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Purslane protects against the reproductive toxicity of carbamazepine treatment in pilocarpine-induced epilepsy model

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ABSTRACT

Objective: To investigate the protective effect of purslane with carbamazepine treatment.
Methods: Male albino rats were modulated by pilocarpine to be epileptic. Both the normal and epileptic rats were treated with carbamazepine, purslane or carbamazepine plus purslane, with separate non-treated control groups for both normal and epileptic rats.
Results: The data from the current study showed amelioration in amino acids and electrolytes in the epileptic rats treated with purslane and carbamazepine, with this amelioration occurring without decreasing the fertility hormones (testosterone, dehydroepiandrosterone, luteinizing hormone and follicle stimulating hormone). Purslane treatments also prevented the increase in estradiol. The decreased epileptic hyperexcitability with purslane was evidenced by decreased glial fibrillary acidic protein and lipid peroxidation.
Conclusions: Natural products like purslane could be used with the highly repetitive drugs like carbamazepine to reduce or prevent its side-effects.

1. Introduction

Epilepsy is one of the most common neurological disorders, with an incidence of approximately 0.3%–0.5%. An imbalance between excitatory and inhibitory neurotransmission in the brain, which could be produced by a decrease in gamma-aminobutyric acidergic (GABAergic) and/or an increase in glutamatergic transmission, is associated with the development of epilepsy in patients, as well as in animal models including pilocarpine-induced seizures in rodents [1]. The most commonly observed alterations are extensive neural loss, hippocampal mossy fibre sprouting, tissue hyperexcitability, and changes in receptor subunit composition. Antiepileptic drugs (AEDs) are the primary option for the management of epilepsy, with these exerting their anticonvulsant activity by a potentiation of inhibitory and/or inhibition of excitatory neurotransmission by many mechanisms, pre- and post-synaptically [2].

Glial fibrillary acidic protein (GFAP), first described by Eng *et al.* in 1971, is a member of the cytoskeletal protein family [3] and is widely expressed in astroglial cells and in neural stem cells [4,5]. Glial cells contribute to epileptogenesis through the release of inflammatory proteins, predominantly interleukins and chemokines, which can facilitate hyperexcitability. Any change in the proper astrocyte renders these new-born neurons susceptible to abnormal synapses, which may contribute to a hyperexcitable condition [6].

Pilocarpine is a cholinergic agonist used to induce epilepsy. This model shows similarity to human temporal lobe epilepsy from the view of neuropathological damage. Neurochemical studies performed after pilocarpine-induced convulsive processes show that it affects not only neurotransmitters (adenosine, nor-

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The animals were reared according to the principles of the "Guide for the Care and Use of Laboratory Animals" prepared by Beni-Suef University. The Institutional Ethics Committee of Beni-Suef University approved the study. All efforts were made to minimize animal suffering.

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epinephrine, dopamine, serotonin, glutamate, and GABA) but also muscarinic or dopaminergic receptor densities [7].

Carbamazepine (CBZ) is an anticonvulsant drug that was first approved in 1974 for the treatment of seizures. CBZ is one of the most common and effective of the older AEDs for these seizure types and is chosen for monotherapy due to its high effectiveness and low incidence of side-effects. It is rapidly distributed into the body and about 75%–78% binds to plasma proteins. In the central nervous system (CNS), CBZ reduces neuronal hyperexcitability and elicits its action mainly by inhibition of neuronal Na^+ and also, to a lesser extent, other channels and transmitter systems. Inhibition of Na^+ channels reduces basal release of monoamine and acetylcholine [8].

Epilepsy, AEDs, and the reproductive system have complex interactions. One such interaction appears to affect the reproductive endocrine system, since reproductive endocrine disorders are more common among men with epilepsy than in the general population [9]. The brain regulates hormonal secretion and it is sensitive to hormonal feedback; the neuroendocrine feedback system includes the hypothalamus, pituitary and gonads, and also the amygdala, which is linked to the hypothalamic–pituitary axis and is involved in the regulation, production and secretion of sex hormones. Many experimental and human studies have demonstrated that reproductive endocrine and sexual dysfunction is more common in partial epilepsy than in generalized epilepsy, particularly epilepsy which is of temporal lobe origin, since the limbic system is extensively interconnected with the hypothalamic nuclei involved in regulating gonadal function [10]. Disturbances in sex hormones may have implications for fertility, sexuality and, ultimately, general wellbeing. The possible endocrine side-effects of AEDs may therefore be of importance for a large group of men as AEDs are increasingly used in men of fertile age, not only in epilepsy, but also in psychiatry and pain treatment [11].

