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## **Royal Jelly Pretreatment Can Either Protect or Aggravate Brain Damage Induced by Hypoxia-Ischemia in Mice, Depending on its Dose**

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### **Abstract**

Royal jelly is a bee-derived product with potent antioxidative and many other biological properties. The present study was designed to examine the effect of Royal jelly (RJ) on brain damage induced by hypoxia-ischemia in mice. Mice were given RJ orally one dose / day (0-300 mg/kg body wt./ day for 13 consecutive days, survival time of mice were recorded following decapitation (Total brain ischemia model; model<sub>-1</sub>) and i. p. injection of NaNO<sub>2</sub> to induce brain hypoxia (model<sub>-2</sub>). RJ in the dose of 75 mg/kg body wt. /day for 13 consecutive days (RJ<sub>75</sub>) showed no significant effect in both experimental models compared to control animals. However, the doses of 150mg/kg body wet/day (RJ<sub>150</sub>) and 300mg/kg body wet. /day (R<sub>300</sub>) showed potent pharmacological activity in both experimental models. In model<sub>-1</sub> the survival time of mice by decapitation was significantly (p<0.001) prolonged after administration of RJ<sub>150</sub>, but was significantly (p<0.001) shortened after administration of RJ<sub>300</sub> compared to control groups. However, in model<sub>-2</sub>, 3 out of 10 mice with RJ<sub>150</sub> survived after i.p. NaNO<sub>2</sub>-induced hypoxia, an incident which was not observed in control mice that received only i.p. NaNO<sub>2</sub>. The RJ<sub>150</sub>-treated animals that died showed a significant (p<0.001) prolongation in survival time after NaNO<sub>2</sub>-induced hypoxia compared to control animals.

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On the other hand, the R<sub>300</sub> treated animals showed a significantly ( $p < 0.001$ ) shortened of survival time after i.p. NaNO<sub>2</sub>-induced hypoxia compared to control group. Taken together; the results of this study shows that RJ may protect or aggravate brain damage induced by hypoxia-ischemia in mice, depending on its dose.

**Keywords:** Royal jelly; brain hypoxia; brain ischemia; sodium dinitrate; mice.

## **1. Introduction**

The brain is extremely sensitive to both reductions in blood flow and deficiency in the amount of oxygen supply i.e. the brain significantly depends on uninterrupted delivery of oxygen and glucose, more than any other organ [1]. This is due to brain's high-energy demand, together with its incomplete ability to store energy [2]. The major pathogenic mechanism causing neurological injury resulting from ischemia and/or hypoxia is due to the deficiency of the glucose and oxygen supply to the brain, initiating a cascade of biochemical events leading to cell dysfunction and eventually to cell death [3,4]. Accumulating evidences show that oxidative stress, which is an imbalance between free radicals and antioxidant system, is the main biochemical event leading to brain damage [5, 6, 7]. Enhanced formation of reactive oxygen species (ROS) reduces membrane fluidity through lipid peroxidation resulting in cell injury [8]. As oxidative stress is considered to be an early feature in the procedure of brain damage after ischemia or hypoxia, this makes it attractive target for neuroprotective strategies [9]. Therefore, inhibition of free radical damage with antioxidants and free radical scavengers could be a viable intervention to protect neuronal damage after ischemia and hypoxia [5]. These antioxidants and free radical scavengers can be either synthetic or natural ones. However, it has been showed that inhibition of the formation of reactive oxygen species by synthetic antioxidants is not always completes [9, 10, 11, 12]. In addition, antioxidant must be safe and nontoxic, and must easily penetrate the blood–brain barrier.

