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REVIEW

Neurophysiological study on possible protective and therapeutic effects of Sidr (*Zizyphus spina-christi* L.) leaf extract in male albino rats treated with pentylenetetrazol

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Abstract this study, anti-convulsant effect of Sidr leaf extract was examined by using pentylenetetrazol (PTZ) model on male albino rat by evaluating the changes in norepinephrine (NE), dopamine (DA) and serotonin (5-HT) contents in different brain regions (cerebellum, brainstem, striatum, cerebral cortex, hypothalamus and hippocampus). The administration of subconvulsive dose of PTZ (40 mg/kg i.p.) every other day for 9 days caused a significant decrease in monoamine content in different brain areas, this is may be due to the increase in nitric oxide levels, although antagonized the GABAA receptors which led to neurotransmitter release so the content is decreased. Administration of PTZ after treatment with Sidr (50 mg/kg i.p.) leaf extract for 3 weeks as a protective group and administration of Sidr leaf extract for 3 weeks after treatment of PTZ as a therapeutic group caused significant increase in NE, DA, and 5-HT contents in all tested brain regions at most of the time intervals studied. This may be due to the presence of peptide and cyclopeptide alkaloids in the extract which inhibit neurotransmitter activity which led to the inhibition of

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neurotransmitter release. From these results, we can say that the Sidr leaf extract has neuroprotective and therapeutic roles against pentylenetetrazol convulsant effect.

Contents

1. Introduction

Convulsion, sudden, violent, involuntary contraction of the muscles of the body is often accompanied by loss of consciousness. It is not known what causes the abnormal impulses from the brain that result in convulsive seizures, since the disturbance may arise in normal brain tissue as well as in diseased or injured tissue. Convulsion may occur in such conditions as epilepsy, poisoning, high fever (especially in young children), disturbances of calcium or phosphorus metabolism, alkalosis, diabetes, oxygen insufficiency, and low blood-sugar content, as well as in local irritation and injury of the brain (Bornstein et al., 1998).

Chemical kindling is widely used as an experimental model of convulsion. This phenomenon is characterized by progressive intensification of seizure activity after repeated administration of doses of different central nervous system (CNS) stimulants, including pentylenetetrazol (PTZ) (Racine, 1972). Pentylenetetrazol is commonly used as a convulsant drug, acting as a $GABA_A$ antagonist. Studies have shown that the mechanisms involved in PTZ kindling may include a decrease in central GABAergic function (Berman et al., 2000). The enhanced seizure susceptibility induced by kindling is probably attributable to plastic changes in the synaptic efficacy (Morimoto et al., 2004).

Zizphus spina-christi (L.), locally known as Sidr, is a multipurpose tree species belonging to the botanical family Rhamnaceae. It is an important cultivated tree and one of the few truly native tree species of Arabia that is still growing along with many newly introduced exotic plants (Mandavillae, 1990). Flowering and fruiting occur in this species during September–November. The flowers are important for the production of wild bee honey (Ghazanfar, 1994). The winter honey (i.e. nabk honey) collected during November from the flowers of the Sidr is in high demand among the citizens for its medicinal qualities in addition to its excellent taste and fragrant smell. 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. From the different species of genus Zizyphus, peptide and cyclopeptide alkaloids, flavonoids, tannins, betulinic acid and triterpenoidal saponin glycosides have been isolated and chemically identified (Ikram and Tomlinson, 1976; Duke, 1985; Morishita et al., 1987; Cheng et al., 2000; Shahat et al., 2001).

Zizyphus has been used in folk medicine as a demulcent, depurative, anodyne, emollient, stomachic, for tooth aches, astringents and as a mouth wash (Nazif, 2002). 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 *Z. spina-christi* (L.) leaf extract as a anti-convulsant effect in the pentylenetetrazol (PTZ)-induced seizure in male albino rat by evaluating the changes in norepinephrine (NE), dopamine (DA) and serotonin (5-TH) contents in different brain regions (cerebellum, brainstem, striatum, cerebral cortex, hypothalamus and hippocampus) of male albino rats.

2. Materials and methods

2.1. Chemicals and plant extract

Pentylenetetrazol (PTZ) of highest purity supplied from Sigma chemical company was used in the 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), Dhir et al. (2007).

Fresh *Z. spina-christi* (L.) leaf were collected from trees growing in Taif (Saudi Arabia) between September and November of 2008. Zizyphus leaf were extracted according to the method described by Adzu et al. (2001). The plant was dried under shade at 25 °C and the dried leaves of the plant were grounded 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 was poured into it to give a final volume of 200 ml as reported previously (Abdel-Wahhab et al., 2007).

2.2. 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 h light and dark cycles. The rats were arranged into six groups, six per group.