Vegetables, fruits, flowers and grain products are natural sources of antioxidants and other phytochemicals. *Portulaca oleracea* L. (Portulacaceae) (*P. oleracea*), commonly known as purslane, is listed by the World Health Organization as one of the most used medicinal plants and has even been termed as "global panacea" [12]. The plant has muscle relaxant, anticonvulsive, analgesic and anti-inflammatory properties, and also a potential anti-anxiety effect. It has further been shown to exhibit hepatoprotective activity in rats with hepatic injuries. Recent research indicates that purslane offers better nourishment than the major cultivated vegetables due to its shoot that is a rich source of ω-3-fatty acids, α-tocopherols, ascorbic acid, β-carotene and glutathione. Its seeds also contain a high percentage of α-linolenic acid [13]. These features contribute to the antioxidative properties of purslane. Antioxidants reduce oxidative stress by scavenging free radical species, and since the majority of antioxidants are phenolic compounds, they are known to be responsible for the antioxidant activity of plants. Experimental evidence reveals purslane to be effective as an antioxidant agent, as well as providing nourishment for the liver, kidneys, testes, and heart tissues [14].

The primary aim of the current study was to investigate the influence of long-term use of the AED, CBZ on fertility hormones. The second aim was to reduce those side-effects of CBZ that are related to reproductive endocrine function by using purslane as an antioxidant antiepileptic plant in combination with a low dose of CBZ.

2. Materials and methods

2.1. Animals

White male albino rats (*Rattus norvegicus*) weighing 170–200 g were obtained from the animal house of the National Research Institute, Eldoki, El-Giza, Egypt. They were housed in polypropylene cages and maintained on a natural light/dark cycle with free access to food and water. The animals were reared according to the principles of the "Guide for the Care and Use of Laboratory Animals" prepared by Beni-Suef University. The Institutional Ethics Committee of Beni-Suef University approved the study. All efforts were made to minimize animal suffering.

2.2. Chemicals

Pilocarpine hydrochloride (99%) was purchased from Acros Organics (Newark, New Jersey, USA). CBZ [5-carbamyl-5*H*-dibenzo[*b,f*] azepine, 5*H*-dibenzo*[b,f]* azepine-5-carboxamide] has a chemical formula of $C_{15}H_{12}N_{20}$. CBZ known commercially as tegretol was reproduced with permission from Novartis Corporation (Basel, Switzerland).

2.3. Preparation of extract

One liter of boiled distilled water was added to 100 g of grinded purslane seeds, cooled and filtered. The extract was then concentrated to the desired volume.

2.4. Experimental designs

Eighty adult male rats were used in the experiment, and were divided into two main groups, the negative non epileptic one, which subdivided into four subgroups, normal control (NC), normal treated with CBZ, normal treated with purslane and normal treated with half CBZ and half purslane (n = 8 each subgroup) and the positive epileptic one, which subdivided into four subgroups, epileptic control, epilepsy treated with CBZ, epilepsy treated with purslane and epilepsy treated with half CBZ and half purslane (n = 12 each subgroup).

2.5. Induction of epilepsy

Epilepsy was experimentally induced according to the method of Turski *et al.* [15]. Rats were injected with methylscopolamine nitrate (1 mg/kg in saline, *s.c.*) 30 min before pilocarpine injection to minimize the peripheral effects of pilocarpine. Animals were then injected with pilocarpine hydrochloride (300 mg/kg in saline, *i.p.*). The course of pilocarpine-induction was described previously [16]. Once initiated, the epileptic behaviours occurred every 2–5 min and developed epilepsy 1 h after pilocarpine injection. Seizures were terminated with diazepam (4 mg/kg, *i.p.*) delivered every 20 min as needed. Animals were lethargic for up to 12 h and were often ataxic and slightly dehydrated for 24 h post pilocarpine. To prevent severe dehydration, animals were given 5 mL of lactated Ringer's solution as needed in the 2 days after pilocarpine treatment.

2.6. Treatment schedule

Treatments started 3 days after pilocarpine injection for both the negative and positive groups and continued for 3 weeks. Purslane extract was orally administered using intragastric intubation at a dose of 10 mL/kg body weight per day. CBZ was suspended in distilled water and given orally at a dose of 55 g/kg body weight per day.

2.7. Blood and tissue homogenate sampling

At the end of the experiment, rats were sacrificed. Blood samples were collected and sera were separated for hormonal analysis, electrolytes, and GFAP. The hippocampus was sliced from the brain on ice containing plates and homogenized in saline (1% w/v) for amino acids (glutamic, glycine, GABA and asparagine) determination. The testis tissues were cleaned from the fatty parts and homogenized in ice-cold 50 mmol/L sodium phosphate buffer (pH 7.4). The supernatant was separated by centrifugation at 2 000 r/min for 10 min for determination of cholesterol content, lipid peroxidation and peroxidase activity.

