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Anticonvulsant activity of *Cotyledon orbiculata* L. (Crassulaceae) leaf extract in mice

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Abstract

The anticonvulsant activity of *Cotyledon orbiculata* L. (Crassulaceae) was investigated by studying the effects of both aqueous and methanol extracts of the plant species on seizures induced by pentylenetetrazole, bicuculline, picrotoxin and *N*-methyl-DL-aspartic in mice. Aqueous extract of *Cotyledon orbiculata* (50–400 mg/kg, i.p.) and methanol extract (100–400 mg/kg, i.p.) significantly prolonged the onset of tonic seizures induced by pentylenetetrazole (95 mg/kg, i.p.). Methanol extract (400 mg/kg, i.p.) also significantly reduced the incidence of the seizures. One hundred to two hundred milligrams/kilogram (i.p.) of aqueous extract of *Cotyledon orbiculata* significantly delayed the onset of the tonic seizures induced by bicuculline (40 mg/kg, i.p.), picrotoxin (12 mg/kg, i.p.) and *N*-methyl-DL-aspartic acid (NMDLA, 400 mg/kg, i.p.). Similarly, methanol extract (100–400 mg/kg, i.p.) significantly delayed the onset of the tonic seizures induced by bicuculline (40 mg/kg, i.p.) and picrotoxin (12 mg/kg, i.p.) while 100 mg/kg (i.p.) significantly delayed the onset of *N*-methyl-DL-aspartic acid (NMDLA, 400 mg/kg, i.p.)-induced seizures. Methanol extract (200 mg/kg, i.p.) significantly reduced the incidence of the seizures induced by bicuculline (40 mg/kg, i.p.). Phenobarbitone (12 mg/kg, i.p.) and diazepam (0.5 mg/kg, i.p.) effectively antagonized only seizures induced by PTZ (95 mg/kg, i.p.), bicuculline (40 mg/kg, i.p.) and picrotoxin (12 mg/kg, i.p.). Phenytoin (30 mg/kg, i.p.) did not affect any of the seizures to any significant extent. The data obtained suggest that both aqueous and methanol extracts of *Cotyledon orbiculata* have anticonvulsant property and may probably be affecting both gabaergic and glutaminergic mechanisms to exert its effect. The phytochemical analysis carried out revealed the presence of cardiac glycosides, saponins, tannins, reducing sugar and triterpene steroids in the plant extract.

Keywords: *Cotyledon orbiculata* leaf; Aqueous and methanol extracts; Anticonvulsant activity; Phytochemical analysis; Mice

1. Introduction

Southern Africa, in particular South Africa, has a rich floral biodiversity containing about 4000 species of plants with medicinal properties and use (Van Wyk and Gericke, 2000). This has also lead to greater reliance of mainly the rural communities in South Africa on plant medicines for their daily healthcare needs. One of such plants with wide medicinal use is *Cotyledon orbiculata* L. It belongs to the family, Crassulaceae. It is a small shrub with fleshy leaves and widely distributed in Southern Africa. It is known locally as "Seredile" in Sotho and Tswana, "Plakkie" in Afrikaans and "Imphewula" in Xhosa (Watt and Breyer-Brandwijk, 1962; Van Wyk et al., 1997). *Cotyledon orbiculata* is used in the treatment of various ailments in different parts of South Africa. The fleshy leaves have been used to treat corn and warts. The juice of the leaves is used as drops for earache and toothache, and as hot poultice for boils and inflammation (Watt and Breyer-Brandwijk, 1962; Van Wyk et al., 1997). According to Watt (1967), the juice has been used to treat epilepsy. However, traditional medicine practitioners in the Western Cape Province, South Africa use the infusion of the fleshy leaves for the treatment of epilepsy (oral communication). Little information about the plant exists. The claim of therapeutic success of the plant in the treatment of epilepsy has not been scientifically scrutinized. The main aim of this project was, therefore, to investigate the anticonvulsant effect of *Cotyledon orbiculata* L. leaf aqueous and methanol extracts in mice.