Royal jelly (RJ), a bee-derived product, posses potent antioxidative properties. The antioxidant actions of this product have been extensively verified, and previous studies have revealed that RJ can ameliorate a variety of disease processes in different model [13, 14, 15, 16, 17, 18, 19]. RJ inhibits lipid peroxidation and protein oxidation by acting as a scavenger [14, 15, 16, 20]. To date, no study has investigated the neuroprotective effects of RJ in models of ischemia or hypoxia-induced brain damage. Therefore, this study was carried out to determine the neuroprotective effects of RJ in two experimental models i.e. complete brain ischemia and brain hypoxia induced by NaNO<sub>2</sub>.

## **2. Materials and Methods**

### **2.1. Materials**

Egyptian RJ was purchased from Wadii Alnaheel Company, Hail, Saudi Arabia, and was stored at -20°C until used. Sodium dinitrate was obtained from Sigma (St. Louis, MO, USA). Male Swiss albino mice, 20-25 g, were obtained from King Saud University, Riyadh, Saudi Arabia. The animals were housed in colony cages with free access to standard commercial food and tap water. The experiments started after 7- day acclimatization to standardized laboratory conditions (22-24°C and a 12-hour light/dark cycle). The protocol of this study was

approved by the ethics board of animal experiments, University of Hail. All experimental procedures were conducted in accordance with the guidance of care and use of laboratory animals.

**2.2. Complete brain ischemia & Brain hypoxia induced by NaNO<sub>2</sub>.**

The method described by Xiao-Dong et al was to induce complete brain ischemia & brain hypoxia [21]. Mice were randomly assigned to 4 main groups consisting of 20 animals each according to their experimental treatment. The three experimental groups (RJ<sub>300</sub>, RJ<sub>150</sub> and RJ<sub>75</sub>) received royal jelly dissolved in normal saline at 300, 150, or 75 mg/kg/day orally respectively. The control group received only normal saline. This procedure was repeated for 13 days. Standard diet was given to during this period but no food was allowed to the mice during night (a period of 12 hours), only water was allowed. Twenty –four hours after the final royal jelly and normal saline treatment, every group was divided into two subgroups A and B consisting of 10 mice each. All animals of A subgroups were killed by decapitation to induce complete brain ischemia and the time from decapitation to breath stop was recorded. The animals of B subgroups were given a single dose of NaNO<sub>2</sub> (250 mg/kg) i.p. to induce brain hypoxia and the survival time of the mouse was recorded.

**3. Results**

None of the animals in the control and experimental groups died during the experiment. The effects of RJ on mouse complete brain ischemia are presented in Fig.1. The effects of this substance on mouse brain hypoxia are shown in Fig.2.

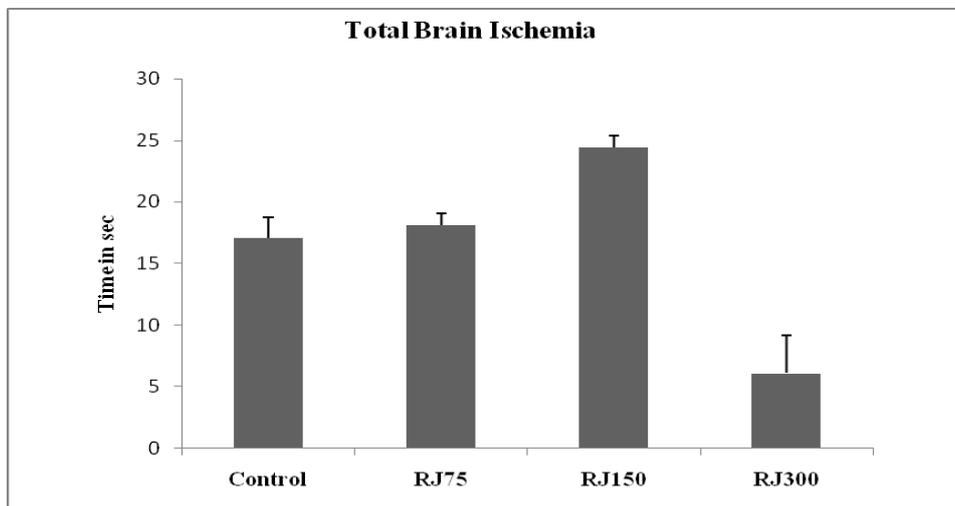


Fig.1. Effects of RJ on the survival time (in seconds) of mice following decapitation

