Original Article

Anticonvulsant Activity of *Capsicum Annum* (Red Pepper) Fruit Extract using Pentylentetrazole and Maximum Electroshock Induced Seizure Tests in Rats

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ABSTRACT

Background: Epilepsy is a disorder of brain function characterized by periodic and unpredictable occurrence of seizures that refers to as a transient alteration of behavior due to disordered, synchronous and rhythmic firing of populations of brain neurons. Modern drug therapy of epilepsy is complicated by side effects and inability to control seizures in some patients.

Objectives: The present study was conducted to test the anticonvulsant activity of *Capsicum annum* fruit extract in rat models.

Materials and Methods: The methanolic extract of *Capsicum annum* fruit was tested for its potential anticonvulsant activity using pentylentetrazole (PTZ) and maximum electroshock (MES) induced seizure models in rats. The simple activity meter was used to determine the sedative activity of *Capsicum annum* fruit extract. Thereafter, the protective index was calculated.

Results: Results obtained showed that the extract at concentrations of 5, 9, 10, 20 and 40 mg/kg produced dose dependent anticonvulsant activity. *Capsicum annum* (40 mg/kg, i.p.) caused 100% protection from seizure induced by PTZ and sedation in 50% of rats (TD_{50}). Fifty percent anticonvulsant activity was produced by 9mg/kg *Capsicum annum*. Moreover, the protective index was calculated to be more than four. When tested on seizure induced by MES, *Capsicum annum* extract at doses of 10, 20 and 40 mg\kg showed no seizure protection.

Conclusion: It could be concluded that *Capsicum annum* fruit could be a source for potential antiepileptic agent(s). Further studies are needed to determine the active constituents responsible for the anticonvulsant activity as well as to elucidate their mechanism(s) of action.

Key words: Epilepsy, Pentylenetetrazole, electroshock, *Capsicum annum*, Sudan.

Epilepsy is a disorder of brain function characterized by periodic and unpredictable occurrence of seizures that refers to as a transient alteration of behavior due to disordered, synchronous and rhythmic firing of populations of brain neurons[1].

Modern drug therapy of epilepsy is complicated by the inability of drugs to control seizure in some patients and by the side effects that range in severity from minimal impairment of the central nervous system (CNS) to death from aplastic anemia or hepatic failure. Thus their effectiveness and safety remain challenges[2]. Medicinal plants used in traditional medicine for the treatment of epilepsy have been scientifically shown to possess promising anticonvulsant activities in animal models and they could be considered as a good source of newer anticonvulsants[3]. *Capsicum annum* (red pepper) and many other plants were reported to be used traditionally as anti-seizure[4]. *Capsicum annum* is a species native to South America and it is cultivated worldwide. Capsicumoleoresins contain a complex mixture of essential oils, waxes, colored materials (mainly capsanthin, capsorubin, zeaxanthin, cryptoxanthin, and lutein). Several capsaicinoids are commonly used as pungent flavors in food, natural plant colors, and pharmaceutical ingredients[5].Capsicum also contains fixed oils, proteins, carotenoids, ascorbic acid and thiamine[6].The main medicinal properties of capsicum are derived from capsaicin. Capsicum has been used to treat asthma, pneumonia, diarrhea, cramps, colic, toothache, flatulent, dyspepsia without inflammation, insufficiency of peripheral

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circulation, as a gargle for sore throat, chronic pharyngitis and laryngitis and when used for cold, the ground pepper has been used with water and honey[7].

This study aimed to investigate the anticonvulsant activity of methanolic extract of *Capsicum annum* fruit.

MATERIALS AND METHODS

Materials:

Plant material: The dried fruits of *Capsicum annum* were purchased from the local market in Wad-Medani, Sudan. The plant material was identified by the Department of Pharmacognosy, Faculty of Pharmacy, University of Gezira, Sudan.

Preparation of working solutions of chemicals: Freshly prepared solutions of pentylentetrazole (Sigma-Aldrich, UK) dissolved in normal saline and sodium valproate (Sigma-Aldrich, UK) dissolved in distilled water were used.

Experimental animals: Albino rats of both sexes, weighing 150-200 g, were used. Rats were kept in the animal house, Faculty of Pharmacy, University of Gezira at room temperature. The animals have free access to water and fasted for an overnight before the experiment.

Methods:

Extraction of plant material: One hundred grams of the coarsely powdered *Capsicum annum* fruits were extracted by maceration using pure methanol in a conical flask for 72 hours, filtered and evaporated at 60 ^{o}C using a rotary evaporator. The dried extract powder was placed into an amber glass container and kept in a refrigerator until use.

Anticonvulsant tests: Pentylenetetrazole (PTZ) and the maximal electroshock (MES) induced seizure tests wereused for assessing the anticonvulsive effects of *Capsicum annum*in rats.