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2. Materials and methods

2.1. Plant material

The fleshy leaves of *Cotyledon orbiculata* were collected from Kirstenbosch National Botanical Garden, Cape Town. The plant material was identified by Mr. Franz Weitz, a taxonomist in the Department of Biodiversity and Conservative Biology, University of the Western Cape and the voucher specimen (GEORGE 01) deposited in the University's Herbarium.

2.2. Preparation of plant extract

The fleshy leaves (16.8 kg) of *Cotyledon orbiculata* were washed with water, sliced into pieces and dried in a ventilated oven at 40 °C for 120 h. The dried plant material (880.2 g) was ground into fine powder using Waring Commercial laboratory blender and passed through 850 μm sieve. For the preparation of the aqueous extract, 63 g of dried powder was refluxed in 1 l of boiled water, allowed to cool over 24 h and filtered. The resultant filtrate was frozen at −80 °C and freeze-dried (LSL Secfroid SR, Model 3021, Switzerland) for 72 h to obtain a yield of 15.9 g of dried aqueous plant extract. For the preparation of the methanol extract, the dried powder (800.6 g) was extracted in a soxhlet extractor with methanol for 72 h. The methanol filtrate was evaporated to dryness using a Buchi RE11 rotavapor and Buchi 461 water bath. A yield of 283.8 g of crude methanol extract was obtained and preserved in a desiccator. Fresh solution of the aqueous crude extract was prepared on each day of the experiment by dissolving a given quantity of the dried extract in an appropriate volume of physiological saline while that of the crude methanol extract was prepared by dissolving a given quantity of the methanol extract in a small volume of dimethylsulfoxide (DMSO) and made up to the appropriate volume with physiological saline. Either the aqueous or methanol solution was administered intraperitoneally (i.p.) to mice in a volume of 1 ml/100 g of animal.

2.3. Animals

Male albino mice bred in the Animal House of the Discipline of Pharmacology, School of Pharmacy, University of the Western Cape, South Africa, weighing 18–30 g were used in groups of eight per dose of plant extract or drug. They had access to food and water *ad libitum*. Each animal was used for one experiment only.

2.4. Drugs and chemicals

Pentylenetetrazole (PTZ, Sigma Chemical Co.), picrotoxin (Sigma Chemical Co.), *N*-methyl-DL-aspartic acid (NMDLA, Sigma Chemical Co.), phenobarbitone (Gardenal, Rhonepoulenc Rorer, South Africa) and 5,5-diphenylhydantoin sodium salt (Phenytoin, Sigma Chemical Co.) were all dissolved in physiological saline. +Bicuculline (Sigma Chemical Co.) was suspended in 0.5 ml of Tween 80 and adjusted to an appropriate volume with physiological saline. Diazepam (Valium®, Roche, South Africa) was also suspended in a minimum amount of polyethylene glycol 400 (Fluka AG, Buchs) and adjusted to an appropriate volume with physiological saline. Fresh drug solutions were prepared on each day of the experiments. Drugs were administered intraperitoneally (i.p.) in a volume of 1 ml/100 g of animal. Control animals received equal volume injections of the appropriate vehicle. The doses and pretreatment times of the aqueous or methanol extract and the standard antiepileptic drugs used were obtained from preliminary studies in our laboratory. The pretreatment times following the administration of either PTZ, bicuculline, picrotoxin or NMDLA were plant extract (15 min), phenobarbitone (10 min), diazepam (20 min) and phenytoin (20 min).

2.5. Anticonvulsant assessment

Modified method of Vellucci and Webster (1984) was used to assess the anticonvulsant effect of the aqueous or methanol extract of *Cotyledon orbiculata*. Mice were kept individually in transparent mice cages (25 cm × 15 cm × 15 cm) for 30 min to acclimatize to their new environment before the commencement of the experiment. Seizures were induced in mice with PTZ (95 mg/kg, i.p.), bicuculline (40 mg/kg, i.p.), picrotoxin (12 mg/kg, i.p.) or NMDLA (400 mg/kg, i.p.) and the animals were observed for convulsion for a period of 30 min. Hind limb extension was taken as tonic convulsion.