**3.1. Effects of RJ on mouse complete brain ischemia induced by decapitation**

In order to test the effects of RJ pre-treatments against complete brain ischemia induced by decapitation, RJ was administered to mice in three increasing doses (75mg/day, 150mg/day, and 300mg/day) for 13 consecutive days

[21]. As shown in Fig.1 RJ in the dose of 75mg/kg/day prolonged the duration of breathe of mouse from 17.1 seconds (for control group) to 18.1 seconds, which was not statistically significant. The dose of RJ of 150mg/kg/day resulted in the significant ( $P < 0.001$ ) prolonged this value to 24.4 seconds. In contrast, RJ in the dose of 300 mg/kg/day leads to significant ( $P < 0.001$ ) shorten of the duration of breath of mouse to 6.1 seconds.

### 3.2. Effects of RJ on mouse brain hypoxia induced by $\text{NaNO}_2$

Figure. 2. shows effects of RJ pre-treatments against brain-hypoxia induced by  $\text{NaNO}_2$  in royal jelly treated mice. RJ in the dose of 75mg/kg/day for 13 consecutive days led to prolongation of the survival time of mouse after injection of  $\text{NaNO}_2$ , form 32.86 minutes (for control group) to 33.4 minutes , which was not statistically significant. The dose of 150mg/kg/day RJ protected 3 animals out of 10 from death. The dead animals of this group showed significant ( $P < 0.001$ ) prolongation of survival time to 39.3 minutes. Conversely, RJ in the dose of 300 mg/kg/day resulted in significant ( $P < 0.001$ ) shortening of survival time of the animals after giving the  $\text{NaNO}_2$  to 26.0 minutes.

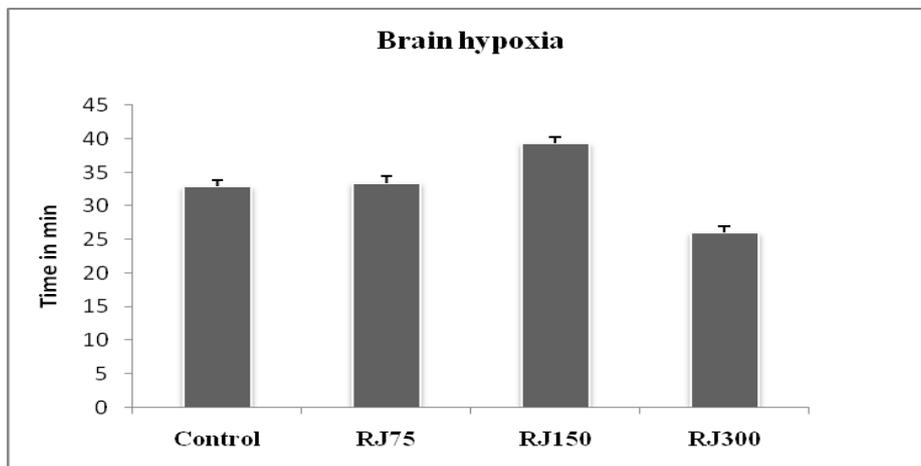


Fig.2. Effects of RJ on the survival time (in minutes) of mice following i.p.  $\text{NaNO}_2$

### 3.3. Statistical analyses

The SPSS version 15 was used for data analysis. Data were expressed as mean  $\pm$  SD. Mean values were compared using unpaired t-test. P-values less than 0.05 were considered significant.