2.8. Biochemical measurements

The amino acids were determined in serum by the method of Heinrikson and Meredith [17]. Na^+, K^+, Ca^{2+} and Mg^{2+} were measured using reagent kits purchased from Reactivos Spinreact (Girona, Spain).

The fertility hormones [testosterone, dehydroepiandrosterone (DHEA), luteinizing hormone (LH) and follicle stimulating hormone (FSH)] were tested by enzymatic immunoassay method. The IMMULITE 1000 reagent kits were purchased from Siemens Healthcare Diagnostics (Tarrytown, NY, USA).

GFAP was assayed by ELISA based on a double-antibody sandwich technique.

The testes tissues were cleaned from the fatty parts and homogenized in ice-cold 50 mmol/L sodium phosphate buffer (pH 7.4). The supernatant was separated by centrifugation at 2 000 r/min for 10 min. The supernatant was used for the measurement of cholesterol level oxidative stress. Lipid peroxidation level was estimated according to the methods of Preuss *et al.* [18], and peroxidase activity was estimated according to the method of Kar and Mishra [19].

2.9. Statistical analysis

The data were analysed using Tukey–Kramer method for *post hoc* analysis to compare various groups with each other. The results were expressed as mean ± SE. Statistical significance interval is considered as $P < 0.05$ for all data. All results were analyzed using SPSS version 20.0 software.

3. Results

3.1. Epileptic indicators

Following injection with pilocarpine, epilepsy was detected in this study by a continuous stationary phase lasting for 5–10 min followed by oro-facial movements (facial automatisms), whole body vibration, hair erection and limbic motor seizures, all of which continued for about 2 h, as described in pilocarpine model of Turski *et al.* [20].

The biochemical epileptic changes in the pilocarpine model were tested by some excitatory and inhibitory amino acids. As depicted in Table 1, there was no significant change between the normal control and the normal treated groups. Glutamate and glycine showed the highest level in epileptic animals compared to the control ones (12.57 μmol *vs*. 8.76 μmol for glutamate and 1.02 μmol *vs*. 0.61 μmol for glycine), but this increased level was ameliorated significantly ($P < 0.001$) after treatment with CBZ, purslane and their combination (7.95 μmol, 10.05 μmol and 8.05 μmol, respectively). In comparison to the normal control group, epileptic rats showed a significantly ($P < 0.001$) decreased level of GABA (0.31 μmol *vs*. 1.41 μmol) and asparagine ($P < 0.01$) (1.29 μmol *vs*. 2.58 μmol). Treatment with CBZ (1.36 μmol) and CBZ-purslane mixture (1.13 μmol) significantly increased the GABA level compared to the epileptic group, while treatment with purslane alone appeared to have no significant effect. The asparagine level, however, was not significantly affected by any of these treatments (Table 1).

Table 1
The effect of CBZ and *P. oleracea* on the tested amino acids of epileptic rats (μmol/g tissue).

Group		Amino acids in hippocampus			
		Glutamate	Glycine	GABA	Asparagine
Normal	NC	8.76 ± 0.32^{a}	0.61 ± 0.06^{a}	1.41 ± 0.27^{bc}	2.58 ± 0.21^{b}
	N + CBZ	8.19 ± 0.12^{a}	0.55 ± 0.02^{a}	1.76 ± 0.24^{c}	2.81 ± 0.08^{b}
	N + PO	8.52 ± 0.18^{a}	0.59 ± 0.04^{a}	1.75 ± 0.25^{c}	2.65 ± 0.15^{b}
	N + 1/2 CBZ + 1/2 PO	8.31 ± 0.35^{a}	0.56 ± 0.03^{a}	1.76 ± 0.23^{c}	2.76 ± 0.12^{b}
Epileptic	EP	12.57 ± 0.91^{b}	1.02 ± 0.09^{b}	0.31 ± 0.02^{a}	1.29 ± 0.08^{a}
	EP + CBZ	7.95 ± 0.72^{a}	0.64 ± 0.07^{a}	1.36 ± 0.10^{bc}	2.15 ± 0.28^{ab}
	EP + PO	10.05 ± 0.75^{a}	0.53 ± 0.07^{a}	0.70 ± 0.11^{ab}	2.25 ± 0.40^{ab}
	EP + 1/2 CBZ + 1/2 PO	8.05 ± 0.70^{a}	0.63 ± 0.08^{a}	1.13 ± 0.09^{bc}	2.06 ± 0.36^{ab}
F-probability		$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.01$

Values with the different superscript letter are significantly different ($P < 0.05$). NC: Normal control; N: Normal; EP: Epileptic; PO: *P. oleracea*.

As shown in Table 2, the tested treatments had no significant effect on the electrolytes of the normal groups, but epileptic rats showed a significant decrease with 3.87 mEq/L for K^+, 1.38 mg/dL for Ca^{2+} and 0.63 mg/dL for Mg^{2+} ($P < 0.05$, $P < 0.001$ and $P < 0.001$, respectively). While the highest recorded levels varied, it was in the normal group treated with *P. oleracea* for

Table 2
The effect of CBZ and *P. oleracea* on the tested electrolytes of epileptic rats.