The onset of tonic convulsion and the number of animals convulsing or not convulsing within the observation period were noted. Experiments were repeated following the pretreatment of animals with either aqueous or methanol extract of *Cotyledon orbiculata*, phenobarbitone, diazepam, phenytoin or control vehicle prior to the administration of any of the convulsant agents used. The ability of the plant extract to prevent or delay the onset of the hind limb extension exhibited by the animals was taken as an indication of anticonvulsant activity (Vellucci and Webster, 1984; Amabeoku and Chikuni, 1993). All experiments were carried out between 8.40 h and 16.00 h in a quiet room with an ambient temperature of 22 ± 1 °C.

2.6. Phytochemical analysis

The methods of Harbone (1984) and Ikhiri et al. (1992) were used to screen the plant extract for chemical constituents as shown in Table 1.

Table 1
Phytochemical screening of leaf extract of *Cotyledon orbiculata*

Compounds	Tests/reagents
Alkaloids	Dragendorf's reagent/Meyer's reagent
Saponins	Frothing test
Cardiac glycosides	Lieberman's test/Keller–Killiani test
Tannins	Ferric chloride reagent
Reducing sugars	Fehling's reagent
Anthraquinones	Bornträger's test, BPC
Triterpene steroids	Sulfuric acid reagent
Flavonoids	Acid-alcohol/solid magnesium/amy-alcohol

2.7. Statistical analysis

The data on the onset of tonic convulsions were analysed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. The analysis of the number of animals convulsing was done using the Chi-squared test (Amabeoku and Chikuni, 1993; Amabeoku et al., 1998).

2.8. Ethical considerations

The experimental protocol used in this study was approved by the Ethics Committee of the University of the Western Cape, Bellville 7535, South Africa, and conforms with the University's Regulations Act concerning animal experiments.

3. Results

3.1. Anticonvulsant activity

3.1.1. Effect of aqueous extract of Cotyledon orbiculata on pentylenetetrazole-induced seizures

PTZ (95 mg/kg, i.p.) elicited tonic convulsion in 100% of the animals used. *Cotyledon orbiculata* (50–400 mg/kg, i.p.) delayed the onset of PTZ (95 mg/kg, i.p.)-induced tonic convulsion significantly. One hundred to two hundred milligrams/kilogram (i.p.) of aqueous extract of *Cotyledon orbiculata* protected 25% of mice against the convulsion. Phenobarbitone (12 mg/kg, i.p.) and diazepam (0.5 mg/kg, i.p.) completely protected the animals against the tonic convulsion elicited by PTZ (95 mg/kg, i.p.). Phenytoin (30 mg/kg, i.p.) neither affected the onset nor the incidence of PTZ-induced convulsion to any significant extent (Table 2).

3.1.2. Effect of aqueous extract of Cotyledon orbiculata on bicuculline-induced seizures

Bicuculline (40 mg/kg, i.p.) induced tonic convulsion in all the animals used. Aqueous extract of *Cotyledon orbiculata* (100–200 mg/kg, i.p.) significantly delayed the onset of bicuculline (40 mg/kg, i.p.)-induced convulsion. *Cotyledon orbiculata* (100 mg/kg, i.p.) did not affect the incidence but 200 mg/kg (i.p.) protected 37.5% of mice against the convulsion. Phenobarbitone (12 mg/kg, i.p.) and diazepam (0.5 mg/kg, i.p.) profoundly antagonized the tonic convulsion elicited by bicuculline (40 mg/kg, i.p.). All the animals were protected from the convulsion. Phenytoin (30 mg/kg, i.p.) did not affect the onset or the incidence of bicuculline-elicited tonic convulsion to any significant extent (Table 3).

