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# Effect of Sidr (*Zizyphus spina-christi* ) Fruit Extract on the Central Nervous System in Male Albino Rats

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**Abstract:** Sidr (*Zizyphus spina-christiL* ) is medicinal plant with many traditional uses. The aim of the present study is to investigate the chronic effect of Sidr (*Zizyphus spina-christiL* ) fruit extract on norepinephrin (NE), dopamine (DA), serotonin (5-HT) and gamma-aminobutyric acid (GABA) content in different brain areas (cerebellum, brain stem, striatum, cerebral cortex, hypothalamus and hippocampus ) of male albino rat. The daily i.p. administration of Sidr fruit extract ( 50mg/kg body wt ) for 3 weeks caused a significant decrease in neurotransmitter content in all the tested brain areas at most of the time intervals studies. This may be to the presence of ascorbic acid which caused neurotransmitter release from presynaptic by enhanced Ca2+ influx and the content is decreased, also ascorbic acid increase in nitric oxide (NO) levels so the content of neurotransmitter is decreased.

**Key word:** Sidr · Zizuphus · Ascorbic acid · Brain · Neurotransmitter

## INTRODUCTION

*Zizyphus 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 [1].

Sidr has been used in folk medicine as demulcent, depurative, anodyne, emollient, stomachic for toothaches, astringents and as a mouth wash [2]. The decoction of bark and fresh fruits is used to promote the healing of fresh wounds and also used as a body wash, while fruits are used for dysentery [3,4].

Sidr is cultivated mainly as a dry crop for its nutritious fruits, honey production and landscaping purposes. Flowering and fruiting occur in this species during September-November. The flower are important for the production of wild bee honey [5]. The winter honey (i.e. nabk honey) collected during November from the flowers of the sidr is in high demand by 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. The dry fruit (i.e., per 100 g) contains 314 calories, 9.3% $H_2O$, 4.8% protein, 0.9% fat, 80.6% total carbohydrate, 4.4% ash, 140 mg Ca, 3 mg Fe, 0.04 mg thiamin, 0.13 mg riboflavin, 3.7 mg niacin and 30 mg ascorbic acid [2]. It is consumed fresh, dried and candied [6].

Mahran *et al.* [7] reported that the butanol extract of Z. *Spina-christi* leaves contains four saponin glycosides: christanin A (jujubagenin), christanin B, C and D. It was shown to contain beutic acid and ceanothic acid, cyclopeptides, as well as saponin glycoside and flavonoids lipids, protein, free sugar and mucilage [8].

Since *Zizyphus spina-christi (L)* is a wild tree commonly available in Saudi Arabia and its fruits are used in folk medicine for treatment, it is therefore deemed interesting to examine the effect of daily administration of *Zizyphus spina-christi (L)* fruits extract on norepinephrine (NE), dopamine (DA), Serotonin (5-HT) and gamma-aminobutyric acid (GABA) contents in different brain areas of male albino rats.

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## MATERIALS AND METHODS

The fruits of *Zizyphus spina-christi (L)* were collected from trees growing in Taif (Saudi Arabia) collected between September and November of 2008.

**Preparation of the Plant Extract:** Aqueous extract: *Zizyphus* fruit was extracted according to the method described by Adzu *et al.* [9]. The plant was dried under shade at 25°C and the dried leaves of 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 poured into it to give a final volume of 200 ml as reported previously [10].

**Animals:** Forty-eight 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 two groups.

The first group (n = 24) was divided into four subgroups each of 6 rats. The animals were daily injected (i.p.) with 50 mg/kg [11,12] of *Zizyphus spina-christi (L)* fruit extract and one subgroup was decapitated at the end of each week up to three weeks. To examine the withdrawal effect, the remaining one subgroups were decapitated after one week from the withdrawal of extract. The second group was divided as the first group, they were injected with saline vehicle and served as control.

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 [13] cerebellum, brain stem, striatum, cerebral cortex, hypothalamus and hippocampus. The brain tissues were wiped dry, weighed and wrapped into plastic films and quickly frozen in dry ice pending analysis. NE. DA and 5-HT were extracted and estimated according to the method of Chang [14] and modified by Ciarlone [15]. The GABA was estimated according to the method of Sutton and Simmondes [16]. The fluorescence was measured in Jenway 6200 fluorometer.