Group		Electrolytes			
		Na (mEq/L)	K (mEq/L)	Ca (mg/dL)	Mg (mg/dL)
Normal	NC	136.83 ± 0.79^{a}	5.11 ± 0.39^{b}	3.10 ± 0.13^{d}	1.91 ± 0.08^{c}
	N + CBZ	138.67 ± 0.33^{a}	4.55 ± 0.30^{ab}	2.83 ± 0.19^{cd}	2.00 ± 0.07^{c}
	N + PO	137.33 ± 0.67^{a}	5.28 ± 0.19^{b}	2.78 ± 0.27^{cd}	2.03 ± 0.18^{c}
	N + 1/2 CBZ + 1/2 PO	137.83 ± 1.30^{a}	4.98 ± 0.29^{b}	3.17 ± 0.11^{d}	2.25 ± 0.22^{c}
Epileptic	EP	152.00 ± 4.04^{b}	3.87 ± 0.11^{a}	1.38 ± 0.06^{a}	0.63 ± 0.09^{a}
	EP + CBZ	138.33 ± 1.86^{a}	5.03 ± 0.15^{b}	2.07 ± 0.03^{b}	1.27 ± 0.08^{b}
	EP + PO	141.17 ± 1.24^{a}	4.83 ± 0.31^{ab}	2.00 ± 0.07^{b}	1.19 ± 0.08^{b}
	EP + 1/2 CBZ + 1/2 PO	138.50 ± 0.43^{a}	4.93 ± 0.17^{ab}	2.28 ± 0.09^{bc}	1.16 ± 0.07^{b}
F-probability		$P < 0.001$	$P < 0.05$	$P < 0.001$	$P < 0.001$

Values with the different superscript letter are significantly different ($P < 0.05$). NC: Normal control; N: Normal; EP: Epileptic; PO: *P. oleracea*.

K^+ and in the normal treated with CBZ plus *P. oleracea* mixture for Ca^{2+} and Mg^{2+}. On the other hand, Na^+ was significantly increased ($P < 0.001$) compared to the normal control group (136.83 mEq/L), with its highest level recorded in the epileptic group (152.00 mEq/L).

Glial cell changes have become a hallmark of the sclerotic hippocampus, involved astrocyte hypertrophy, and increased expression of GFAP. Figure 1 shows that while GFAP was increased significantly in the epileptic rats compared to the normal control group (50.23 pg/mL *vs.* 16.33 pg/mL), all the treatments decreased its level significantly, with the highest decrease being after CBZ treatment (24.68 pg/mL) followed by the treatment with *P. oleracea* (29.08 pg/mL) and finally the CBZ plus *P. oleracea* mixture (32.52 pg/mL). These treatments didn't show significant difference in GFAP level of the normal groups.

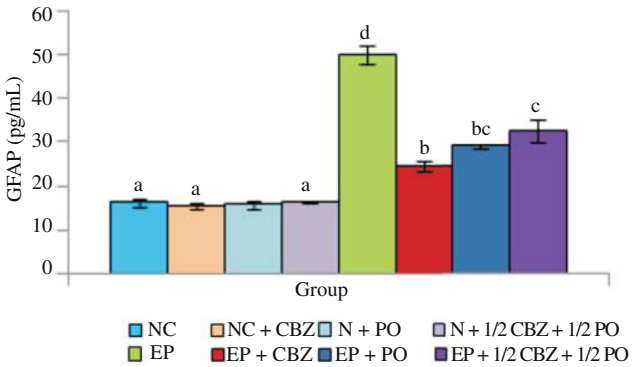

Figure 1. The effect of CBZ and *P. oleracea* on GFAP of epileptic rats. Data are expressed as mean ± SE, $n = 6$. Values with the different superscript letter are significantly different ($P < 0.05$). NC: Normal control; N: Normal; EP: Epileptic; PO: *P. oleracea*.

3.2. Fertility indicators

Considering now the effects of the various treatments on the fertility of the normal rats, the CBZ treatment decreased testosterone (T) (2.55 ng/mL *vs.* 6.25 ng/mL) and increased estradiol (E) (16.44 pg/mL *vs.* 11.28 pg/mL) levels significantly, while it had no significant effect when combined with *P. oleracea*. Table 3 also shows that testosterone, DHEA, the pituitary hormones (LH & FSH) and the fertility ratios (T/E & T/LH) were significantly decreased in epileptic animals and showed more decrease in epileptic animals treated with CBZ compared to the normal control group. Estradiol was increased significantly in these two groups, while progesterone was decreased and prolactin was increased significantly in the epileptic CBZ treated group. Purslane with CBZ (the CBZ and *P. oleracea* mixture) ameliorated these changes significantly (although the amelioration was not significant in the case of FSH and progesterone).