## 4. Discussion

The current study revealed the neuroprotective effects of RJ, which was given to the mice in the dose of 150mg/kg/day for 13 consecutive days, against brain damage caused by both decapitation induced ischemia as well as sodium nitrite induced hypoxia. To my knowledge, this effect has not been previously observed. These findings indicated that RJ pretreatment increased survival rates of animals after hypoxia as well as after ischemia, demonstrating that RJ may be a promising agent to prevent hypoxia-ischemia brain damage in patients in a clinical setting. These findings may have extreme importance, because ischemic damage is main

cause of death and disability over the world and, there is no definite reasonable and effective treatment for this disorder yet [22, 23].

These results agree with recent findings that RJ in the dose of 50 mg/kg per day for 7 days protected radiation-induced brain damages in rats [24]. The results of this study are also in concordance with the previous report which described the neuroprotective effect of 100 mg/ kg RJ on traumatic spinal cord injury- induced neuronal damage in rabbits [25].

A surprising observation in the present study was the detection of harmful effect of RJ in the dose of 300mg/kg/day for 13 consecutive days, against brain damage for both ischemic and hypoxic animals. It turned out that this dose of RJ significantly shortened survival rates of both ischemic and hypoxic animals. However, it is known from the literature that the toxic dose of RJ is 3000mg/kg/day in mice and rats [30]. Moreover, RJ in the dose of 300 mg/kg/day or more showed anti oxidative effects and thus, many benefit protective effects in other organs than brain [17, 26, 27, 28, 29], despite the fact that the main constituents of royal jelly (water, protein, sugars, lipids and mineral salts) are relatively constant when comparing different colonies, bee races and time (Although they occur with notable variations) [30]. Therefore, further investigations are needed to determine safe doses of this product when used clinically in those suffering from ischemic and/or hypoxic disorders. The finding of this study that RJ exhibits neuroprotective may be attributed to antioxidant and radical scavenging activity of this product. On the other hand, namely the deleterious effect of RJ against ischemic and hypoxic brain at 300 mg /kg body wet. /day cannot be easily explained based on the available literature data. RJ has been reported to aid the differentiation of all types of brain cells including neurons from cultured neural stem/progenitor cells [31] and facilitate *in vivo* neurogenesis in the hippocampal dentate gyrus [32]. In addition, it has been demonstrated that RJ may play neurotrophic and/or neuroprotective roles in the adult mouse brain via expression of glial cell line-derived neurotrophic factor, a potent neurotrophic factor acting in the brain [31, 33, 34]. RJ has also been shown to assist restoration of the cognitive ability in trimethyltin-intoxicated mice [32]. Thus, RJ may protect brain damage-induced by ischemia and/or hypoxia through multiple mechanisms.

The deleterious effect of chronic pretreatment of animals with RJ300 may involve many mechanisms. It has been shown that acidosis is one of the factors that aggravate ischemic brain injury during cerebral ischemia [35, 36]. Results from other studies in the rat and mice stroke models induced by transient middle cerebral artery occlusion proposed that activation of acid-sensing channels (ASICs) during cerebral ischemia contributed to ischemic brain injury [37, 4]. Activation of the ASICs by the lower extracellular pH as a result of acidosis allows influx of calcium ions through the channels into the neuronal cells; the intracellular calcium-overload induces cell injury and cell death [4, 38]. Meanwhile, the pH of RJ is 3.6 to 4.2 [30].

Therefore, aggravation of brain damage induced by large doses of RJ pretreatment could be ascribed to acidosis of extracellular fluid due to RJ acidity. In view of the above, it may be hypothesized that RJ (or its more acidic components) in brain possess two opposite mechanisms i.e.; neuroprotective effects via activation of the antioxidant way (and other mechanisms) along with enhanced neuronal damage mechanism through decreasing the pH of the extracellular fluid. The higher the RJ dose the lower the pH in brain extracellular fluid, the more predominate the second harmful mechanism.