**Statistical Analysis:** SPSS for windows version 10.0 computer program was used for statistical analysis. All numeric data were expressed as mean ± SE. Data were analyzed using the student *t*-test to compare means before and after treatment. The changes between the means were considered significant when the value of <0.01 (17). All statically analysis were computed by SPSS version 14. Percentage difference is representing the percent of variation with respect to the control.

## RESULTS

Tables 1, 2, 3 and 4 illustrated norepinephrine (NE), dopamine (DA), serotonin (5-HT) and gamma aminobutyric acide (GABA) content (µg/g fresh tissue ) of brain regions (cerebellum, brainstem, striatum, cerebral cortex, hypothalamus and hippocampus) of *zizyphus Spina-christi* fruit -treated and withdrawal male albino rats respectively.

The single daily i.p injection of 50mg/kg of Zizyphus fruit extract significantly decreased the NE content at all time intervals except in striatum and hypothalamus after one and 2 weeks and in cerebral cortex after one week.After the withdrawal of the extract NE content returned to the normal values an all tested areas (Table 1).

Table 1: Effect of chronic administration of *zizyphus Spina-christi* fruit extract ( 50mg/kg i.p ) on norepinephrine (NE) content in different brain areas of albino rat

| Time of area Decapitation (week) | Cerebellum µg/g±S.E | Brain stem µg/g±S.E | Striatum µg/g±S.E | Cerebral cortex µg/g±S.E | Hypothalamus µg/g±S.E | Hippocampus µg/g±S.E |
|---|---|---|---|---|---|---|
| Treatment 1st % C | 306.91±9.13 | 157.31±12.41 | 719.83±8.59 | 107.64±0.82 | 650.72±7.57 | 613.66±9.34 |
| T | 220.00+8.22 | 120.74+7.12 | 680.90+32.09 | 105.37+1.02 | 670.29+11.15 | 441.73+13.99 |
| | -28.31* | -23.24* | -5.40 | -2.10 | 3.00 | -28.01* |
| 2nd % C | 282.64±16.49 | 171.22±7.67 | 717.18±9.16 | 107.32±1.46 | 658.80±12.91 | 611.32±5.23 |
| T | 163.37+8.17 | 112.93+5.19 | 655.75+10.61 | 69.21+1.41 | 608.80+16.59 | 543.63+3.49 |
| | -42.19* | -34.04* | -8.56 | -35.51* | -7.58 | -11.07* |
| 3rd % C | 290.93±3.98 | 174.55±7.88 | 746.95±15.18 | 101.03±2.08 | 653.59±13.23 | 616.94±6.55 |
| T | 157.58+7.35 | 108.44+2.22 | 570.32+9.22 | 64.84+2.24 | 436.15+7.58 | 430.23+7.26 |
| | -45.83* | -37.87* | -23.64* | -35.82* | -33.26* | -30.26* |
| Withdrawal 1st % C | 268.14±10.20 | 174.56±5.87 | 745.04±10.14 | 100.38±1.57 | 676.64±6.43 | 612.36±8.74 |
| T | 280.17+9.94 | 173.96+9.72 | 774.72+12.73 | 99.25+2.06 | 672.14+14.47 | 672.25+13.72 |
| | 4.48 | -0.34 | 3.98 | -1.12 | -0.66 | 9.78 |

Statistical analyses were performed between control (C=6) and treated (T=6) animals by using paired T' test. %: percentage of change from control.
* significant at P <0.01.

