

## Full Length Research Paper

# Neuronal activities of *Portulaca oleracea* in adult rats

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***Portulaca oleracea* (purslane) belonging to the family "Portulacaceae" was considered to be an anti-magic herb used to protect against variety of diseases. Here, we investigated the neuronal activities of *P. oleracea* in adult rats. Animals were treated with 1.5 ml/kg of aqueous extract of purslane (leaves and stem) for 12 days. Cerebellum, pons and medulla, cerebral cortex, thalamus and hypothalamus, midbrain and spinal cord were isolated to estimate the concentration of calcium, acetyl cholinesterase, dopamine, norepinephrine and serotonin in them. Purslane was able to induce a significant decrease in calcium concentration of the brain cortex. Dopamine, norepinephrine and serotonin were significantly altered in the studied brain regions after treatment of rats with purslane. Acetyl cholinesterase was increased in all brain regions except in the cerebellum. Our results suggest the potential role of purslane mediated changes in the neuronal tissues.**

**Key words:** *Portulaca oleracea*, leaves and stem, neurotransmitters, acetylcholinesterase, calcium, brain, rats.

## INTRODUCTION

A healthy, balanced diet, including an adequate intake of micronutrients, essential fatty acids, amino acids and antioxidants, is essential for both physical and mental well-being. Nutrients which are particularly important for brain development and function include n-3 PUFA (Mechan et al., 2011). The monoamine neurotransmitters, serotonin (5-HT), dopamine (DA) and norepinephrine (NE) are synthesized from dietary amino acids; specifically, 5-HT is synthesized from the essential amino acid, tryptophan, and DA and NE are synthesized from tyrosine. The rate of synthesis of these neurotransmitters is therefore sensitive to the supply of their respective precursors (Fernstrom, 1990). Following their synthesis, 5-HT, DA and NE are released into the synaptic cleft to act at post-synaptic receptor sites. Neurotransmitter action is terminated by reuptake into the pre-synaptic neuron through the activity of high-affinity Na<sup>+</sup>/Cl<sup>-</sup>-dependent monoamines reuptake transporters and enzymatic degradation by monoamine oxidase

(MAO; all three neurotransmitters) or catechol-O-methyltransferase (DA and NE) (Jayanthi and Ramamoorthy, 2005; Frederick and Stanwood, 2009). Activity of the monoamine neurotransmitters can thus be enhanced by inhibition of MAO and/or by inhibition of the reuptake transporters.

Stimulation of cholinergic nerves occurs by inhibiting the cholinesterase enzyme, thus permitting a buildup of acetylcholine on the nerve receptor sites. As a result, acetylcholine increases in quantity with successive nerve impulses so that large amounts of acetylcholine can accumulate and repetitively stimulate receptors.

Plants secondary metabolites have been used as inhibitors of various classes of enzymes. Several thousand plant extracts have been screened against acetylcholinesterase (AChE) from different parts of the world (Orhan et al., 2004; Mukherjee et al., 2007; Ashraf et al., 2011). Recently, Shahwar et al. (2010) and Lee et al. (2011) have demonstrated the AChE inhibition activities from various plants. *Portulaca oleracea* belonging to the family "Portulacaceae" is listed in the World Health Organization as one of the most used medicinal plants and it has been given the term 'Global Panacea'. It is a warm-climate annual and has a

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cosmopolitan distribution. The stems and leaves of the plant are succulent and edible with a slightly acidic and salty taste similar to spinach. It is available commercially in both ornamental and culinary cultivars. Widely used as a potherb in the Mediterranean, Central European and Asian countries, it is also referred to as the common purslane. The aerial parts of the plant are used medicinally for alleviating pain and swelling and as an antiseptic (Chan et al., 2000). The dried herb can be boiled and is made into tea/soups in China (Cai et al., 2004).

Recent research has shown that *P. oleracea* (purslane) is a rich source of omega-3 ( $\omega$ -3) fatty acids, which is important in preventing heart attacks and strengthening the immune system (Simopoulos, 2004). It was reported to contain gallotannins (Lewis and Lewis, 2003), kaempferol, quercetin and apigenin (Cai et al., 2004). The water extracts of *P. oleracea* show no cytotoxicity or genotoxicity, and have been certified safe for daily consumption as a vegetable (Yen et al., 2001). This plant was reported to have neuropharmacological actions, wound healing activities and bronchodilatory effects (Malek et al., 2004). Dietary glutathione, normally occurring in high amounts in fresh meat and in moderate amounts in some fruits and vegetables, is found in *P. oleracea* (Simopoulos, 2004).

