

NEUROPHYSIOLOGICAL STUDY ON POSSIBLE PROTECTIVE AND THERAPEUTIC EFFECTS OF SIDR (*ZIZYPHUS SPINACHRISTIL*) FRUIT EXTRACT IN MALE ALBINO RATS TREATED WITH PENTYLENETETRAZOL

¹Abeer M. Waggas and ²Reem H. Al-Hasani

¹Department of Biology (Zoology), Faculty of Girls Education, Scientific Department, (King Abdulaziz University), Jeddah, Saudi Arabia ,
Email :ombaderm@yahoo.com

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ABSTRACT

Sidr (*Zizyphus spina-christi* L) has several physiological and morphological characteristics . In this study , anticonvulsant effect of sidr fruit extract was examined by using pentylenetetrazol (PTZ) model on male albino rat , by evaluating the changes in norepinphrine(NE) , dopamine (DA) , serotonin (5-HT) gamma-aminobutyric acid (GABA) contents in different brain regions (cerebellum , brain stem , striatum , cerebral cortex , hypothalamus and hippocampus) . Administration of subconvulsive dose of PTZ (40mg/kg i.p.) every other day for 9 days caused a significant decrease in neurotransmitter content in different brain areas , this is may be due to increased nitric oxide (NO) levels and activation in Na(+)-ATPase activity , which caused neurotransmitter release so the content is decreased . The administration of PTZ after treatment with sidr (50mg/kg i.p.) fruit extract for 3 weeks as a protective group , and administration of sidr fruit extract for 3 weeks after treatment of PTZ as a therapeutic groups caused significant increase in NE , DA , 5-HT and GABA contents in all tested brain regions at most of the time intervals studied which may be related to presence of ascorbic acid (vitamin C)in the extract which inhibit neurotransmitters release by inhibition of Na(+)-K(+)-ATPase activity , which activated by PTZ ,so the content is increased .From these results , we can say that the sidr fruit extract has a neuroprotective and therapeutic role against pentylenetetrazol convulsant effect .

Key word : Sidr , *Zizyphus spina-christi* , Pentylenetetrazol , Neurotransmitter , Brain , Rat .

INTRODUCTION

EPILEPSY affects 1-2% of the world population and is associated with imbalances between excitatory and inhibitory neurotransmission in the brain (Locke *et al.* 2009). In particular, interneurons expressing gamma-aminobutyric acid (GABA), the principal inhibitory neurotransmitter in the human brain, are essential for normal neuronal synchronization and maintenance of a seizure

²Department of Biology (Zoology) , Faculty of Girls Education, Scientific Department,(Umm Al-qura University), Makah, Saudi Arabia

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threshold in humans (Cossette *et al.* 2002) rodents (Delory *et al.* 1998), and zebrafish (Barban *et al.* 2005). A failure of the brain to properly regulate neuronal synchrony can result from ion channel defects (Xu and Clancy, 2008), neuropeptide depletion (BRILL *et al.* 2006), brain malformations (PATEL *et al.* 2004), interneuron loss (Cobos *et al.* 2005), and/or synaptic vesicle recycling failure (Di Paolo *et al.* 2002), all of which may be caused by disrupting the nerve-cell cytoskeleton .

Modern drug therapy of epilepsy is complicated by the inability of drugs to control seizure and side effects that range in severity from minimal impairment of the central nervous system (CNS) to death from aplastic anaemia or hepatic failure (Wannang *et al.* , 2008) . Thus its effective and safe therapy remains a challenge (Smith and Bleck , 1991 ; Roger and Brian , 1998) . Medicinal plants used in traditional medicine for the treatment of epilepsy have been scientifically shown to possess promising anticonvulsant activities in animal models for screening for anticonvulsant activity and can be a source of newer anticonvulsants (Sandabe *et al.* , 2003 ; Salahdeen and Yemitan , 2006) .

Zizphus spina-christi (L) , locally known as Sidr , is a multipurpose tree species belonging to the botanical family *Rhamnaceae* . Flowering and fruiting occur in this species during September-November (Mandavilae , 1990) . Sidr is one of the important fruit crops in the dry parts of tropical Asia and Africa . Its fruit is highly nutritious and rich in vitamin C . The dry fruit contains 314 calories , 9.3% H₂O , 4.8 % protein , 0.9% fat , 80.6% total carbohydrate , 4.4% ash , 140 mg Ca , 3mg Fe , 0.04mg thiamin , 0.13mg riboflavin , 3.7mg niacin and 30mg ascorbic acid (Duke , 1985) .

