds is taken as health drink because of the presence of vitamin A, B,C and E and minerals, like iron, copper, potassium, calcium, in it[11].

Centratherum anthelminticum L. belongs to the family Astraceae. Its local names are 'Kali Ziri', 'Somraj' JangliJiri' and bitter cumin[4]. Seeds are 4.5-6 mm long, pointed from one side and tapered on the other, dark-brown in colour with a characteristic odour and bitter taste. Phytochemicals present in *Centratherum anthelminticum L.* are carbohydrates, phenolic compounds, tannins, flavonoids, proteins, saponins, sterols, lipids and constituents like stearic acid, palmitic acid, mysteric acid, oleic, monohydroxy-oleic acids, linoleic acid, vernolic acid and resins[1,13]. In Travancore and Ceylon, it is used for febrile convulsions. Its seeds are grounded with lime juice and applied to destroy pediculi and also given in abscesses[14,15].

Epilepsy is one of the chronic central nervous system diseases. It is characterised by unpredictable, recurrent, rhythmic electrical firing of neurons of brain called seizures. Epileptic seizures are classified into two categories partial (focal) and generalised depending on the area of the brain involved. Epilepsy may be associated with chemical imbalance, hypoxia or hypoglycaemia etc. After introduction of many new anti-epileptic drugs and combination therapies, still 25-30% cases are resistant and difficult to control. Thus, the toxicity and side effects of these allopathic medicines is attracting the use of herbal medicines. Herbs are usually considered safe since they belong to natural sources. Therefore, there is a need to scrutinize for highly effective as well as safe anti-epileptic therapy in order to minimise drug-related toxicity. In this study, we are investigating and comparing the anti-epileptic potential of methanolic extracts of *Cuminum cyminum L.* and *Centratherum anthelminticum L.* seeds in strychnine-induced seizure model.

Materials and Methods

The seeds of *Cuminum cyminum L*. and *Centratherum anthelminticum L*. were bought from the main merchant market of Saddar, Karachi. Authenticated by a senior scientist of Pharmacy at Hamdard University. The samples of seeds were also submitted in the Museum of Pharmacognosy, University of Karachi with the voucher #00111 and 00112 respectively for future reference.

The seeds of *Cuminum cyminum L.* and *Centratherum anthelminticum L.* were soaked in methanol for fifteen days. After 15 days solvent was filtered through filter paper and evaporated by rotary evaporator under reduced pressure. The concentrated extracts were used for the study.

A total of 40 healthy albino mice (either sex) weighing 25-30 g were selected for the study[16]. All animals were bred locally in the animal house of Department of Pharmacology, University of Karachi, housed in iron cages, fed on regular pellet diet and water ad libitum. Animals were kept under standard environmental conditions that are: temperature 26 ± 2ºC, humidity 55 ± 5% and a cycle of light and

dark 12/12, and were handled with specifications provided in the Helsinki's Resolution, (1964). Board of Advanced Studies and Research (BASR) University of Karachi has approved the study protocol, resolution # 10(P)11 dated 21.02.2014 and 03.03.2014.

Sample size was calculated by Resource equation method[16]. All mice were divided into four groups, each group containing ten mice (n= 10). Methanolic extracts of *Cuminum cyminum L*. and *Centratherum anthelminticum L.* were insoluble in water so it was dissolved in 10% DMSO (dimethlysulphoxide) an organic solvent. Group 01: all mice were given 0.25 ml of DMSO daily (control group). Group 02: methanolic extract of *Cuminum cyminum L*. was administered with a dose of 500 mg/kg[17]. The average weight of the mice was 25 g, by making the solution 500 mg/10 ml, 0.25 ml was given to each daily(12.5 mg/25 g). Group 03: Methanolic extract of *Centratherum anthelminticum L*. was given a dose of 200 mg/kg by making a solution 200 mg/10 ml and 0.25 ml was given to each daily[18]. Group 04: all mice were given diazepam 3 mg/kg i.e. 0.75 mg/25 g[19].

Strychnine is used as a chemical convulsing agent[20]. It is administered intraperitoneally at a dose of 0.1 mg/kg intraperitoneally (IP). Fractional dilution was done to give 0.025 mg/25 g and animals were observed for latency and duration of convulsions and further till 48 h for mortality after 24 h.

The statistical analysis was carried out by using SPSS version 19 for windows. Data was analysed by using one way ANOVA followed by post hoc Tukey's test for comparisons between groups. The results were presented as mean ± SD (n= 10) varies with $p \leq 0.05$ considered to be statistically significant.