The methanol extracts from this plant were found to exhibit moderate antimicrobial activity against *Bacillus subtilis* (Sakai et al., 1996). The inhibitory effect on lipopolysaccharide and interferon- $\gamma$ -induced NO production was shown by the extracts of *P. oleracea* in a concentration dependent manner (Abas et al., 2006). In addition, purslane is reported to be rich in  $\alpha$  linolenic acid and  $\beta$ -carotene and its used as a health food for patients with cardiovascular diseases (Liu et al., 2000). It contains several types of vitamins and minerals especially calcium and potassium. Also, purslane contains *N*-transferuloyltyramine, dopamine, dopa and a high concentration of noradrenaline (Xiang et al., 2005).

In the present investigation, we will examine the effect of purslane aqueous juice on brain's monoamines and acetylcholinesterase activity.

## MATERIALS AND METHODS

### Experimental animals

Adult wistar albino male rats weighing 120 to 150 g were obtained from The Holding Company for Biological Products and Vaccines (VACSERA, Cairo, Egypt). Animals were kept in wire bottomed cages in a room under standard condition of illumination with a 12-h light-dark cycle at  $25 \pm 1^\circ\text{C}$ . They were provided with water and balanced diet *ad libitum*. The experiments were approved by the state authorities and followed Egyptian rules on animal protection.

### Preparation of the plant juice

The fresh purslane herb, mainly stem and leaves which are free of

blemishes or obvious defects, was collected from the Delta of Nile, Egypt during August 2010. An aqueous juice of the purslane herbs was prepared by mashing in a proportion of 1:5 (w/v) and left for about 24 h in a refrigerator. After mashing, the resulting crude extract was filtered and the filtrate was kept at  $-20^\circ\text{C}$  for future use.

### Experimental protocol

To study the effect of purslane, twelve adult male albino rats were randomly divided into two groups, six rats in each group. Group I served as control and received saline (0.2 ml saline/ rat) by oral administration via epi-gastric tube. Group II received oral administration of 1.5 ml/kg purslane aqueous juice for 12 days according to our previous study (Dkhil et al., 2011). The animals of the two groups were cervically dislocated. Brains of rats were carefully removed. Dissection of brains were performed on an ice-cold glass plate for the separation of four brain regions (spinal cord, pons and medulla oblongata, cerebellum, midbrain, thalamus and hypothalamus and cerebral cortex) according to the method described by Glowinski et al. (1966). The six brain regions from 6 rats were longitudinally divided in two equal parts; the first parts were stored frozen for further determination of dopamine, norepinephrine, serotonin and  $\text{Ca}^{2+}$  levels. The second parts were homogenized immediately to give 50% (w/v) homogenate in ice-cold medium containing 50 mM Tris-HCl and 300 mM sucrose and centrifuged at 500 g for 10 min at  $4^\circ\text{C}$ . The supernatant was used for the acetylcholinesterase determination.

### Calcium ions concentration in brain regions and spinal cord

Calcium content was measured in the brain regions and spinal cord according to Jones and Hopkin (1998) and Del Rosario et al. (1982), respectively. Tissue samples of brain regions and spinal cord were oven dried at  $60^\circ\text{C}$  and combusted at  $450^\circ\text{C}$  (24 h). Thereafter, the combusted samples were dissolved in hot solution of 1 M  $\text{HNO}_3$ . The samples were transferred into 50 ml volumetric flasks and adjusted with the deionized water to this volume. The appropriately diluted and digested tissue samples were analyzed at 283.3 nm using flame atomic absorption spectrophotometer (Perkin-Elmer, 3100).