Since *Zizyphus* is a wild tree commonly available in Saudi Arabia and its leaves are used in folk medicine for treatment , it is therefore deemed interesting to examine the neuroprotective and therapeutic effects of *Zizphus spina-christi* (L) fruit extract as an anti-convulsant effect in the pentylenetetrazol (PTZ) - induced seizure in male albino rat by evaluating the changes in norepinephrine (NE) , dopamine (DA) , serotonin (5-HT) and gamma-aminobutyric acid (GABA) content in different brain regions (cerebellum , brain stem , striatum , cerebral cortex , hypothalamus and hippocampus) of male albino rats .

MATERIAL AND METHODS

Chemicals and Plant Extract :

pentylenetetrazol (PTZ) of highest purity supplied from Sigma chemical company was used in present work . The applied dose of 40 mg/kg BW was dissolved in 0.2 ml saline solution The applied dose was selected according to Akula *et al.* (2007) and Dhir *et al.* (2007) .

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Fresh *Zizyphus spina-christi* (L) fruit collected from trees growing in Taif (Saudi Arabia) between September and November of 2008. Zizyphus fruit was extracted according to the method described by Adzu *et al.* (2001) . The plant was dried under shade at 25°C and the dried fruits of plant were ground with a blender. The powder part was kept in nylon bags in deep freezer until the time of use. It was weighted (100 g) and cold distilled water poured into it to give a final volume of 200 ml as reported previously (Abdel-Wahhab *et al.* , 2007) .

Experimental work :

Thirty -six adult male albino rats (*Rattus norvegicus*) weighing approximately 150 g BW were used for experimentation . Free access of standard diet and water was allowed *ad-libitum*. They were kept under good ventilation with 12 hour light and dark cycle. The rats were arranged into six groups , six per each .

- 1- The first group (n=6) , The animals were daily injected with saline vehicle and served as control.
- 2- The 2nd group (n=6) , the rats were injected in a subconvulsive dose (40 mg/kg , i.p.) every other day for 9 days (Dhir *et al.* , 2005 ; Akula *et al.* , 2007).
- 3- The 3rd group(n=6) , the rats were treated with PTZ (40 mg/kg) every other day for 9 days after Zizyphus (50 mg/kg) leave extract treated for 3 weeks .
- 4- 4th ,5th and 6th groups were injected in a subconvulsive dose (40 mg/kg , i.p.) every other day for 9 days then injected with single dose of Zizyphus (50 mg/kg i.p.) for three weeks respectively .

At the end of treatment , the rats of both control and experimental groups were sacrificed , and the brain was rapidly dissected and separated into two equal halves . Each half was then separated into the following regions according to the method of Glowinski and Iversen (1966): cerebellum, brainstem, striatum, cerebral cortex, hypothalamus and hippocampus. The brain tissues were wiped dry , weighed and wrapped into quickly plastic frozen in dry ice pending analysis. NE , DA were extracted and estimated according to the method of Chang (1964) and modified by Ciarlone (1978) , serotonin was estimated according to the method of Miller *et al.* (1970) . The GABA was estimated according to the method of Sutton and Simmonds (1973) . The fluorescence was measured in Jenway 6200 fluorometer.

Statistical Analysis:

The results are reported as the mean + S.E.M. of values obtained from multiple observation . Differences between means were considered statistically significant at P<0.01 variance (Woolson, 1987) . All statistical analysis were computed by SPSS version 10 .

RESULT

Figures 1 - 4 illustrated norepinephrine (NE) , dopamine (DA) , serotonin (5-TH) and gamma-aminobutyric acid (GABA)content ($\mu\text{g/g}$ fresh tissue) of brain regions (cerebellum , brain stem , striatum , cerebral cortex , hypothalamus and hippocampus) of *Zizyphus* fruit (50mg/kg)protective and therapeutic in albino rats treated with pentylenetetrazol (PTZ) respectively .

Figure 1 shows that the injection of PTZ (40 mg/kg i.p.)every other day for 9 days (G2) caused a significant decrease in NE content in all investigated brain regions compared to control group (G1) . There was a significant increase in NE content in protective group G3 (rats were treated with PTZ for 9 days after *Zizyphus* fruit extract treated for 3 weeks) in all tested brain regions compared to G2 (control rats were treated with PTZ) only . A significant increase in NE content was also found in therapeutic groups G4 , G5 and G6 (rats were treated with *Zizyphus* fruit extract after PTZ injection) in all brain regions compared to G2 only .