Table 2: Effect of chronic administration of *zizyphus Spina-christi* fruit extract ( 50mg/kg i.p ) on dopamine (DA) content in different brain areas of albino rat.

| Time of area Decapitation (week) | Cerebellum µg/g±S.E | Brain stem µg/g±S.E | Striatum µg/g±S.E | Cerebral cortex µg/g±S.E | Hypothalamus µg/g±S.E | Hippocampus µg/g±S.E |
|---|---|---|---|---|---|---|
| Treatment 1st % C | 301.10±4.22 | 175.18±7.65 | 733.85±8.91 | 105.17±1.19 | 665.18±9.60 | 621.86±6.78 |
| T | 225.50+8.52 | 180.97+4.74 | 800.30+12.99 | 106.82+1.67 | 676.76+12.51 | 681.26+10.73 |
| | -25.10* | 3.30 | 9.05 | 1.56 | 1.74 | 9.55 |
| 2nd % C | 304.17±5.30 | 159.51±9.70 | 730.09±10.50 | 105.98±0.99 | 702.51±4.97 | 646.18±10.92 |
| T | 173.33+10.96 | 111.44+3.10 | 621.34+9.77 | 67.54+1.33 | 508.56+14.07 | 526.17+14.64 |
| | -43.01* | -30.13* | -14.89* | -36.27* | -27.60* | -18.57* |
| 3rd % C | 287.84±4.40 | 159.00±10.57 | 747.65±11.55 | 101.03±2.08 | 660.13±16.28 | 608.27±7.54 |
| T | 141.89±8.50 | 85.98±2.90 | 494.68±11.87 | 62.34±0.81 | 388.87±10.38 | 410.90±10.63 |
| | -50.70* | -45.92* | -33.83* | -38.29* | -41.09* | -32.44* |
| Withdrawal 1st % C | 293.17±3.59 | 159.49±8.07 | 725.51±9.04 | 103.74±1.88 | 696.67±6.04 | 691.70±13.64 |
| T | 320.20±9.92 | 172.71±5.12 | 755.69±8.78 | 105.30±2.15 | 684.97±15.46 | 663.81±13.33 |
| | 7.51 | 8.28 | 4.15 | 1.50 | -1.67 | -4.03 |

Statistical analyses were performed between control (C=6) and treated ( T=6) animals by using paired T' test. % : percentage of change from control.
* significant at P <0.01.

Table 3: Effect of chronic administration of *zizyphus Spina-christi* fruit extract (50mg/kg i.p) on serotonin (5-HT) content in different brain areas of albino rat

| Time of area Decapitation (week) | Cerebellum µg/g±S.E | Brain stem µg/g±S.E | Striatum µg/g±S.E | Cerebral cortex µg/g±S.E | Hypothalamus µg/g±S.E | Hippocampus µg/g±S.E |
|---|---|---|---|---|---|---|
| Treatment 1st % C | 306.62±6.30 | 155.06±10.57 | 742.92±11.53 | 108.02±0.51 | 666.38±9.60 | 667.61±4.23 |
| T | 230.87+7.14 | 161.52+2.10 | 780.33+15.12 | 106.39+1.50 | 685.11+11.68 | 640.65+16.90 |
| | -24.70* | 4.16 | 5.03 | -1`.50 | 2.81 | -32.49* |
| 2nd % C | 312.14±9.72 | 155.49±10.13 | 742.01±12.04 | 106.31±1.16 | 676.85±7.79 | 641.67±11.50 |
| T | 190.69+8.08 | 120.14+0.06 | 546.35+10.33 | 60.69+1.04 | 376.39+4.27 | 331.16+8.62 |
| | -38.90* | -22.73* | -26.36* | -42.91* | -44.39* | -48.39* |
| 3rd % C | 302.08±9.30 | 140.41±6.11 | 726.31±6.72 | 105.75±0.93 | 659.93±10.17 | 648.40±10.16 |
| T | 164.23+7.30 | 111.58+2.38 | 580.28+11.86 | 66.10+1.35 | 446.11+14.98 | 491.44+16.45 |
| | -45.63* | -20.53* | -20.10* | -37.49* | -32.40* | -24.20* |
| Withdrawal 1st % C | 298.98±10.67 | 136.59±5.92 | 718.68±4.10 | 103.25±0.58 | 650.26±8.51 | 624.65±4.51 |
| T | 310.07+6.29 | 144.67+5.92 | 780.60+12.88 | 108.75+2.09 | 558.92+15.55 | 658.12+14.12 |
| | 3.70 | 5.91 | 8.61 | 5.32 | -14.04* | 5.35 |

Statistical analyses were performed between control (C=6) and treated ( T=6) animals by using paired T' test. % : percentage of change from control.
* Significant at P <0.01.