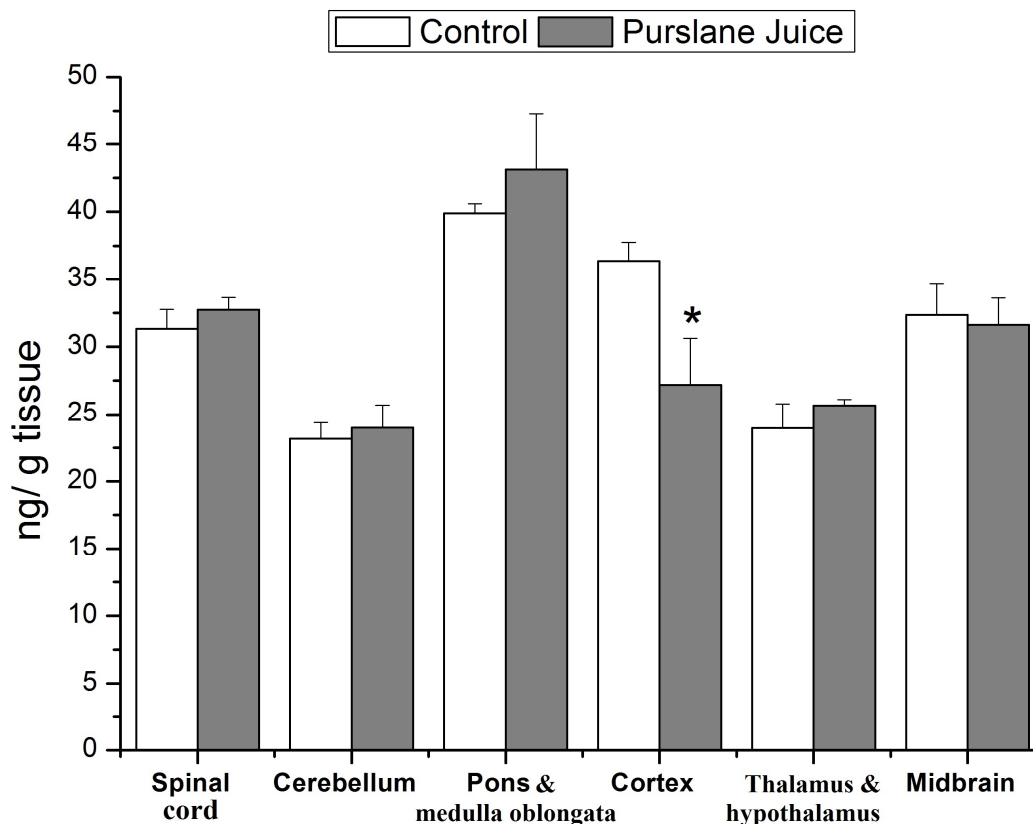
### Monoamines analysis by HPLC

Dopamine (DA), norepinephrine (NE) and serotonin (5-HT) were assayed by means of HPLC with UV detection. The tissue samples were weighed and homogenized in ice-cold 0.1 M trichloroacetic acid containing 0.05 mM ascorbic acid. After centrifugation ( $10,000 \times g$  for 5 min), the supernatants were filtered through RC 58 0.2  $\mu\text{m}$  cellulose membranes.

The chromatograph (Hewlett-Packard 1050) was equipped with C18 columns. The mobile phase consisted of 0.05 M citrate-phosphate buffer, pH 3.5, 0.1 mM EDTA, 1 mM sodium octyl sulfonate and 3.5% methanol. The flow rate was maintained at 0.8 ml/min. Monoamines were quantified by peak height comparisons with standards run on the day of analysis.

### Acetylcholinesterase activity in brain regions

Acetylcholinesterase assay is based on an improved Ellman method (Ellman et al., 1961), in which thiocholine produced by the action of acetylcholinesterase forms a yellow color with 5,5'-dithiobis(2-nitrobenzoic acid). The intensity of the product color, measured at 412 nm, is proportionate to the enzyme activity in the sample.



**Figure 1.** Effect of purslane aqueous juice (Group II) on  $\text{Ca}^{2+}$  levels (ng/mg tissue) in the different brain regions and spinal cord of adult male albino rats after 12 days of oral administration. Values are means  $\pm$  SE (n = 6). \*, Significant change at  $p < 0.05$  with respect to Group I.

**Table 1.** Effect of purslane aqueous juice (Group II) on dopamine content ( $\mu\text{g}/\text{mg}$  tissue) in the different brain regions of adult male albino rats after 12 days of oral administration.

Group	Spinal cord	Cerebellum	Pons and medulla oblongata	Cerebral cortex	Thalamus and hypothalamus	Midbrain
I	5.39 $\pm$ 0.32	4.48 $\pm$ 0.17	7.38 $\pm$ 0.60	5.41 $\pm$ 0.31	5.22 $\pm$ 0.33	4.22 $\pm$ 0.27
II	3.71 $\pm$ 0.21*	8.05 $\pm$ 0.49*	7.20 $\pm$ 0.57	8.91 $\pm$ 0.19*	6.46 $\pm$ 0.29*	4.71 $\pm$ 0.25

Values are means  $\pm$  SE (n = 6). \*, Significant change at  $p < 0.05$  with respect to the Group I.