The cholesterol level in the testes showed a significant decreased in the epileptic group (11.69 mg/dL) compared to the normal control (14.10 mg/dL) and the normal treated with purslane (14.28 mg/dL) groups. The three different treatments used, however, induced a non-significant effect in the level of testis cholesterol in both the normal and the epileptic groups (Figure 2).

Oxidative stress was detected by lipid peroxidation and measured by malondialhyde (MDA) level. As illustrated in Figure 3, the MDA level significantly increased in epileptic rats (126.15 nmol MDA/g tissue) and ameliorated after treatments, but was still elevated in the rats treated with either CBZ (113.74 nmol MDA/g tissue) or *P. oleracea* (93.17 nmol MDA/g tissue), compared to the control group (67.75 nmol MDA/g tissue). The CBZ-*P. oleracea* treated rats (73.26 nmol MDA/g

Table 3
The effect of CBZ and *P. oleracea* on the fertility hormones of epileptic rats.

Group		T (ng/mL)	DEHA (ng/mL)	LH (IU/L)	FSH (IU/L)	E (pg/mL)	PG (ng/mL)	PL (ng/mL)	T/E*1 000	T/LH/1 000
Normal	NC	6.25 ± 0.63^{cd}	4.32 ± 0.46^{cd}	2.20 ± 0.08^{cde}	3.85 ± 0.31^{c}	11.28 ± 0.45^{ab}	3.72 ± 0.57^{b}	2.13 ± 0.18^{a}	0.490 ± 0.050^{c}	2.58 ± 0.35^{cd}
	N + CBZ	2.55 ± 0.06^{a}	4.11 ± 0.31^{cd}	1.86 ± 0.13^{bcd}	3.68 ± 0.19^{c}	16.44 ± 0.27^{de}	2.88 ± 0.22^{ab}	2.32 ± 0.26^{a}	0.400 ± 0.030^{c}	1.88 ± 0.13^{bc}
	N + PO	7.08 ± 0.61^{d}	5.36 ± 0.17^{d}	2.55 ± 0.16^{e}	3.78 ± 0.32^{c}	10.53 ± 0.19^{a}	2.55 ± 0.24^{ab}	1.85 ± 0.08^{a}	0.500 ± 0.020^{c}	2.91 ± 0.20^{d}
	N + 1/2 CBZ + 1/2 PO	5.58 ± 0.40^{bcd}	4.79 ± 0.36^{bc}	2.28 ± 0.08^{de}	3.68 ± 0.32^{c}	11.69 ± 0.51^{ab}	2.79 ± 0.26^{ab}	2.05 ± 0.22^{a}	0.460 ± 0.030^{c}	2.37 ± 0.26^{bcd}
Epileptic	EP	4.01 ± 0.38^{b}	2.51 ± 0.31^{ab}	1.07 ± 0.04^{a}	1.07 ± 0.07^{a}	17.14 ± 0.44^{e}	2.42 ± 0.35^{ab}	2.26 ± 0.14^{a}	0.090 ± 0.010^{a}	1.66 ± 0.08^{ab}
	EP + CBZ	1.66 ± 0.10^{a}	1.98 ± 0.22^{a}	0.99 ± 0.01^{a}	1.00 ± 0.04^{a}	22.07 ± 0.63^{f}	1.90 ± 0.29^{a}	3.09 ± 0.09^{b}	0.120 ± 0.001^{a}	1.11 ± 0.06^{ac}
	EP + PO	5.50 ± 0.27^{bcd}	3.30 ± 0.19^{bc}	1.69 ± 0.22^{bc}	2.19 ± 0.13^{b}	13.31 ± 0.94^{bc}	2.58 ± 0.32^{ab}	2.37 ± 0.16^{a}	0.250 ± 0.010^{b}	2.02 ± 0.14^{bc}
	EP + 1/2 CBZ + 1/2 PO	4.95 ± 0.25^{bc}	3.24 ± 0.36^{bc}	1.57 ± 0.20^{b}	1.79 ± 0.30^{ab}	14.86 ± 0.68^{cd}	3.36 ± 0.13^{ab}	2.52 ± 0.06^{ab}	0.110 ± 0.009^{a}	2.19 ± 0.06^{bcd}
F-probability		$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.05$	$P < 0.001$	$P < 0.001$	$P < 0.001$

Values with the different superscript letter are significantly different ($P < 0.05$). NC: Normal control; N: Normal; EP: Epileptic; PO: *P. oleracea*; T: Testosterone; E: Estradiol; PG: Progesterone; PL: Prolactin.