1. The 1st group ($n = 6$), the animals were injected daily 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 (Akula et al., 2007 and Dhir 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) leaf extract treated for 3 weeks.
4. The 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 the 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 and serotonin were extracted and estimated according to the method of Chang (1964) and modified by Ciarlone (1978). The fluorescence was measured in Jenway 6200 fluorometer.

2.3. Statistical analysis

The results are reported as the mean ± SEM of values obtained from multiple observations. Differences between means were considered statistically significant at $P < 0.01$ variance (Woolson, 1987). All statistical analyses were computed by SPSS version 10.

3. Result

Figs. 1–3 illustrate protective and therapeutic effects of Zizyphus on norepinephrine (NE), dopamine (DA) and serotonin (5-TH) contents (μg/g fresh tissue) in brain regions (cerebellum, brainstem, striatum, cerebral cortex, hypothalamus and hippocampus) of albino rats treated with pentylenetetrazol (PTZ), respectively.

Fig. 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 leaf extract treated for 3 weeks) in all tested brain regions compared to G1 (control rats were treated with saline) and G2 (control rats were treated with PTZ). A significant increase in NE content was also found in therapeutic groups G4, G5 and G6 (rats were treated with Zizyphus leaf extract after PTZ injection) in all brain regions compared to G1 and G2 except in cerebral cortex and hypothalamus in G4 which significantly increased compared to G2 only.

A result from Fig. 2 revealed that PTZ treatment led to the inhibition of dopamine content in the investigated brain regions compared to control group (G1). Whereas there was a significant increase in DA content in protective group (G3) in all tested brain regions compared to G1 and G2. A significant increase was also found in therapeutic groups (G4, G5 and G6) in investigated brain regions compared to G1 and G2.

As shown in Fig. 3, treatment with 40 mg/kg of PTZ caused depletion of 5-HT content in investigated brain regions compared to control group (G1). However, protective group significantly increases 5-HT content in all brain regions compared to G1 and G2. A significant increase was also found in therapeutic groups (G4, G5 and G6) compared to G1 and G2 except in

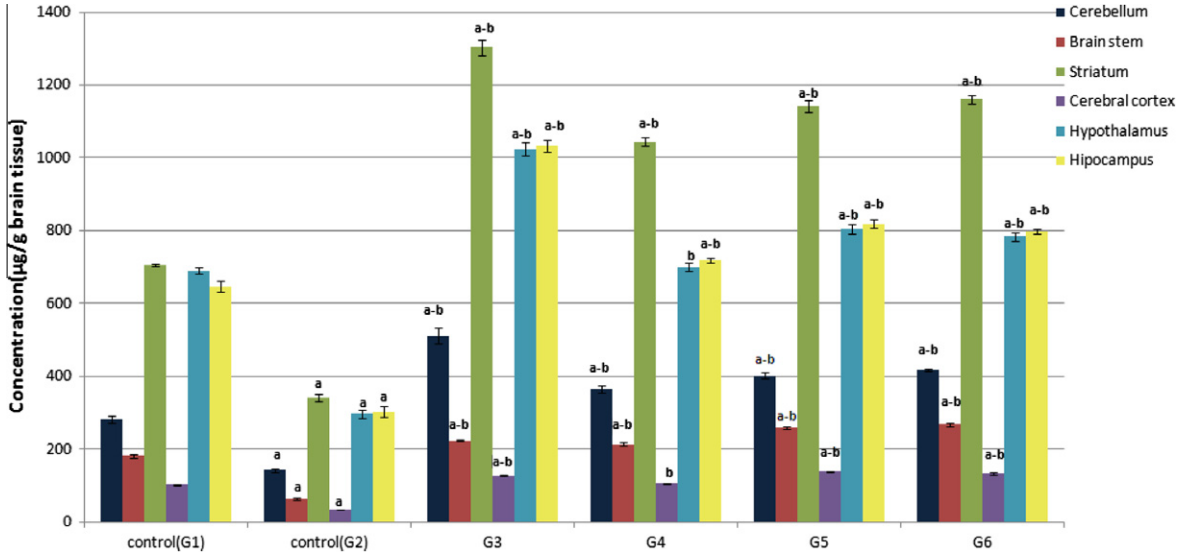

Figure 1 Protective and therapeutic effects of *Zizphus spina-christi* (L.) leaves on norepinephrine (NE) content in different brain regions of male albino rats treated with pentylenetetrazol (PTZ). (a) Statically significant ($P < 0.01$) compared to group 1. (b) Statically significant ($P < 0.01$) compared 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 leave extract treated for 3 weeks; G4, rats were treated with Zizyphus leave extract treated for 1 weeks after PTZ injection for 9 days; G5, rats were treated with Zizyphus leave extract treated for 2 weeks after PTZ injection for 9 days; G6, rats were treated with Zizyphus leave extract treated for 3 weeks after PTZ injection for 9 days.