## 5. Conclusion

Based on the findings of this study it may be suggested that RJ possesses opposite effects depending on its dose, i.e. either protect or aggravate brain damage induced by hypoxia-ischemia in mice. Further studies on these effects of RJ may help in designing optimal effective and safe doses for better therapeutic regimes for both ischemia and hypoxia injury. Additional investigations are also of utmost importance to find out the mechanism of detrimental action of large doses of RJ against brain damage induced by ischemia and/or hypoxia.

## References

- [1] Sharp, F.R., Bernaudin, M. "HIF1 and oxygen sensing in the brain". *Nature Reviews Neuroscience*, vol. 5, pp.437-448, 2004.
- [2] Zaidi, Z.F. "Sodium nitrite-induced hypoxic injury in rat hippocampus". *Pakistan Journal of Medical Sciences*, vol. 26, pp. 532-537, 2010.
- [3] Perlman, J.M. "Summary proceedings from the neurology group on hypoxic- ischemic encephalopathy". *Pediatrics*, Vol.117, pp. 28–33, 2006.
- [4] Tai, K.K., Truong, D.D. "Amiloride but not memantine reduces neurodegeneration, seizures and myoclonic jerks in rats with cardiac arrest-induced global cerebral hypoxia and reperfusion". *PLoS ONE*, DOI: 10.1371/journal.pone.0060309, 2013.
- [5] Chen. H., Yoshioka, H., Kim, G.S., Jung, J.E., Okami, N., Sakata H, Maier CM, Narasimhan P, Goeders CE, Chan PH. (, Apr. "Oxidative stress in ischemic brain damage: mechanisms of cell death and potential molecular targets for neuroprotection". *Antioxid Redox Signal*, vol.14, pp.1505-1517, 2011
- [6] Li, Q., Bi, M.J., Bi, W.K., Kang, H., Yan, L.J., Guo. Y.L. (2014, Oct.). "Edaravone attenuates brain damage in rats after acute CO poisoning through inhibiting apoptosis and oxidative stress". *Environmental Toxicology*. doi: 10.1002/tox.22052. Available: <http://onlinelibrary.wiley.com/doi/10.1002/tox.22052/abstract> [Jan.10, 2015].
- [7] Tonni, G., Leoncini, S., Signorini, C., Ciccoli, L.D.e., Felice, C. "Pathology of perinatal brain damage: background and oxidative stress markers". *Archives of Gynecology and Obstetrics*, vol. 290, pp. 13-20, 2014.
- [8] Coban, J., Doğan-Ekici, I., Aydın, A.F., Betül-Kalaz, E., Doğru-Abbasoğlu, S., Uysal, M. "Blueberry treatment decreased D-galactose-induced oxidative stress and brain damage in rats". *Metabolic Brain Disease*. Doi: 10.1007/s11011-014-9643-z, 2014.
- [9] Zhang, X., Song, L., Cheng, X., Yang, Y.d., Luan, B., Jia, L., Xu, et al. "Carnosine pretreatment protects against hypoxia–ischemia brain damage in the neonatal rat model". *The European Journal of Pharmacology*, vol. 667, pp. 202-207, 2011.