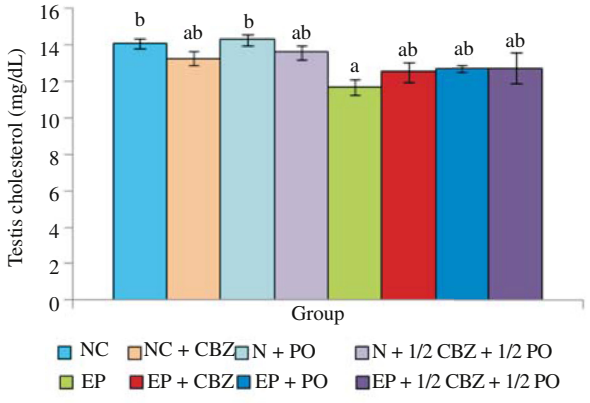

Figure 2. The effect of CBZ and *P. oleracea* on cholesterol content in the testis homogenate of epileptic rats.
Data are expressed as mean ± SE, $n = 6$. Values with the different superscript letter are significantly different ($P < 0.05$). NC: Normal control; N: Normal; EP: Epileptic; PO: *P. oleracea*.

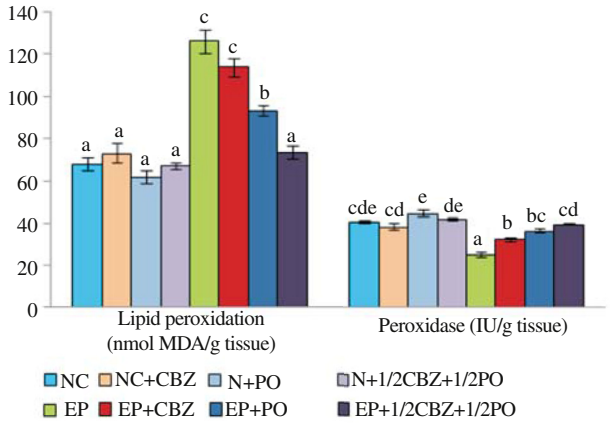

Figure 3. The effect of CBZ and *P. oleracea* on lipid peroxidation and peroxidase activity in the testis of normal and epileptic rats.
Data are expressed as mean ± SE, $n = 6$. Values with the different superscript letter are significantly different ($P < 0.05$). NC: Normal control; N: Normal; EP: Epileptic; PO: *P. oleracea*.

tissue), however, showed a non-significant difference in MDA level in comparison to the normal ones. Antioxidant activity was represented here by peroxidase activity. The peroxidase activity was significantly decreased in epileptic rats (24.84 IU/g tissue) compared to the normal control group (40.50 IU/g tissue). All the tested treatments significantly ameliorated the decreased peroxidase activity with the CBZ-*P. oleracea* mixture showing the highest increase in antioxidant activity.

4. Discussion

4.1. Epilepsy changes

An epileptic seizure is the result of functional disorders of the brain and is caused by abnormal, excessive bioelectrical discharges in the nerve cells.

Temporal lobe epilepsy has been related to excessive excitability in limbic structures, low function of inhibitory pathways or a combination of both events [21]. The animal model of temporal lobe epilepsy induced by pilocarpine represents one of the most successful and widely used models, not only for the study of the pathogenesis of temporal lobe epilepsy in humans, but also for the evaluation of the potential antiepileptogenic drugs [22].

As indicated in our results, an epileptic seizure is a sudden imbalance between excitatory and inhibitory processes in the neural network. The low level of GABA in pilocarpine treatment is produced by the down-regulation of GABAergic receptors in several brain regions, and different receptors have been implicated in the mechanism of pilocarpine-induced seizures. Thus, activation of M1 muscarinic receptors is involved in the first step of seizure activity, while serotonin, glutamate, dopamine and GABA systems appear to mediate the propagation and/or maintenance of seizure activity. Potentiation of GABAergic inhibition is the main mechanism of action of many AEDs [23]. It has been reported that during epileptic activity, there is a greater increase in cerebral metabolic rate. Once glucose enters the brain, it is metabolized into different substrates including amino acids [24]. Consequently, the significant increase in the amino acid neurotransmitters of pilocarpinized rats observed in the present study may be mediated by the increased rate of cerebral glucose utilization [25]. Stress also increases extracellular glutamate concentrations and alters glutamate receptor binding profiles in several brain regions [26]. In addition, the glutamate released by activated microglia, induces excitotoxicity and may contribute to neurodegeneration in numerous neurological diseases, including epilepsy [26]. The significant increase in glycine levels after pilocarpine injection might be linked to the increase of excitatory glutamatergic neurotransmission via *N*-methyl-D-aspartate (NMDA) receptors [27].