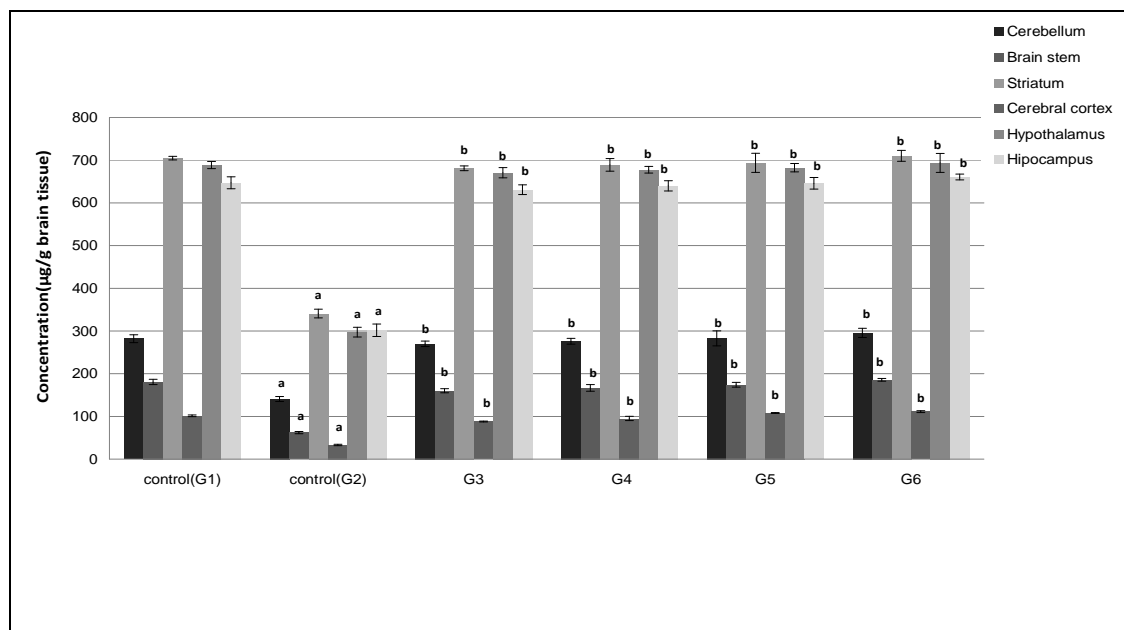


Fig . 1 : Protective and therapeutic effect of *Zizyphus spina-christi* (L) fruit on norepinephrine (NE) content in different brain regions in male albino rats treated with pentylenetetrazol (PTZ) .

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As shown in Figure 3 , treatment with 40 mg/kg of PTZ caused depletion of 5-HT content in investigated brain regions compared to control group (G 1). However , protective group significantly increase in 5-HT content in all brain regions compared to G2 . A significant increase was also found in therapeutic groups (G4 , G5 and G6) compared to G2 in all tested brain regions .