### Statistical analysis

Results were expressed as the means  $\pm$  standard error of the mean (SEM). Statistical analysis was performed using an unpaired student's *t*-test using a statistical package program (SPSS version 17.0).

## RESULTS

Treatment of rats with purslane aqueous juice for 12 days significantly decreased the  $\text{Ca}^{2+}$  level in cerebral cortex by about -25.2% at  $p < 0.05$ . However, there was no significant change in  $\text{Ca}^{2+}$  ions in the remaining brain regions due to purslane aqueous juice treatment

compared to the control group (Figure 1). Purslane aqueous juice administration induced significant increase ( $p < 0.05$ ) in DA content in cerebellum, cerebral cortex and thalamus and hypothalamus of rats by, 79.7, 64.7 and 23.7%, respectively (Table 1). However, purslane aqueous juice administration caused significant decrease (-31.2%) in DA content of spinal cord compared to the control group.

Purslane induced significant increase ( $p < 0.05$ ) in NE content in cerebellum, pons and medulla oblongata, cerebral cortex, and thalamus and hypothalamus of rats by, 160.1, 140.2, 99.6 and 241.9%, respectively (Table 2). However, purslane aqueous juice administration caused significant decrease in NE content of spinal cord

**Table 2.** Effect of purslane aqueous juice (Group II) on norepinephrine content ( $\mu\text{g}/\text{mg}$  tissue) in the different brain regions of adult male albino rats after 12 days of oral administration.

Group	Spinal cord	Cerebellum	Pons and medulla oblongata	Cerebral cortex	Thalamus and hypothalamus	Midbrain
I	34.09 $\pm$ 1.52	12.08 $\pm$ 0.82	35.32 $\pm$ 1.34	10.43 $\pm$ 0.69	7.74 $\pm$ 0.53	22.32 $\pm$ 1.17
II	16.71 $\pm$ 0.87*	31.42 $\pm$ 1.21*	84.83 $\pm$ 2.71*	20.82 $\pm$ 1.06*	26.46 $\pm$ 1.34*	13.84 $\pm$ 0.73*

Values are means  $\pm$  SE (n = 6). \*, Significant change at  $p < 0.05$  with respect to the Group I.

**Table 3.** Effect of purslane aqueous juice (Group II) on serotonin content ( $\mu\text{g}/\text{mg}$  tissue) in the different brain regions of adult male albino rats after 12 days of oral administration.

Group	Spinal cord	Cerebellum	Pons and medulla oblongata	Cerebral cortex	Thalamus and hypothalamus	Midbrain
I	6.12 $\pm$ 0.24	12.94 $\pm$ 0.78	9.56 $\pm$ 0.54	12.76 $\pm$ 0.76	12.43 $\pm$ 0.92	10.34 $\pm$ 0.74
II	4.14 $\pm$ 0.19*	11.51 $\pm$ 0.69	13.66 $\pm$ 0.91*	15.23 $\pm$ 0.69*	25.34 $\pm$ 1.08*	9.77 $\pm$ 0.52

Values are means  $\pm$  SE (n = 6). \*, Significant change at  $p < 0.05$  with respect to the Group I.

and midbrain (-51.0 and -38.0%, respectively) compared to control group. Table 3 summarized the effect of purslane aqueous juice treatment in 5-HT content in different neuronal regions of rats. Purslane aqueous juice administration induced significant increase ( $p < 0.05$ ) in 5-HT content in pons and medulla oblongata, cerebral cortex and thalamus and hypothalamus of rats by, 42.9, 19.4 and 103.9%, respectively (Table 3). However, purslane caused significant decrease in 5-HT content of spinal cord by -32.4% compared to the control group. Treatment of rats with purslane aqueous juice for 12 days significantly increased the acetyl cholinesterase activity in spinal cord and all brain regions except cerebellum by about 83.3% in spinal cord, 504.8% in pons and medulla oblongata, 233.3% in cerebral cortex, 97.0% in thalamus and hypothalamus and by 119.6% in midbrain respectively, at  $p < 0.05$ . However, no significant change in AChE activity was noticed in cerebellum due to purslane aqueous juice treatment compared to the control group (Figure 2).