Castilla-Guerra *et al.* [28] and Victor *et al.* [29] pointed out that hypernatremia, as indicated in our study, is more likely to be a result of seizure activity. Intracellular glycogen is metabolized to lactate in muscle during seizures. As lactate is more osmotically active than glycogen, the intracellular osmolality of muscle fibres increases and water moves into cells, causing hypernatremia. Generally, the deficiency in K^+ is correlated to hypomagnesaemia and hypocalcaemia [30]. Mg is essential in neuronal excitability, which alters calcium mobilization and stabilizes excitable membranes and also exerts a voltage-dependent blockage of the NMDA receptor channel. Many studies have reported low Mg levels in epileptics, particularly in association with hypocalcaemia and hypokalaemia, with this being attributed to the hyperexcitability in uncontrolled epileptic patients and increased seizure frequencies [31]. Ca^{2+} has been implicated in the pathophysiology of seizures, characterized by a chronic shift in the balance of excitation and inhibition such that brain tissue manifests spontaneously recurrent seizures [32]. It has frequently been documented that low levels of Ca^{2+} are responsible for the initiation of convulsions. This is also due to a massive influx of calcium ions via voltage gated and NMDA-dependent ion channels [33].

Over the last two decades, several lines of evidence have suggested that glial cells are potential therapeutic targets for the treatment of epilepsy and other CNS diseases [34]. Astrocytes play an established role in the removal of glutamate at synapses and the sequestration and redistribution of K^+ during neural activity [35]. During most, if not all, neuropathology, astrocytes exhibit alterations in morphology, number and distribution. Such changes can be investigated by assaying the accumulation of GFAP, an intermediate filament protein expressed by astrocytes [36] as indicated in this experiment, which is in accordance with the changes in glial specific

proteins shown by Ransom and Behar [37]. Direct stimulation of astrocytes leads to prolonged neuronal depolarization and epileptiform discharges [38]. Glial cells can release neuroactive molecules and also modulate synaptic transmission through modifications in channels, gap junctions, receptors and transporters [39,40].

AEDs like CBZ act on the CNS by decreasing the neuronal activity [41]. GABA is the main target of these drugs; it has two types of receptors, GABAA and GABAB. The activation of GABAA receptors induces an inward entry of chloride ions, while activation of GABAB receptors induces an outflow of K^+ ions, with both actions leading to an inhibition of neuronal activity. These two complementary and contradictory neurotransmission pathways constitute the targets for most AEDs [42].

The significantly higher levels of Ca^{2+} among our CBZ-treated group of epileptics could be a marker for better seizure control with AED therapy [43]. Singh and Asconapé [44] reported that CBZ-induced hyponatraemia may occur by inducing excessive water reabsorption in the collecting tubule, since the capacity of some patients to excrete the water load was found to be grossly impaired. Hyponatraemia is also caused by increasing the secretion of the antidiuretic hormone from the pituitary gland or heightening sensitivity of antidiuretic hormone receptors in the kidneys.

In our study, purslane indicated hypoexcitable and antioxidant effects in the treated rats. Its neuroprotective effect against neurotoxicity may, at least in part, be caused by an increase in the activities of antioxidant enzymes with a reduction in lipid peroxidation with its radical scavenging activity [45].

Purslane has been described as a "power food of the future" because of its high nutritive and antioxidant properties. Antioxidants work by neutralizing or scavenging free radicals by hydrogen donation before they are able to attack cells and other biological components [14]. Purslane has the propensity to attenuate oxidative stress by reversing the inhibition of Na^+/K^+ ATPase activity [46]. It has been argued that the extract may act in part on postsynaptic α-adrenoceptors and by interference with transmembrane calcium influx [47]. Also, purslane contains a moderate amount of L-norepinephrine which increases the epileptic threshold [48]. By influencing the GFAP level it has a positive effect on epilepsy at a cellular level and using it with CBZ may ameliorate the side-effects of the latter.

4.2. Fertility changes

Epilepsy, AEDs and the reproductive system have complex interactions, and reproductive disorders are more common among men with epilepsy than in the general population. Epilepsy itself may affect sex hormone secretion. Evidence from preclinical investigations showed that induction of temporolimbic seizures in animals can cause changes in reproductive hormones levels and reproductive function [49].

Epileptiform discharges tense the temporolimbic modulation of hypothalamo-pituitary function, and this is supported by disruptions in the secretion of prolactin, FSH and LH [50]. Testosterone deficiency is an unusually common clinical observation [51]. Diminished levels of biologically active testosterone have been attributed to many factors, including: 1. Elevated amounts of sex hormone binding globulin synthesis which are raised by increased estradiol [52]; 2. Increased negative feedback on the hypothalamo-pituitary axis due to elevated levels of serum estradiol; although constituting only 1% of the total reproductive steroid, estradiol exerts one-half of the negative feedback on the hypothalamo-pituitary axis [53]; 3. Altered patterns of hypothalamic gonadotropin-releasing hormone secretion induced by temporal lobe epileptiform discharges, a dopaminergic system and GABA in the zona incerta. These stimulate LH release and may mediate the positive feedback effects of the gonadal steroids on LH release, so their decrease may alter this feedback [54]; and 4. Oxidative damage directed at the tissues that synthesize testosterone [9]. All these changes lead to hypogonadism, *i.e.* diminished gonadal function as determined by low serum testosterone level and/or decreased testosterone: estradiol or testosterone: luteinizing hormone ratio, leading in turn to abnormal sperm production [55].