3.1.3. Effect of aqueous extract of Cotyledon orbiculata on picrotoxin-induced seizures

Picrotoxin (12 mg/kg, i.p.) elicited seizures in all the eight mice used. Aqueous extract of *Cotyledon orbiculata* (100–200 mg/kg, i.p.) significantly prolonged the onset of picrotoxin (12 mg/kg, i.p.)-elicited tonic convulsion in mice. Two hundred milligrams/kilogram (i.p.) of *Cotyledon orbiculata* protected 50% of the animals against the seizures while 100 mg/kg

Table 2
Effect of aqueous extract of *Cotyledon orbiculata* (CO) on pentylenetetrazole (PTZ)-induced seizures in mice

Dose (mg/kg)					No. of animals convulsed/used	Onset of tonic convulsion (mean ± S.E.M.) (min)
PTZ	CO	Phenobarbitone	Diazepam	Phenytoin		
95	–	–	–	–	8/8	2.00 ± 0.38
95	50	–	–	–	8/8	6.13 ± 0.97*
95	100	–	–	–	6/8	11.83 ± 0.72**
95	200	–	–	–	6/8	10.17 ± 1.17**
95	400	–	–	–	8/8	7.13 ± 0.99*
95	–	12	–	–	0/8+	0
95	–	–	0.50	–	0/8+	0
95	–	–	–	30	8/8	2.88 ± 0.44

* $p < 0.02$ vs. PTZ (95 mg/kg, i.p.) control.
** $p < 0.005$ vs. PTZ (95 mg/kg, i.p.) control.
\+ $p < 0.001$ vs. PTZ (95 mg/kg, i.p.) control, Chi-squared test.

Table 3
Effect of aqueous extract of *Cotyledon orbiculata* (CO) on bicuculline (BC)-induced seizures in mice

Dose (mg/kg)					No. of animals convulsed/used	Onset of tonic convulsion (mean ± S.E.M.) (min)
BC	CO	Phenobarbitone	Diazepam	Phenytoin		
40	–	–	–	–	8/8	7.38 ± 0.46
40	100	–	–	–	8/8	13.25 ± 0.59*
40	200	–	–	–	5/8	14.13 ± 0.52*
40	–	12	–	–	0/8+	0
40	–	–	0.50	–	0/8+	0
40	–	–	–	30	8/8	7.50 ± 0.42

* $p < 0.005$ vs. bicuculline (40 mg/kg, i.p.) control.
\+ $p < 0.001$ vs. bicuculline (40 mg/kg, i.p.) control, Chi-squared test.

Table 4
Effect of aqueous extract of *Cotyledon orbiculata* (CO) on picrotoxin (PC)-induced seizures in mice

Dose (mg/kg)					No. of animals convulsed/used	Onset of tonic convulsion (mean ± S.E.M.) (min)
PC	CO	Phenobarbitone	Diazepam	Phenytoin		
12	–	–	–	–	8/8	10.75 ± 0.53
12	100	–	–	–	8/8	15.50 ± 0.68*
12	200	–	–	–	4/8	15.75 ± 0.70*
12	–	12	–	–	4/8	22.25 ± 0.60**
12	–	–	0.50	–	2/8+	24.00 ± 0.50**
12	–	–	–	30	8/8	10.88 ± 0.77

* $p < 0.02$ vs. picrotoxin (12 mg/kg, i.p.) control.
** $p < 0.001$ vs. picrotoxin (12 mg/kg, i.p.) control.
\+ $p < 0.01$ vs. picrotoxin (12 mg/kg, i.p.) control, Chi-squared test.

(i.p.) did not affect the number of animals convulsing. Phenobarbitone (12 mg/kg, i.p.) significantly protected 50% of mice against the convulsion. 0.5 mg/kg (i.p.) of diazepam significantly prolonged the onset of tonic convulsion elicited by picrotoxin (12 mg/kg, i.p.) and also significantly increased the incidence of the convulsion by protecting 75% of the animals. Phenytoin (30 mg/kg, i.p.) neither affected the onset nor the incidence of picrotoxin (12 mg/kg, i.p.)-elicited convulsion to any significant extent (Table 4).