Result

Table 1 on comparison of Group 02 and 03 with Group 01 (the control group), regarding seizure protection, latency of seizures was significantly ($p \leq 0.001$) increased in both groups i.e. Cuminum group and *Centratherum anthelm-inticum L*. group. While the duration of seizures was significantly ($p \leq 0.01$) decreased in *Cuminum cyminum L*. group and non-significantly decreased in *Centratherum anthelminticum L*. group. Mortality rate was reduced significantly ($p \leq 0.05$) in both groups 02 and 03 *Cuminum cyminum L.* and *Centratherum anthelminticum L.* groups. While on comparison to diazepam group (04), latency was significantly decreased ($p \leq 0.05$) and ($p \leq 0.01$) respectively in *Cuminum cyminum L*. and *Centratherum anthelminticum L*. groups.

Table 1. Effect of treatment on strychnine-induced seizures

Groups	Latency (sec)	Duration (of seizure)	Mortality after 24h
Control(DMSO)	115.86 ± 4.40	31.00 ± 3.86	8/10
Cuminum cyminum	203.93 ± 10.24$^{***\#}$	24.54 ± 1.94**	5/10$^{*\#}$
Centratherum anthe -lminticum	181.24 ± 4.56$^{***\#\#}$	30.62 ± 1.54$^{\#\#}$	5/10$^{*\#}$
Diazepam (standard)	229.30 ± 7.96***	23.18 ± 2.79***	0/10***

n=10, Values are mean ± SD, significance calculated by using one way ANOVA. Followed by post hoc Tukey's test and LSD, according to which $^{*\#}p \leq 0.05$, $^{**\#\#}p \leq 0.01$, $^{***\#\#\#}p \leq 0.001$ were considered significant, very significant and highly significant as compared to control and standard, respectively.

Discussion

The results of present study demonstrate that methanolic extracts of Cuminum cyminum L. and Centratherum anthelminticum L. possess anticonvulsant effect triggered by strychnine, a glycine-receptor antagonist. Strychnine is a poisonous alkaloid, obtained from Strychnous nux-vomica seeds. It is a neurotoxin and acts on spinal cord stimulation. Strychnine exerts its effects by blocking the binding of glycine to the glycine-sensitive chloride channel within the spinal cord. Normally, when glycine binds to this channel, it causes increased inward flow of chloride resulting in hyperpolarisation and blocking cells to propagate nerve signals. These inhibitory effects of glycine are blocked by strychnine intoxication, which consequently result in increased nerve signal transmission. Strychnine induced excitation leads to unregulated muscle spasm, extensor muscle con-

tractions, convulsions followed by death due to respiratory paralysis[25].

Pre-experiment dosing with methanolic extracts showed its protective effects against convulsions. The measured parameters, the latency to seizure onset and duration of seizures seemed to be most sensitive parameters when comparing with anticonvulsant effects, previous studies showed depressed seizure activity in animal model of epilepsy after exogenous glycine administration[21,26]. Furthermore, other studies suggested synergistic anticonvulsant effect when glycine was administered with $GABA_A$ (gamma-aminobutyric acid) receptor agonist[27]. Anticonvulsant activity of methanolic extracts of *Cuminum cyminum L.* and *Centratherum anthelminticum L.* may be attributed due to inhibition of strychnine sensitive glycine receptors alone or by concomitant potentiation of glycinergic and GABAergic activities which in turn may amplify its anti-epileptic activity.

Neuroprotective effects of vitamin E have already been identified previously due to its antioxidant characteristics. Vitamin E could exert anti-epileptic effect by inhibiting free radical formation[28,29]. Results of another study showed that vitamin E administration reduces seizure frequency and intensity by decreasing oxidative stress in brain cells of experimental animals[29]. The phytochemicals of *Cuminum cyminum L.* i.e. vitamins and minerals are also responsible for its antioxidant role[11]. However, previous studies indicate that neurons involved in oxidative injury create a toxic environment within the hippocampus as a result of induction of reactive oxygen species activation and propagation to apoptosis which ultimately destroy hippocampal neurons[22,30].

Anticonvulsant effect of methanolic extracts of *Cuminum cyminum L.* and *Centratherum anthelminticum L.* could be attributed to its protective effect against cellular injury induced by strychnine in hippocampal neurons. Exact mechanism of this resistance to hippocampal injury is currently unclear it can be assessed by measuring superoxide dismutase and glutathione levels. The anticonvulsant activity of diazepam (benzodiazepines) is by modulating the activity on $GABA_A$ receptors, which also causes muscle relaxation, sedative effects and amnesia. The disadvantage of diazepam is its tolerance, depression of CNS functions i.e. mental alertness and motor co-ordination[23,24].

Conclusion

The results of our study indicate that methanolic extracts of *Cuminum cyminum L.* and *Centratherum anthelminticum L*.possess anticonvulsant activity, which may justify its use as an alternative medicine in order to prevent drug related toxicities and drug resistance. The proposed anticonvulsant may involve potentiation of both GABAergic and glycinergic stimulatory mechanism. Antioxidant activity can amplify its anti-epileptic activity. The exact mechanism of action requires detailed molecular study.

Conflict of Interest

Authors have no conflict of interests and no grant/funding from any organisation.

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