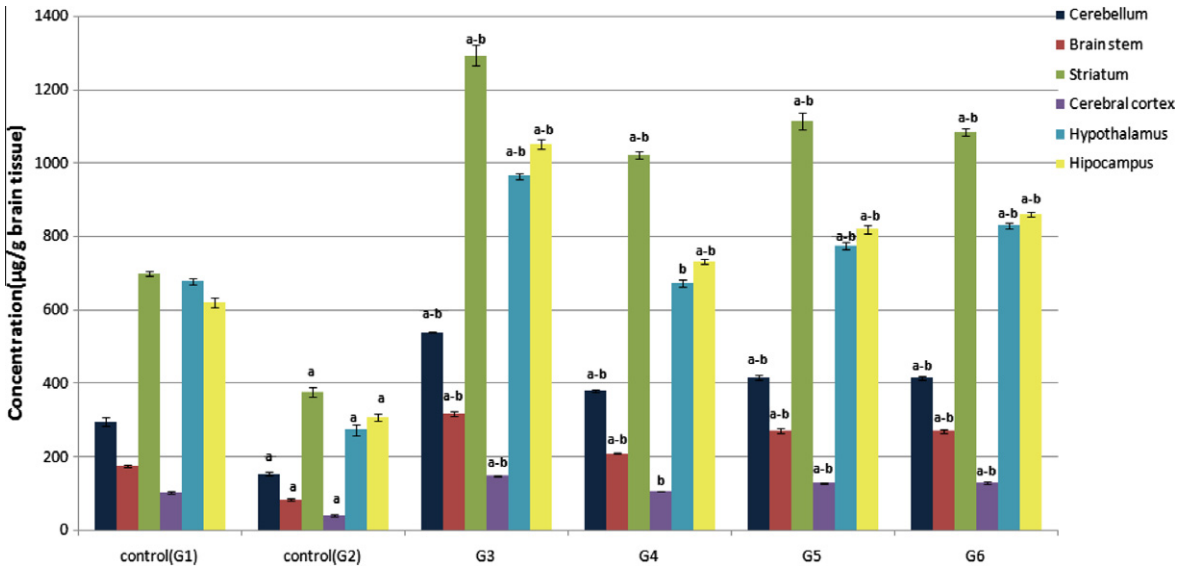

Figure 2 Protective and therapeutic effects of *Zizphus spina-christi* (L.) leaves on dopamine (DA) content in different brain regions of male albino rats treated with pentylenetetrazol (PTZ). (a) Statically significant ($P < 0.01$) compared to group 1. (b) Statically significant ($P < 0.01$) compared 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 leave extract treated for 3 weeks; G4, rats were treated with Zizyphus leave extract treated for 1 weeks after PTZ injection for 9 days; G5, rats were treated with Zizyphus leave extract treated for 2 weeks after PTZ injection for 9 days; G6, rats were treated with Zizyphus leave extract treated for 3 weeks after PTZ injection for 9 days.