3.1.4. Effect of aqueous extract of Cotyledon orbiculata on N-methyl-DL-aspartic acid-induced seizures

NMDLA (400 mg/kg, i.p.) elicited tonic convulsion in 100% of mice used. One hundred to two hundred milligrams/kilogram (i.p.) did not affect the incidence of NMDLA (400 mg/kg, i.p.)-induced convulsion but significantly delayed the onset. Phenobarbitone (12 mg/kg, i.p.), diazepam (0.5 mg/kg, i.p.) and phenytoin (30 mg/kg, i.p.) did not significantly affect the incidence or the onset of NMDLA (400 mg/kg, i.p.)-elicited tonic convulsion in mice (Table 5).

3.1.5. Effect of methanol extract of Cotyledon orbiculata on pentylenetetrazole-induced seizures

Pentylenetetrazole (95 mg/kg, i.p.) produced tonic seizures in all the animals used. A dose of 50 mg/kg (i.p.) of *Cotyledon orbiculata* did not protect any of the animals against PTZ-induced seizures. *Cotyledon orbiculata* (100–200 mg/kg, i.p.) protected 50% of the animals against PTZ-induced tonic seizures and significantly delayed the onset of the seizures. A dose of 400 mg/kg (i.p.) of *Cotyledon orbiculata* protected 62.5% of mice against PTZ-induced seizures. The dose significantly reduced the number of animals convulsing and significantly delayed the onset of PTZ-induced seizures in mice. The standard antiepileptic drugs, phenobarbitone (12 mg/kg, i.p.) and diazepam (0.5 mg/kg, i.p.) profoundly antagonized the tonic seizures produced by pentylentetrazole. DMSO (0.25 ml, i.p.) used as a vehicle, did not alter PTZ-induced seizures in mice (Table 6).

3.1.6. Effect of methanol extract of Cotyledon orbiculata on bicuculline-induced seizures

Bicuculline (40 mg/kg i.p.) induced seizures in all the animals used. *Cotyledon orbiculata* (100 mg/kg, i.p.) protected 25% of mice and significantly delayed the onset of bicuculline (40 mg/kg, i.p.)-induced seizures. A dose of 200 mg/kg (i.p.) of *Cotyledon orbiculata* protected 75% of animals against seizures induced by bicuculline (40 mg/kg, i.p.). It significantly reduced the incidence and significantly delayed the onset of the seizures. *Cotyledon orbiculata* (400 mg/kg, i.p.) did not affect the incidence of the seizures induced by bicuculline (40 mg/kg, i.p.), but significantly delayed the onset of the seizures. The standard antiepileptic drugs, phenobarbitone (12 mg/kg, i.p.) and diazepam (0.5 mg/kg, i.p.) profoundly antagonized seizures produced by bicuculline (40 mg/kg, i.p.). All the animals used were protected against bicuculline seizures, either by phenobarbitone or diazepam. DMSO (0.25 ml, i.p.) which was used as a vehicle, did not alter bicuculline (40 mg/kg, i.p.)-induced seizures in mice (Table 7).

Table 5
Effect of aqueous extract of *Cotyledon orbiculata* (CO) on *N*-methyl-DL-aspartic acid (NMDLA)-induced seizures in mice

Dose (mg/kg)					No. of animals convulsed/used	Onset of tonic convulsion (mean ± S.E.M.) (min)
NMDLA	CO	Phenobarbitone	Diazepam	Phenytoin		
400	–	–	–	–	8/8	2.75 ± 0.45
400	100	–	–	–	8/8	6.00 ± 0.46*
400	200	–	–	–	8/8	6.13 ± 0.44*
400	–	12	–	–	8/8	2.88 ± 0.44
400	–	–	0.50	–	8/8	3.00 ± 0.57
400	–	–	–	30	8/8	2.75 ± 0.31

* $p < 0.02$ vs. NMDLA (400 mg/kg, i.p.) control. There was no significant difference in the incidence of seizures between control and test animals, Chi-squared test.