## DISCUSSION

Dietary phytochemical antioxidants are capable of removing free radicals. Among them, phenolic and polyphenolic compounds, such as flavonoids in edible plants, exhibit potent antioxidant activities (Fang et al., 2002). A large body of the literature has documented the beneficial effects of polyphenolic compounds on scavenging free radicals and on their role in the prevention and therapy of disease. First, polyphenols enhance red blood cell resistance to oxidative stress *in vitro* and *in vivo* (Youdim et al., 2000). Second, polyphenols effectively scavenge superoxide and

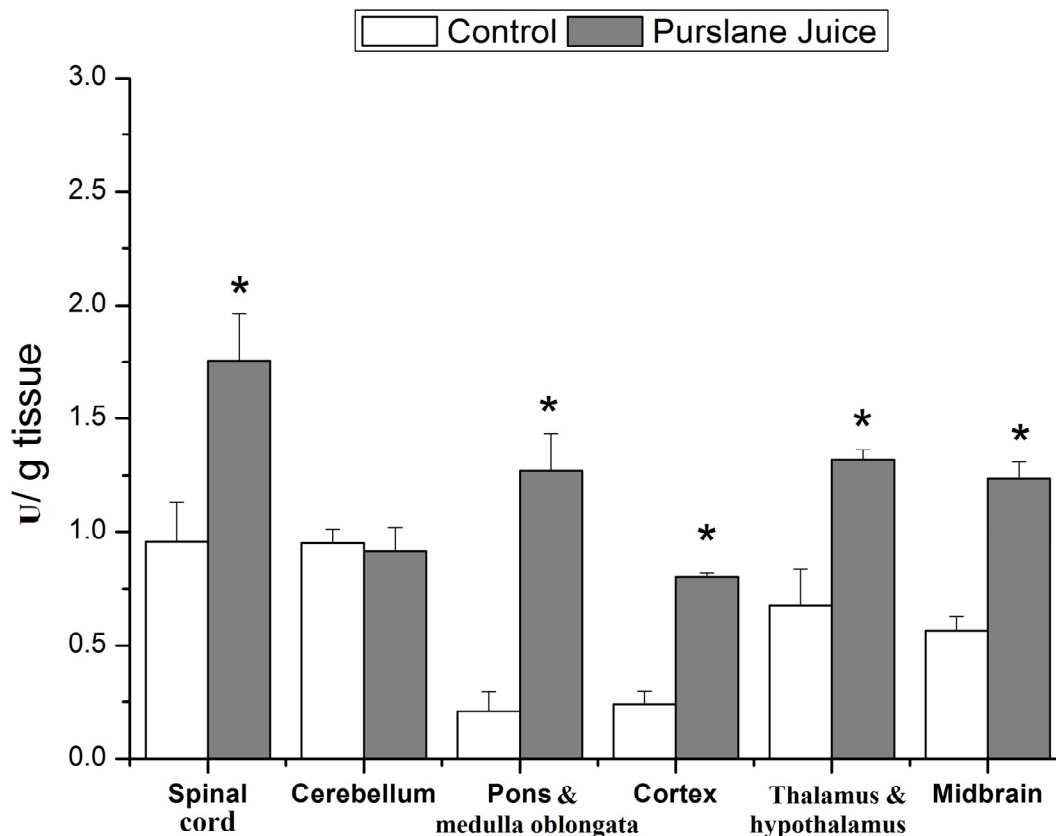
hydroxyl radicals and inhibit oxidative modification of low density lipoprotein (Fang et al., 1998). Third, polyphenols inhibit the growth and induce apoptosis of several human cancer cell lines *in vitro* (Fang et al., 2002). Fourth, polyphenols enhance Cu, Zn-SOD activity and decrease malondialdehyde (Cui et al., 2000).

Plants, vegetables, herbs and spices used in folk and traditional medicine have been accepted currently as one of the main sources of chemo preventive drug discovery and development (Aruoma, 2003). It has been observed that many plant polyphenols, such as ellagic acid, catechins, and chlorogenic, caffeic and ferulic acids act as potent antioxidant, antimutagenic and anticarcinogenic agents (Ayrton et al., 1992).