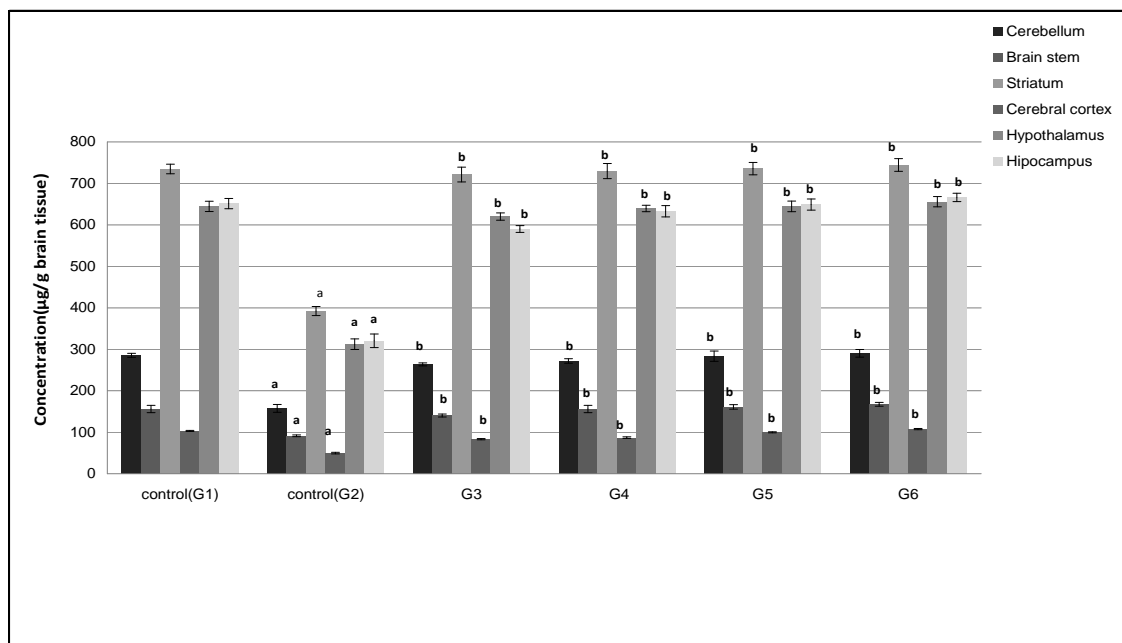


Fig . 3 : Protective and therapeutic effect of *Zizyphus spina-christi* (L) fruit on serotonin (5-HT) content in different brain regions in male albino rats treated with pentylenetetrazol (PTZ) .

a : Statically significant ($P < 0.01$) compared to group 1 . b : Statically significant ($P < 0.01$) compared to group 2 .

G1: control rats were treated with saline .

G2 : control rats were treated with PTZ .

G3 : rats were treated with PTZ for 9 days after Zizyphus fruit extract treated for 3 weeks .

G4 : rats were treated with Zizyphus fruit extract treated for 1 weeks after PTZ injection for 9 days .

G5 : rats were treated with Zizyphus fruit extract treated for 2 weeks after PTZ injection for 9 days .

G6 : rats were treated with Zizyphus fruit extract treated for 3 weeks after PTZ injection for 9 days .

Results from Figure 4 shows that PTZ-treatment(40 mg/kg i.p.) led to inhibition of gamma-aminobutyric acid content in the investigated brain regions compared to control group (G1) . Whereas there was a significant increase in GABA content in protective group (G3) in all tested brain regions compared to G2 only .A significant increase was also found in therapeutic groups (G4,G5 and G6) in investigated brain regions compared to G2 only.

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nitric oxide (NO) levels and activation in Na(+)-K(+)-ATPase activity (Schneider Oliveira *et al.*, 2004).

From the present result it is clear that the administration of subconvulsant doses of Pentylene-tetrazol (40 mg/kg) caused a significant decrease in NE, DA, 5-HT and GABA content in all tested brain regions, and the rats showed myoclonic jerks and clonus. From the previous studies and present results it is clear that Pentylene-tetrazol decrease neurotransmitter (NE, DA, 5-HT and GABA) in all tested brain areas this may be due increased nitric oxide (NO) levels and activation in Na(+)-ATPase activity, which caused neurotransmitter release so the content is decreased.

Zizyphus spina-christi has several physiological and morphological characteristics. Previous study shown that the crude aqueous extract of *Zizyphus spina-christi* exhibited anti-nociceptive potency with both central and peripheral effect (Adzu *et al.*, 2002). Duke (1985) reported that *Zizyphus* fruit is highly nutritious and rich in ascorbic acid (vitamin C). Previous studies suggested that ascorbic acid is an antioxidant vitamin that is found in high concentrations in the brain which seems to have neuroprotective properties in some experimental models of excitotoxic neurological disorders, including convulsive behavior and reactive species-related damage, ascorbic acid in high doses protected against PTZ-induced convulsion and inhibition of Na(+)-K(+)-ATPase activity (Richard *et al.*, 1984; Schneider Oliveira *et al.*, 2004; Souza *et al.*, 2009). From the present result and earlier studies it could be concluded that, the Pentylene-tetrazol caused a decrease in NE, DA, 5-HT and GABA content which can be minimized by Sidr (*Zizyphus spina-christi*) fruit extract which improve content of these neurotransmitters in all deferent brain regions, which may be related to the presence of ascorbic acid which inhibition of Na(+)-K(+)-ATPase activity, So the content is increased. we can say that the sidr fruit extract has a neuroprotective and therapeutic role against pentylene-tetrazol convulsant effect.

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