Table 6
Effect of methanol extract of *Cotyledon orbiculata* (CO) on pentylenetetrazole (PTZ)-induced seizures in mice

Dose (mg/kg)					No. of animals convulsed/used	Onset of tonic convulsion (mean ± S.E.M.) (min)
PTZ	CO	Phenobarbitone	Diazepam	DMSO		
95	–	–	–	–	8/8	4.75 ± 0.98
95	50	–	–	–	8/8	4.63 ± 1.41
95	100	–	–	–	4/8	13.00 ± 2.87*
95	200	–	–	–	4/8	13.25 ± 3.30**
95	400	–	–	–	3/8+	14.12 ± 2.10***
95	–	12	–	–	0/8++	0
95	–	–	0.50	–	0/8++	0
95	–	–	–	0.25 ml	8/8	4.70 ± 1.02

DMSO, dimethylsulfoxide.
* $p < 0.025$ vs. PTZ (95 mg/kg, i.p.) control.
** $p < 0.05$ vs. PTZ (95 mg/kg, i.p.) control.
*** $p < 0.01$ vs. PTZ (95 mg/kg, i.p.) control.
+ $p < 0.05$ vs. PTZ (95 mg/kg, i.p.) control, Chi-squared test.
++ $p < 0.001$ vs. PTZ (95 mg/kg, i.p.) control, Chi-squared test.

Table 7
Effect of methanol extract of *Cotyledon orbiculata* (CO) on bicuculline (BC)-induced seizures in mice

Dose (mg/kg)					No. of animals convulsed/used	Onset of tonic convulsion (mean ± S.E.M.) (min)
BC	CO	Phenobarbitone	Diazepam	DSMO		
40	–	–	–	–	8/8	4.50 ± 0.53
40	100	–	–	–	8/8	10.50 ± 3.40*
40	200	–	–	–	2/8+	16.50 ± 1.75**
40	–	12	–	–	0/8++	0
40	–	–	0.50	–	0/8++	0
40	–	–	–	0.25 ml	8/8	5.50 ± 0.50

DMSO, dimethylsulfoxide.
* $p < 0.05$ vs. bicuculline (40 mg/kg, i.p.) control.
** $p < 0.005$ vs. bicuculline (40 mg/kg, i.p.) control.
+ $p < 0.01$ vs. bicuculline (40 mg/kg, i.p.) control, Chi-squared test.
++ $p < 0.001$ vs. bicuculline (40 mg/kg, i.p.) control, Chi-squared test.

3.1.7. Effect of methanol extract of Cotyledon orbiculata on picrotoxin-induced seizures

Picrotoxin (12 mg/kg, i.p.) produced tonic seizures in all animals used. Doses of 100 and 400 mg/kg (i.p.) of *Cotyledon orbiculata* protected 12.5% of mice against picrotoxin (12 mg/kg, i.p.)-induced seizures and significantly delayed the onset of the seizures. *Cotyledon orbiculata* (200 mg/kg, i.p.) protected 25% of the animals against picrotoxin (12 mg/kg, i.p.)-induced seizures and also significantly delayed the onset of the seizures. The standard anticonvulsant drug, phenobarbitone (12 mg/kg, i.p.) protected 50% of mice against picrotoxin (12 mg/kg, i.p.)-induced seizures and significantly delayed the

Table 8
Effect of methanol extract of *Cotyledon orbiculata* (CO) on picrotoxin (PC)-induced seizures in mice

Dose (mg/kg)					No. of animals convulsed/used	Onset of tonic convulsion (mean ± S.E.M.) (min)
PC	CO	Phenobarbitone	Diazepam	DMSO		
12	–	–	–	–	8/8	10.75 ± 2.01
12	100	–	–	–	7/8	20.57 ± 0.98*
12	200	–	–	–	6/8	18.00 ± 1.72**
12	400	–	–	–	7/8	17.29 ± 1.30**
12	–	12	–	–	4/8	24.50 ± 0.94***
12	–	–	0.50	–	2/8+	24.25 ± 0.67***
12	–	–	–	0.25 ml	8/8	11.75 ± 0.45