Table 4: Effect of chronic administration of *zizyphus Spina-christi* fruit extract (50mg/kg i.p) on gamma-aminobutyric acid (GABA) content in different brain areas of albino rat

| Time of area Decapitation (week) | Cerebellum µg/g±S.E | Brain stem µg/g±S.E | Striatum µg/g±S.E | Cerebral cortex µg/g±S.E | Hypothalamus µg/g±S.E | Hippocampus µg/g±S.E |
|---|---|---|---|---|---|---|
| Treatment 1st % C | 300.95±9.94 | 151.05±10.83 | 691.27±15.82 | 127.54±1.24 | 736.24±9.20 | 715.94±5.66 |
| T | 213.95±2.42 | 137.06±2.25 | 589.08±18.78 | 64.77±0.49 | 432.65±3.93 | 459.90±5.85 |
| | -28.90* | 13.99* | -14.78* | -49.21* | -41.23* | -35.76* |
| 2nd % C | 294.85±4.37 | 159.33±7.86 | 735.89±11.48 | 100.38±2.70 | 667.42±12.42 | 565.73±13.48 |
| T | 220.87±14.24 | 164.32±4.49 | 770.76±9.97 | 88.71±0.82 | 647.36±18.49 | 622.74±22.68 |
| | -25.09* | 3.13 | 4.73 | -11.62* | -3.00 | 10.07* |
| 3rd % C | 298.70±8.92 | 151.05±10.83 | 772.02±10.02 | 106.40±1.05 | 647.36±7.48 | 652.89±13.32 |
| T | 305.89±6.45 | 148.49±9.43 | 767.02±13.70 | 99.52±2.51 | 632.35±7.68 | 622.54±3.12 |
| | 2.40 | -1.69 | -0.64 | -6.40 | -2.31 | -4.64 |
| Withdrawal 1st % C | 307.26±6.45 | 164.33±9.34 | 758.18±7.90 | 105.43±1.60 | 708.24±5.66 | 667.16±18.71 |
| T | 315.43±7.93 | 177.09±9.75 | 773.63±10.41 | 112.18±0.61 | 650.13±16.09 | 656.46±6.93 |
| | 2.65 | 7.76 | 2.03 | 6.40 | -8.20 | -1.60 |

Statistical analyses were performed between control (C=6) and treated ( T=6) animals by using paired T' test. % : percentage of change from control.
* Significant at P <0.01.

Table 2 shows that 50mg/kg of *Zizyphus* fruit extract significant decrease in DA content in cerebellum after one week, whereas there was a significant decrease in DA content in all tested areas after 2 and 3 weeks. After the withdrawal of the extract NE content returned to the normal values an all tested areas (Table 2).

Results from table 3 shows that *Zizyphus* fruit extract caused, a significant decrease in 5-HT content in all tested areas after one, 2, 3 weeks except in : brain stem striatum cerebral cortex and hypothalamus after one week. The significant decrease in 5-HT content persisted for one week of the withdrawal in hypothalamus only (Table 3).

However, the treatment significantly decreased the GABA content in all tested areas after one except in brainstem. A significant decreased in GABA content was observed in cerebellum and cerebral cortex after 2 weeks and there was insignificant changes in all tested areas after 3weeks. GABA content returned to the normal level in all tested areas after the withdrawal (Table 4).

## DISCUSSION

*Zizyphus spina-christi* has several physiological and morphological characteristics [8]. Previous study shown that the crude aqueous extract of *Zizyphus spina-christi* exhibited anti-nociceptive potency with both central and peripheral effect [9].

Adzu *et al.* [18] studied the effect of *Zizyphus spina-christi* aqueous extract on central nervous system in mice. It was observed that the aqueous extract of *Zizyphus spina-christi* root bark may have some sedative activity.

From the present result it is clear that the daily injection of 50mg/kg (i.p) of *Zizyphus spina-christi* fruit extract caused a significant decrease in norepinephrine (NE), dopamine (DA), serotonin (5-HT) and gamma-aminobutyric acid (GABA) content in most of the tested brain areas at the different time intervals used.