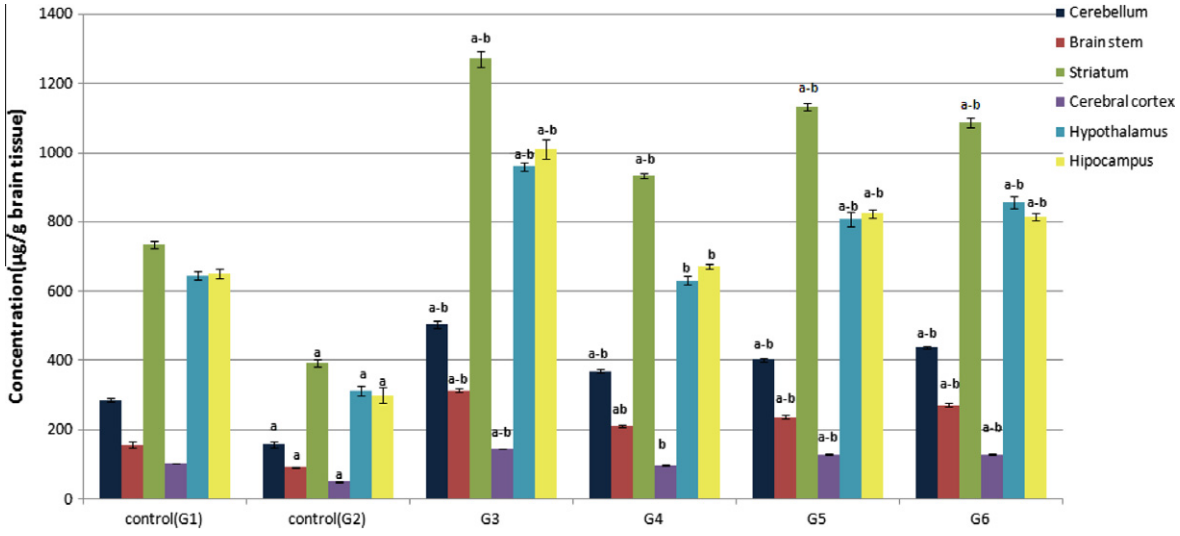

Figure 3 Protective and therapeutic effects of *Zizphus spina-christi* (L.) leaves on serotonin (5-HT) content in different brain regions of male albino rats treated with pentylenetetrazol (PTZ). (a) Statically significant ($P < 0.01$) compared to group 1. (b) Statically significant ($P < 0.01$) compared 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 leave extract treated for 3 weeks; G4, rats were treated with Zizyphus leave extract treated for 1 weeks after PTZ injection for 9 days; G5, rats were treated with Zizyphus leave extract treated for 2 weeks after PTZ injection for 9 days; G6, rats were treated with Zizyphus leave extract treated for 3 weeks after PTZ injection for 9 days.

cerebral cortex, hypothalamus and hippocampus in G4 which significantly increases compared to G2 only.

4. Discussion

Pentylenetetrazol (PTZ) is commonly used as a convulsant drug. The enhanced seizure susceptibility induced by kindling is probably attributable to plastic changes in the synaptic efficacy (Oses et al., 2007). Dazzi et al. (1997) indicated that PTZ kindling enhances the basal activity and sensitivity of dopamine neurons in rat brain and suggested that mesocortical, mesoaccumbens and nigrostiatal dopaminergic neurons contribute to the central alterations associated with experimental epilepsy.

From the present result it is clear that the administration of pentylenetetrazol (40 mg/kg) caused a significant decrease in NE, DA and 5-HT contents in all tested brain regions. The earlier studies indicated that PTZ is a potent competitive antagonist at inhibitory $GABA_A$ receptors that can induce seizure activity by altering potassium permeability of the cell membrane via a voltage-dependent mechanism, also, treatment with PTZ significantly increased nitric oxide (NO) levels in brain (Berman et al., 2000; Huang et al., 2001; Dhir et al., 2005).

Sidr (*Z. spina-christi*) has been used as an anti-convulsant and hypnotic in oriental countries due to its CNS inhibitory activity (Yu-ching, 1983; Han et al., 1986; Zhang et al., 2003; Park et al., 2004). Adzu et al. (2002) studied the effect of *Z. spina-christi* aqueous extract (100–200 mg/kg, i.p.) on the central nervous system in mice. It was observed that the aqueous extract of *Z. spina-christi* root bark may have some sedative activities, this is evident from the marked inhibition of the exploratory behavior in mice spontaneous motor activity (SMA) and prolonged sleeping time. These results suggest that the extract contained some constituents that depress the central nervous system. These findings correlate with those observed by Morishita et al. (1987) on the aqueous extract of Zizyphus seeds.

The protective and therapeutic effects of Zizyphus leaf extract against Pentylenetetrazol-induced seizure were clear in the present study. The extract improves the content of NE, DA and 5-HT in all brain regions compared with animal group that received pentylenetetrazol.

In 2006 Waggas reported that the daily i.p. injection of Sidr (*Z. spina-christi*) leaf extract (50 mg/kg BW) for 15 days and subsequent withdrawal caused a significant increase in epinephrine (E), norepinephrine (NE), dopamine (DA), serotonin (5-HT), 5-hydroxyindolacetic acid (5-HIAA) and gamma-aminobutyric acid (GABA) contents in different brain areas. This study suggested that the increase in neurotransmitter content in CNS areas may be due to the inhibition of calcium-ATPase and phosphodiesterase, at the same time inhibition of Ca2+/calmodulin binding which plays an important role in the release of these neurotransmitters, which may be due to the presence of peptide and cyclopeptide alkaloids in the extract. These agents share the ability to depress excitable tissue at all levels of the CNS, leading to a decrease in the amount of transmitter released by the nerve impulse, as well as to general depression of postsynaptic responsiveness and ion movement (Levin and Weiss, 1979; Bloom, 2001; Han et al., 2005).

In conclusion, the present study showed that the Pentylenetetrazol caused a decrease in NE, DA and 5-HT contents which can be minimized by Sidr (*Z. spina-christi*) which improves the contents of these neurotransmitters in different brain regions, this may be due to the presence of peptide and cyclopeptide alkaloids in the Sidr leaf extract.

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