DMSO, dimethylsulfoxide.
* $p < 0.005$ vs. picrotoxin (12 mg/kg, i.p.) control.
** $p < 0.05$ vs. picrotoxin (12 mg/kg, i.p.) control.
*** $p < 0.001$ vs. picrotoxin (12 mg/kg, i.p.) control.
+ $p < 0.01$ vs. picrotoxin (12 mg/kg, i.p.) control, Chi-squared test.

Table 9
Effect of methanol extract of *Cotyledon orbiculata* (CO) on *N*-methyl-DL-aspartic acid (NMDLA)-induced seizures in mice

Dose (mg/kg)					No. of animals convulsed/used	Onset of tonic convulsion (mean ± S.E.M.) (min)
NMDLA	CO	Phenobarbitone	Diazepam	DMSO		
400	–	–	–	–	8/8	2.13 ± 0.30
400	100	–	–	–	8/8	4.38 ± 0.46*
400	200	–	–	–	8/8	3.63 ± 1.24
400	400	–	–	–	8/8	2.88 ± 0.55
400	–	12	–	–	8/8	2.45 ± 0.74
400	–	–	0.50	–	8/8	2.90 ± 1.83
400	–	–	–	0.25 ml	8/8	2.37 ± 0.84

DMSO, dimethylsulfoxide.
* $p < 0.01$ vs. NMDLA (400 mg/kg, i.p.) control. There was no significant difference in the incidence of seizures between control and test animals, Chi-squared test.

onset of the tonic seizures. Diazepam (0.5 mg/kg, i.p.) protected 75% of the animals and also significantly delayed the onset of picrotoxin (12 mg/kg, i.p.)-induced seizures. DMSO (0.25 ml, i.p.), used as a vehicle, did not alter picrotoxin-induced seizures in mice (Table 8).

3.1.8. Effect of methanol extract of Cotyledon orbiculata on N-methyl-DL-aspartic acid-induced seizures

N-Methyl-DL-aspartic acid (400 mg/kg, i.p.) elicited seizures in all the animals used. Methanol extract of *Cotyledon orbiculata* (100 mg/kg, i.p.) did not affect the incidence of NMDLA (400 mg/kg, i.p.)-induced seizures, but significantly delayed the onset of the seizures. *Cotyledon orbiculata* (200 and 400 mg/kg, i.p.) neither affected the incidence nor the onset of seizures induced by NMDLA (400 mg/kg, i.p.). Similarly, phenobarbitone (12 mg/kg, i.p.), diazepam (0.5 mg/kg, i.p.) and DMSO (0.25 ml, i.p.) did not protect any of the animals against NMDLA (400 mg/kg, i.p.)-induced seizures or affect the onset of the seizures (Table 9).

3.2. Phytochemical analysis

The phytochemical tests carried out using dried powder of the fleshy leaves of *Cotyledon orbiculata* showed positive results for cardiac glycosides, saponins, tannins, reducing sugars and triterpene steroids. Negative results were obtained for alkaloids, flavonoids and anthraquinones.