Duke [2-19] reported that Zizyphus fruit is highly nutritious and rich in ascorbic acid (vitamin C). Ascorbic acid is antioxidant vitamin that the brain accumulates from the blood supply and maintains at relatively high concentration under widely varying conditions. Although neuron are known to use this vitamin in many different chemical and enzymatic reactions [20]. Various investigators [21,22,23] have previously demonstrated that ascorbic acid caused neurotransmitter release from presynaptic by enhanced $Ca^{2+}$ influx. The influx of $Ca^{+}$ promotes fusion between the axoplasmic membrane and vesicles. The content of the vesicles are discharged to the exterior by exocytosis [24,25].

Kytzia *et al.* [26] demonstrated that ascorbic acid induced release of nitric oxide. Nitric oxide (NO) is a short lived small molecule free redical produced from L-arganine, it has many biological functions involved in vasodilation, neurotransmission and tissue homeostasis [27]. N-methyl-D-aspartate (NMDA) receptor stimulation also causes neurotransmitter release through NO formation [28]. NMDA receptor stimulation is known to depolarization neuronal membrane and consequently to increase Ca2+ influx into neurons in association with opening of voltage-dependent Ca2+ channels. The increase in Ca2+ influx is assumed to contribute to the initiation of Ca2+ dependent exocytotic release of [29,30].

From the previous studies and present results it is clear that *Zizyphus spina-christi* fruit extract decrease neurotransmitter (NE, DA, 5-HT and GABA) in most of tested areas this may be due to the presence of scorbic acid which caused neurotransmitter release from presynaptic by enhanced Ca2+ influx and the content is decreased, also, ascorbic acid increase in nitric oxide (NO) levels so the content of neurotransmitter is decreased. It could be concluded that *Zizyphus spina-christi* fruit extract caused neurotransmitters release which is probably related to presence of ascorbic acid.

## REFERENCES


1. Mandavillae, J.P., 1990. Flora of Eastern Saudi Arabia. London: Kegan Paul International.
2. Duke, J.A., 1985. Handbook of Medical Herbs. Boca Raton: CRC Press.
3. Irvin, R.F., 1961. Woody plants of Ghana. Oxford University Press.
4. Blatter, E., 1978. Flora Arabica, Bishen Singh Mohendra, Pal Singh, Dehra Dun.
5. Ghazanfar, S.A., 1994. Handbook of Arabian medicinal plants. Boca Raton: CRC Press.
6. Bender, A. and A. Kumar, 1973. Economic Botany. Meerut, India : Educational Publishers.
7. Mahran, E.D.H., K.W. Glombitza, Y.M. Mirhom, R. Hartmann and C.G. Michel, 1996. Noval saponins from Zizyphus spina-christi growing in Egypt. Planta. Medica., 62(2): 163-165.
8. Adzu, B., S. Amos, M.B. Amizan and K. Gamaniel, 2003. Evaluation of the antidiarrhoeal effects of *Zizyphus spina-christi* stem bark in rats. Acta. Trop., 87(2): 245-250.
9. Adzu, B., S. Amos, C. Wambebe and K. Gamaniel, 2001. Antinociceptive activity of *Zizyphus spina-christi* root bark extract. *Fitoterapia*., 72(4): 344-350.