- [10] Ikeda, K., Negishi, H., Yamori, Y. "Antioxidant nutrients and hypoxia/ischemia brain injury in rodents" *Toxicology*, vol.189, pp. 55–61, 2003
- [11] Papaharalambus, C.A., Griendling, K.K.. "Basic mechanisms of oxidative stress and reactive oxygen species in cardiovascular injury". *Trends in Cardiovascular Medicine*, vol. 17, pp. 48-54, 2007.
- [12] Ahmet, I., Spangler, E., Shukitt-Hale, B., Juhaszova, M., Sollott, S.J., Joseph, J.A., et al. "Blueberry-Enriched Diet Protects Rat Heart from Ischemic Damage". *PLoS ONE*, DOI: 10.1371/journal.pone.0005954, 2009.
- [13] Jamnik, P., Goranovic, D., Raspor, P. "Antioxidative action of royal jelly in the yeast cell". *Experimental Gerontology*, vol. 42, pp.594–600, 2007
- [14] Inoue, S.-I., Koya-Miyata, S., Ushio, S., Iwaki, K., Ikeda, M., Kurimoto, M. "Royal Jelly prolongs the life span of C3H/HeJ mice: correlation with reduced DNA damage". *Experimental Gerontology*, vol.38, pp. 965–969, 2003
- [15] El-Nekeety, A.A., El-Kholy, W., Abbas, N.F., Ebaid, A., Amra, H.A., Abdel-Wahhab, M.A. "Efficacy of royal jelly against the oxidative stress of fumonisin in rats". *Toxicon*, vol.50, pp.256–269, 2007.
- [16] Silici, S., Ekmekcioglu, O., Eraslan, G., Demirtas, A. "Antioxidative effect of royal jelly in cisplatin-induced testes damage.". *Urology*.vol.74, pp.545-551, 2009
- [17] Tamura, T., Fujii, A., Kumoyama, N. "Antitumor effect of royal jelly" *Nihon Yakuriqaku Zasshi*, vol. 89, pp.73–80, 1987.
- [18] Sver, L., Orsolich, N., Tadic, Z., Njari, B., Valpotic, I., Basic, I. "A royal jelly as a new potential immunomodulator in rats and mice". *Comparative Immunology, Microbiology and Infectious Diseases*, vol. 19, pp. 31–38, 1996.
- [19] Pyrzanowska, J., Piechal, A., Blecharz-Klin, K., Joniec-Maciejak, I., Graikou, K., Chinou, I., et al. "Long-term administration of Greek royal Jelly improves spatial memory and influences the concentration of brain neurotransmitters in naturally aged wistar male rats". *Journal of Ethnopharmacology*, vol.155, pp.343-51, 2014.
- [20] Guo., H., Ekusa, A., Iwai, K., Yonekura, M., Takahata, Y., Morimatsu, F. "Royal jelly peptides inhibit lipid peroxidation in vitro and in vivo". *Journal of Nutritional Science and vitaminology*, vol. 54, pp. 191-5, July. 2008.
- [21] Xiao-Dong, L., Guo-Yu, P., Lin, X., Yuan-Yuan, H., Wen, L., Qing, S.U., et al. "Cyclosporin A enhanced protection of nimodipine against brain damage induced by hypoxia-ischemia in mice and rats". *Acta Pharmacologica Sinica*, vol.23, pp. 225-229, Mar. 2002.