Pentylentetrazole (PTZ) - induced seizure test: Pentylentetrazoleis known to be used for induction of clonic convulsions[8]. Firstly animals were divided into seven groups, using six rats in each group. Negative control group received distilled water and positive control group was treated with sodium valproate of 200mg\kg intraperitoneally (i.p). The remaining groups were treated with *Capsicum annum* extract 5, 9, 10, 20 and 40mg/kg i.p. Thirty minutes later the rats were treated with PTZ (80mg/kg) subcutaneously. Immediately after the injection of the PTZ, rats were individually placed in glass boxes and observed for the presence or absence of seizure.

Maximal electroshock (MES)-induced seizure test: MES that induces reproducible tonic convulsion characterized by hind limb extension was performed. In this experiment, rats were divided into six groups, using six rats in each group. Negative control group received distilled water and positive control groups were treated with sodium valproate (200 and 400mg\kg i.p). The remaining groups were treated with *Capsicum annum* extract (10, 20 and 40mg/kgi.p).Thirty minutes later rats in all groups received electro convulsive shock (100 pulses\sec, 0.5 s) delivered through a pair of ear clip electrodes (Ugo Basile-ECT unite 5780, Italy) to induce hind limb extension[9]. Thereafter, rats were observed for the presence or absence of seizure.

Measurement of motor activity: The cumulative locomotor activity of rats was measured using a simple activity meter, which is modified from Turner (1965)method[10]. The activity box is divided into equal 25 squares (10 cm x10 cm, for each square). The number of squares crossed by animal was recorded as activity counts. Treated animals were placed in the activity cage after 25 minutes, and motor activity counts were recorded for 5 minutes. Activity counts less than 10 squares/ 5minutes were reported as sedation.

Protective index: To know whether the anticonvulsant activity of a certain compound is selective or due to general CNS depression, value of protective index (PI) for this compound is calculated. Protective index is the ratio of a median sedative (toxic) dose (TD_{50}) to median effective dose (ED_{50}), calculated as follow:

$PI = TD_{50} / ED_{50}$

Where:**TD_{50}:**the median dose which cause sedation in 50% of animals; **ED_{50}:** the median

effective dose which cause anticonvulsant effect in 50% of the tested animals.

RESULTS:

The present study investigated the anticonvulsant potential of *Capsicum annum* fruit. The selection was based on the previous reported traditional use of this plant as anticonvulsant and/or sedative agent[4].

As shown in Table 1, the methanolic extract of Capsicum *annum,* produced a dose dependent anticonvulsant activity. *Capsicum annum* (40 mg/kg) caused 100% protection from seizure induced by PTZ.

The protective index of *Capsicum annum* extract was found to be more than four, since 40mg/kg produced 50% sedation (TD_{50}) and 9mg/kg produced 50% protection from seizure induced by PTZ (ED_{50}).

On the other hand, *Capsicum annum* extract in doses of 10, 20 and 40 mg\kg showed no protection to rats against seizure induced by MES (Table 2).

Table 1: Effect of intraperitonial administration of *Capsicum annum* fruit methanolic extract on locomotor activity and pentylentetrazole induced seizure in rats.

Treatment	Dose	Sedation %	Seizure protection %
Sodium valproate (Positive control)	200mg/kg	0.00	100
Distilled water (Negative control)	10mg/kg	0.00	0.00
Capsicum annum	5 mg/kg	0.00	16.6
	9 mg/kg	16.6	50
	10 mg/kg	16.6	66.6
	20mg/kg	33.3	83.3
	40mg/kg	50	100

Table 2: Effect of intraperitonial administration of *Capsicum annum* fruit methanolic extract on locomotor activity and maximum electroshock induced seizure in rats.

Treatment	Dose	Sedation %	Seizure protection %
Sodium valproate (Positive control)	200mg/kg	0.00	0.00
	400mg/kg	0.00	100
Distilled water (Negative control)	10mg/kg	0.00	0.00
Capsicum annum	10mg\kg	16.6	0.00
	20mg\kg	33.3	0.00
	40mg\kg	50	0.00

DISCUSSION:

A promising anticonvulsant activity was produced by the methanolic extract of *Capsicum annum*. The higher protective index of this extract is a motivating factor for further studies. These results were consistent with those reported by Adesina (1982)[4] who documented that *Capsicum annum* could be used as anticonvulsant agent. Moreover, capsaicin (the biologically active substance in *Capsicum annum*) was found to cause depletion of substance P (or other peptides) and/or glutamate[11], and when capsaicin peripherally administered it was reported to affect the brain electrical activity[12]. A recent study showed that capsaicin (the prototype vanilloid receptor agonist) possesses anticonvulsant activity[13]. Therefore, the anticonvulsant activity produced by *Capsicum annum* extract maybe due to capsaicin and/or other allied phytoconstituents therein through the vanilloid receptors and/or other receptors.

CONCLUSION:

It could be concluded that *Capsicum annum* fruit could be considered as a source for potential antiepileptic agent(s) especially for

absence seizure, or guide to design synthetic or semisynthetic antiepileptic agent(s). Further studies are needed to determine the active constituent(s) responsible for the anticonvulsant activity as well as to elucidate the mechanism(s) of action.

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