10. Abdel-Wahhab, M.A, E.A.Omara, M.M. Abdel-Galil, N.S. Hassan, S.A. Nada, A. Saeed and M.M. ElSayed, 2007. *Zizyphus spina-christi* extract against aflatoxin B1-intitiated hepatic carcinogenicity. Afr. J. Trad. Cam., 4(3): 248-256.
11. Waggas, A.M., 2006. Effect of sidr leaves extract on some neurotra nsmitter content in different brain areas of male albino rats. J. Egypt. Ger. Soc. Zool., 51(A): 297-313.
12. Adzu, B. and A.K. Haruna, 2007. Studies on the use of Zizyphus spina-christi against pain in rats and mice. African J. Biotachnol., 6(11): 1317-1324.
13. Glowinski, J. and L.L. Iversen, 1966. Regional studies of catecholamines in the rat brain I. The disposition of[$^3$H[ dopamine and ]$^3$H[ dopa in various regions of the brain. J. Neurochem., 13: 655-669.
14. Chang, C.C., 1964. A sensitive method for spectrofluorometric assay of catecholamines. Int. J. Neuropharmacol., 4: 643 -649.
15. Ciarlone, A.E., 1978. Further modification of afluoremetric method for analyzing brain amines. Microchem. J., 23: 9-12.
16. Sutton, I. and M.A. Simmodes, 1973. Effect of acute and chronic pentabarbiton on the gamma aminobutyric acid system in rat brain. Biochem. Pharmacol., 23: 1801-1808.
17. Woolson, R.F., 1987. Statistical Method for the Analysis of Biomedical and Mathematical statistics, John Wiley and Sons Inc., New York, pp: 316.
18. Adzu, B., S. Amos, S. Dzma, C. Wambebe and K. Gamanile, 2002. Effect of *Zizyphus spina-christi* wild aqueous extract on the central nervous system in mice. J. Ethnopharmacol., 79(1): 3-16.
19. Keay, R.M.J., C.F.A. Onochie and D.P. Stanfied, 1964. Nigerian trees. Vol II. Department of Forestry, Ibadan, Nigeria, pp: 225-226.
20. Rebec, G.V. and R. Pierce, 1994. A vitamine as neuromodulator: ascorbate release into the extracellular fluid of the brain regulates dopaminergic and glutamatergic transmission. Prog. Neurobiol., 43(6): 537-565.
21. Girbe, F., C. Ramassamy, C. Piton and J. Costentin, 1994. Ascorbic acid increase synaptosomal potassium-induced dopamine release. Neuroreport, 5(9): 1027-1029.
22. Serra, P.A., R. Migheli, G. Rocchitta, M.G. Teras, M.P. Mura, M.R. Delogu, G. Esposito, M.S. Desole, E. Miele and M. Miele, 2003. Role of the nitric oxide/cyclic GMP pathway and ascorbic acid in 3-mprpholinosydonimine (SIN-1)-induced increases in dopamine secretion from PC12 cell. A microdialysis *in vitro* study. Neurosci. Lett., 353(1): 5-8.
23. Dasgupta, J., R.A. Elliott and D.G. Tincello, 2009. Modification of rat detrusor muscle contraction by ascorbic acid and citric acid involving enhanced neurotransmitter release and $Ca^{2+}$ influx. Neurourol Urodyn, 28(6): 542-548.
24. Hille, B., J. Billiard, D.F. Babcock, T. Nguyen and D.S. Koh, 1999. Stimulation of exocytosis without a calcium signal. J. Physiol., 520(1): 23-31.
25. Meir, A., S.A. Ginsburg, I. Butekevich, R. Kaiserman, S. Ahdut, S. Demirgoren and R. Rahamimoff, 1999. Ion channel in presynaptic nerve terminals and control of transmitter release. Physiol. Rev., 79: 1019-1088.
26. Kytzia, A., H.G. Korth, R. Sustman, H. de Groot and M. Kirsch, 2006. On the mechanism of the ascorbic acid-induced release of nitric oxide from N-nitrosated tryptophan derivatives: scavenging of NO by ascorbyl radicals. Chemistry, 12(34): 8786-8797.
27. Montaue, P.R., C.D. Gancayco, M.J. Winn, R.B. Marchase and M.J. Friedlander, 1994. Role of NO production in NMDA receptor - mediated neurotransmitter release in cerebral cortex. Sci., 263: 973-977.
28. Kurlyama, K. and S. Ohkuma, 1995. Role of nitric oxide in central synaptic transmission: effects of neurotransmitter release. Japanese J. Pharmacol., 69(1): 1-8.
29. Nathan, C. and Q.W. Xie, 1994. Regulation of biosynthesis of nitric oxide. J. Biol. Chem., 269: 13725-13728.
30. Silva, J.M. and D.L. Lewis, 2002. Nitric oxide enhances $Ca^{2+}$-dependent $K^+$ channel activity in rat carotid body cells. Eur. J. Physiol., 443: 671-675.