4. Discussion

Pentylenetetrazole, bicuculline, picrotoxin and *N*-methyl-DL-aspartic acid are all convulsant agents (Nicoll, 2001; Rang et al., 2003a). Data from this study show that the onset of tonic convulsion produced by PTZ was significantly delayed by *Cotyledon orbiculata*. The data also show that phenobarbitone and diazepam but not phenytoin antagonized PTZ convulsion. According to De Sarro et al. (1999), PTZ may be exerting its convulsant effect by inhibiting the activity of gamma aminobutyric acid (GABA) at $GABA_A$ receptors. Gamma aminobutyric acid is the major inhibitory neurotransmitter which is implicated in epilepsy. The enhancement and inhibition of the neurotransmission of GABA will attenuate and enhance convulsion, respectively (Meldrum, 1981; Gale, 1992; Westmoreland et al., 1994). Phenobarbitone and diazepam, standard antiepileptic drugs, have been shown to exert their antiepileptic effects by enhancing GABA-mediated inhibition in the brain (Porter and Meldrum, 2001; Rang et al., 2003b). It is possible that both phenobarbitone and diazepam antagonize PTZ convulsion in this study by enhancing GABA neurotransmission. It is also possible that phenytoin, a standard antiepileptic drug, did not alter PTZ convulsion because it is thought to exert its antiepileptic effect by blocking sodium ion channels and preventing the influx of sodium ions into brain cells thus inhibiting generation of repetitive action potential (Porter and Meldrum, 2001). Since the aqueous extract of *Cotyledon orbiculata* delayed the occurrence of PTZ convulsion, it is probable that it may be interfering with gabaergic mechanism(s) to exert its anticonvulsant effect.

In the present study, bicuculline elicited convulsion in mice. Bicuculline, a potent and selective $GABA_A$ receptor antagonist, is thought to produce convulsion by blocking $GABA_A$ receptors to attenuate GABA-mediated inhibition (Rang et al., 2003a). It is significant that phenobarbitone, diazepam and *Cotyledon orbiculata* attenuated bicuculline convulsion in this study. Phenytoin did not affect bicuculline convulsion. This supports the hypothesis that *Cotyledon orbiculata* may be affecting gabaergic mechanism(s) to exert its anticonvulsant activity.

According to Rang et al. (2003a) and Nicoll (2001), picrotoxin exerts its convulsant effect by blocking the $GABA_A$ receptor-linked chloride ion channel which normally opens to allow increased chloride ion conductance into the brain cells following the activation of $GABA_A$ receptors by GABA. Data from this study show that picrotoxin induced convulsion in mice and that phenobarbitone, diazepam and *Cotyledon orbiculata* but not phenytoin attenuated the convulsion. It is probable that *Cotyledon orbiculata* attenuated picrotoxin convulsion by enhancing GABA neurotransmission. This further supports the hypothesis that *Cotyledon orbiculata* may be affecting gabaergic mechanism(s) to exert its anticonvulsant activity.

NMDLA is a specific agonist at NMDLA receptors which are implicated in the pathogenesis of epilepsy. It produces effects similar to glutamic acid at NMDLA receptors and exerts its convulsant effect by activating the receptors to enhance glutaminergic neurotransmission (Watkins and Evans, 1981; Chapman and Meldrum, 1993). In the present study, NMDLA

induced convulsion in mice and *Cotyledon orbiculata* was shown to delay the onset of NMDLA convulsion. However, phenobarbitone, diazepam and phenytoin were shown not to affect the convulsion. It is probable therefore that *Cotyledon orbiculata* may also be affecting glutaminergic mechanisms to significantly prolong the onset of NMDLA convulsion.

Phytochemical tests carried out in the present study show that the leaves of *Cotyledon orbiculata* contain cardiac glycosides, saponins, tannins, reducing sugars and triterpene steroids. It is important to note that Chauhan et al. (1988) reported that plant triterpenoids evaluated for anticonvulsant activity against PTZ-induced convulsion in mice protected 10–40% of the animals. It is possible, therefore, that saponin, which may be of triterpenoid type, and the triterpene steroid present in *Cotyledon orbiculata* might contribute to the anticonvulsant activity of the plant.

In conclusion, the results obtained in the present study suggest that *Cotyledon orbiculata* has anticonvulsant activity and thus, lend pharmacological justification to the use of the plant extract by traditional medicine practitioners in the treatment of epilepsy. It may also not be impossible to suggest that the anticonvulsant activity may be exerted via more than one mechanism especially since the aqueous extract of the plant is thought to affect both gabaergic and glutaminergic mechanisms.

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