- [22] Ansari, S., Azari, H., Caldwell, K. J., Regenhardt, R. W., Hedna, V. S., Waters, M. F., *et al.* (2013). "Endothelin-1 Induced Middle Cerebral Artery Occlusion Model for Ischemic Stroke with Laser Doppler Flowmetry Guidance in Rat". *Journal of Visualized Experiments*. doi:10.3791/50014. Available: <http://www.jove.com/video/50014/endothelin-1-induced-middle-cerebral-artery-occlusion-model-for> [Jan. 13, 2015].
- [23] Tran J, Mirzaei M, Anderson L, Leeder S.R. "The epidemiology of stroke in the middle east and north africa". *Journal of the Neurological Sciences*, vol. 95, pp. 38-40, 2010.
- [24] Yasemin Benderli, C.H., Vedat, A., Erkan, G.C. "Royal Jelly in the prevention of radiation-induced brain damages". *Journal of the Neurological Sciences*, vol. 28, pp.475-486, 2011.
- [25] Aslan, A., Cemek, M., Buyukokuroglu, M.E., Altunbas, K., Bas, O., Yurumez, Y. "Royal jelly can diminish secondary neuronal damage after experimental spinal cord injury in rabbits". *Food and Chemical Toxicology*, vol. 50, pp. 2554-2559, 2012.
- [26] Karadeniz, A., Simsek, N., Karakus, E., Yildirim, S., Kara, A., Can, I., *et al.* "Royal jelly modulates oxidative stress and apoptosis in liver and kidneys of rats treated with cisplatin". *Oxidative medicine and cellular longevity*, vol. 2011, pp.1-10, 2011.
- [27] Ashry, K., Elkady, A.A. "Royal jelly modulates hepatotoxic effect of rats treated with cisplatin". *Arab Journal of Nuclear Science and Applications*, vol. 47, pp.172-180, 2014.
- [28] Hassan AA. "Effect of royal jelly on sexual efficiency in adult male rats". *Iraqi Journal of Veterinary Medicine*, vol. 23, pp.155-160, 2009.
- [29] Azab, K.S., Bashandy, M., Salem. M., Ahmed, O., Tawfik, Z., Helal, H. "Royal jelly modulates oxidative stress and tissue injury in gamma irradiated male wister albino rats." *North American Journal of Medical Sciences*, vol.3, pp. 268-76, 2011.
- [30] Krell, R. 1996. Value-added products from beekeeping. ( 1<sup>st</sup> ). [On-line]. Vol. (124) <http://www.blackburnbeekeepers.com/Royal%20Jelly%20Book.pdf> [ Dec 25, 2014 ].
- [31] Hashimoto, M., Kanda, M., Ikeno, K., Hayashi, Y., Nakamura, T., Ogawa, Y., *et al.* "Oral administration of royal jelly facilitates mRNA expression of glial cell line-derived neurotrophic factor and neurofilament H in the hippocampus of the adult mouse brain". *Bioscience, Biotechnology, and Biochemistry*, Vol. 69, pp.800-805, 2005.
- [32] Hattori, N., Ohta, S., Sakamoto, T., Mishima, S., Furukawa, S. "Royal jelly facilitates restoration of the cognitive ability in trimethyltin-intoxicated mice" *Evidence-Based Complementary and Alternative Medicine*, vol.11, pp.1-6, 2009.

[33] Wang, J., Yang, Z., Liu, C., Zhao, Y., Chen, Y. "Activated microglia provide a neuroprotective role by balancing glial cell-line derived neurotrophic factor and tumor necrosis factor- $\alpha$  secretion after subacute cerebral ischemia". *International Journal of Molecular Medicine*, vol. 31, pp.172-178, 2013.

[34] Yang, C., Zhou, L., Gao, X., Chen, B., Tu, J., Sun, H., et al. "Neuroprotective effects of bone marrow stem cells overexpressing glial cell line-derived neurotrophic factor on rats with intracerebral hemorrhage and neurons exposed to hypoxia/reoxygenation". *Neurosurgery*, vol. 68, pp. 691-704, 2011.

[35] Wang, Y.Z., Xu, T.L. "Acidosis, acid-sensing ion channels, and neuronal cell death". *Molecular Neurobiology*, vol. 44, pp. 350-8, 2011.

[36] Xiong, Z.G., Zhu, X.M., Chu, X.P., Minami, M., Hey, J., Wei, W.L., et al. "Neuroprotection in ischemia: blocking calcium-permeable acid-sensing ion channels" *Cell*, vol. 118, pp. 687–698, 2004.

[37] Duan, B., Wang, Y.Z., Yang, T., Chu, X.P., Yu, Y., Huang, Y. "Extracellular spermine exacerbates ischemic neuronal injury through sensitization of ASIC1a channels to extracellular acidosis". *The Journal of Neuroscience*, vol. 31, pp. 2101–2112, 2011.

[38] Zeng, W.Z., Liu, D.S., Duan, B., Song, X.L., Wang, X., Wei, D., et al. "Molecular mechanism of constitutive endocytosis of acid-sensing ion channel 1a and Its protective function in acidosis-induced neuron death". *The Journal of Neuroscience*, vol. 33, pp.7066–7078, 2013.