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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.

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