Reproductive endocrine disorders can lead not only to reproductive dysfunction but also to exacerbation of epilepsy [56]. An understanding of these relationships and their underlying neurological and neuroendocrine mechanisms is, therefore, important to the comprehensive management of patients with epilepsy. In respect to the effect of hormones on epileptic seizures, there are some reports that suggest that testosterone has anticonvulsant properties when used in experimental animals [57,58]. One possible explanation for this relates to testosterone metabolites: dihydrotestosterone blocks NMDA transmission [59], while androsterone, a powerful GABAA receptor-modulating neurosteroid, and etiocholanolone have been shown to prevent the occurrence of epileptic seizures and may thereby all have anti-seizure effects [60]. Also, another metabolite, estradiol has shown to be raised in epileptics, increases seizure discharges, since it lowers the threshold for seizure in many experimental animal models. Estradiol also increases excitatory neurotransmitters and may alter the structure of the synaptic area of neurons, increasing the number of dendritic spines and the number of spine synapses, thereby increasing cell-to-cell contacts [61].

Both epilepsy itself and AEDs have been implicated in reproductive endocrine pathophysiology [16]. The effects of AEDs (in particular, CBZ) on reproductive functions could be attributed to their central effects on the hypothalamic–pituitary–gonad axis [62]. The use of CBZ is related to a progressive increase in circulating levels of sex hormone binding globulin as a result of the stimulation of the aromatase and cytochrome p450 enzyme system, which in turn accelerates the metabolism of sex steroids [63]. This effect decreases the proportion of free, bioactive testosterone which may result in sexual dysfunction in some men with epilepsy after long term CBZ treatment. Many studies have demonstrated the important role of DHEA in sexual dysfunction. In fact, DHEA probably influences the production of endothelial nitric oxide, which may lead to an improvement in erectile function but this may be altered after long term CBZ treatment [64].

In the interstitial tissue, cholesterol is necessary for the synthesis of testosterone. The regulation of intracellular cholesterol metabolism in the testis involves several enzymes including the hormone-sensitive lipase, which is believed to adjust available free cholesterol supplies to the needs of the cell by hydrolysing esterified cholesterol [65]. Mammalian testes are highly susceptible to oxidative stress. Free radical production and lipid peroxidation are potentially important mediators in testicular physiology and toxicology. Excessive amounts of oxygen free radicals cause lipid peroxidation in the cellular

and mitochondrial membranes. Peroxidation of the lipids in the membranes changes membrane permeability or disrupts membrane integrity and thus cell integrity [66]. Like all cells living under aerobic conditions, spermatozoa produce reactive oxygen species (ROS), mostly originating from normal metabolic activity. High concentrations of ROS play an important role in the pathophysiology of damage to the spermatozoa [67].

The peroxidase measured showed significantly decreased levels, while lipid peroxidation was increased significantly in the testes of the epileptic groups. These results agree with those of Armagan *et al.* [67] and Al-Tonbary *et al.* [68]. Li *et al.* suggested that prolonged exposure to CBZ resulted in excess formation of ROS, eventually resulting in oxidative damage to lipids, proteins and in inhibited antioxidant capacities, leading in turn to reproductive dysfunction in epileptic and CBZ-treated patients [69]. Kosola *et al.* reported that increased levels of lipid peroxidation, and decreased antioxidant capacity with more advanced testicular mitochondrial alterations might play a critical role in regulating the physiological functions of the testis such as steroidogenesis and spermatogenesis [70]. These correlations explained the accompanied decrease in the measured antioxidants and the increased lipid peroxidation.

Adding purslane enhances the fertility measured hormones and decreases the antifertility ones. In the past it was proved to have antifertility effect [71]. This may primarily be due to having CBZ and decreasing its effect, and secondarily refer to its antioxidant effect in preserving the testis composition by decreasing lipid peroxidation. Also purslane contains ω-3 fatty acid as one of its main constituents [72] and its supplementation was proved to restore male fertility and spermatogenesis [73].

In conclusion, using purslane, a powerful antioxidant with CBZ as a complementary AED can decrease its side-effects, especially in respect to reproductive dysfunction.

Conflict of interest statement

We declare that we have no conflict of interest.

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