The genus *Portulaca* was listed as a genus of plants containing alkaloids, coumarins, flavonoids and anthraquinone glycosides. Total betacyanin isolated from *P. oleracea* yielded oleracin I and II. The aqueous extract of the *P. oleracea* leaves and stems might act in part on postsynaptic  $\alpha$ -adrenoceptors and interfere with transmembrane calcium influx (Obied et al., 2003). Recent research demonstrated that purslane is a good source of compounds with a positive impact in human health. The compounds include  $\beta$ -carotene, vitamins and essential amino acids,  $\alpha$ -tocopherols, ascorbic acid and glutathione. Organic acids are also present and alkaloids have been reported to be important chemical constituents of this species (Oliveira et al., 2009).

Also, purslane contains numerous common nutrients including having the highest concentration of  $\omega$ -3 fatty acids among leafy vegetables. Other bioactives found in purslane are dopamine, dopa, coumarins, alkaloids and saponins, polyphenols, flavonoids and anthocyanin (Dkhal et al., 2011).

In the brain  $\omega$ -3, are very rich, plays a crucial role in the



**Figure 2.** Effect of purslane aqueous juice (Group II) on acetylcholinesterase activity (U/mg tissue) in the different brain regions and spinal cord of adult male albino rats after 12 days of oral administration. Values are means  $\pm$  SE (n=6). \*, Significant change at  $p < 0.05$  with respect to the Group I.

regulation of dopaminergic and serotonergic neurotransmission (Zimmer et al., 2000), membrane-bound enzymes ( $\text{Na}^+/\text{K}^+$ -ATPase) (Bowen and Clandinin, 2002), signal transduction via effects on inositol phosphates and protein kinase C (Vaidyanathan et al., 1994), and regulation of glucose uptake via effects on brain glucose transporters (Pifferi et al., 2005, Sinclair et al., 2007). The deficiency of  $\omega$ -3 results in a 30 to 35% reduction in phosphatidylserine in rat brain cortex, brain mitochondria, and olfactory bulb (Hamilton et al., 2000). Phosphatidylserine can activate various enzymes, including protein kinase C,  $\text{Na}^+/\text{K}^+$ -ATPase, and tyrosine hydroxylase, as well as regulating  $\text{Ca}^{2+}$  uptake. It is therefore also suggested that altering phosphatidylserine in cerebral membranes can alter neurotransmission (Logan, 2003). Supplementing the diet of rats with  $\omega$ -3 led to a 40% increase in dopamine levels in the frontal cortex as well as an increase in the binding to the dopamine D2 receptor (Chalon et al., 1998). This may be explaining the increment in monoamines content after the treatment with purslane in this study.

The melatonin concentration in purslane was found to exceed that reported in a number of other fruits and vegetables (Simopoulos et al., 2005). Pineal

neurohormone, melatonin (N-acetyl-5-methoxytryptamine) has a variety of important functions including direct free radical scavenging and antiinflammatory properties (Rodriguez et al., 2004). In addition, melatonin plays an important role, among the different neurohormones and neuropeptides that influence the immune system (Finocchiaro et al., 1991).

Membrane fluidity, which is a key property of the membrane lipid bilayer, has been found to decrease with oxidative stress. AChE activity is inhibited by free radicals and increased oxidative stress. The activity of AChE depends largely on the membrane characteristics. Such changes which may cause alterations in the physical properties of membranes are likely to modify enzymatic activity of membrane bound proteins and lipid-protein interactions. It has also been reported that AChE activity is influenced by membrane surface phenomena (Jha and Rizvi, 2009).

Many literatures described omega-3 fatty acids as AChE inhibitor. However, our results indicated that purslane administration caused significant increase in AChE activity opposite to our knowledge. This increased can be explained by melatonin content in purslane. A large body of evidence links free radical generation

with neuronal degeneration. Reports suggest that the neuroprotective action of pineal melatonin is due to scavenging both reactive oxygen and reactive nitrogen species. It is known from the literature that pineal glands of mammals express cholinesterase activity (Rizvi and Chakravarty, 2011). In addition, Shen et al. (2002) have postulated that melatonin's ability in improving cognitive functions is related to its antioxidant action.

The present study demonstrates that purslane aqueous juice administration significantly increased the different monoamines, acetylcholinesterase activity in rats due its content of melatonin, omega-3 fatty acid, phenolic and flavonoids compounds and other active ingredients, suggesting the potential role of purslane for neurotransmitters which is an integral part of many neurodegenerative disorders.

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