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Research Article

Impact of Pheophytin A, L-Carnitine and Melatonin on Serum Levels of Malondialdehyde, Superoxide Dismutase and Glutathione Peroxidase in Rats with Cerebral Ischemia/Reperfusion Injury

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Abstract

Background and Objective: Cerebral ischemia/reperfusion (I/R) injury is a complex pathological process where the temporary interruption and subsequent restoration of blood flow to the brain cause significant oxidative stress, exacerbating neuronal damage. This study aims to investigate the neuroprotective effects of pheophytin A, L-carnitine and melatonin using a rat model for cerebral ischemia/reperfusion-induced injury, focusing on their influence on oxidative stress markers, specifically malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GPx). **Materials and Methods:** Thirty-six male albino rats were assigned to six groups: Control, sham-operated, saline pretreated ischemia/reperfusion (I/R) and pheophytin A, L-carnitine, or melatonin pretreated I/R groups. The experimental procedure involved anesthetizing animals with thiopental sodium and performing bilateral occlusion of the common carotid arteries to induce ischemia for 30 min, followed by reperfusion for 4 hrs. Blood samples were collected, centrifuged to separate serum and then diluted 1000-fold for analysis. Serum levels of SOD, GPx and MDA, were measured post-surgery using ELISA kits, with absorbance readings taken at 450 nm after several incubation and washing steps. Data were analyzed using one-way ANOVA and *post hoc* test in IBM SPSS v28, with statistical significance set at $p < 0.05$. **Results:** Pheophytin A, L-carnitine and melatonin effectively protect against oxidative stress induced by cerebral ischemia/reperfusion (I/R) injury. Pre-treatment with these compounds led to significant increases in antioxidant enzyme levels (SOD and GPx) and a notable reduction in MDA levels. **Conclusion:** These findings suggest that all three compounds exhibit similar protective effects against oxidative stress in I/R injury, highlighting their potential as therapeutic strategies for ischemic brain disorders.

Key words: Pheophytin A, L-carnitine, melatonin, brain ischemia and reperfusion injury

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cerebral ischemia/reperfusion injury occurs when the blood supply to the brain is reduced and subsequently restored. This phenomenon is a major contributor to neurological disorders such as stroke and traumatic brain injury¹. While the restoration of blood flow is crucial for tissue survival, sudden reoxygenation of tissues after ischemia paradoxically leads to the production of reactive oxygen species and the elevation of oxidative stress. This oxidative damage significantly worsens neuronal injury and is associated with various neurological complications, including neuronal death, blood-brain barrier disruption and inflammation. Therefore, understanding and mitigating the molecular consequences of ischemia/reperfusion injury is crucial for developing effective therapeutic strategies aimed at reducing brain damage.

Oxidative stress is a central feature of I/R injury and arises due to a disruption between ROS production and the body's antioxidant defenses. The ROS including superoxide anion ($O_2^{\bullet-}$) and hydroxyl radicals ($\bullet OH$), are highly reactive species that can damage lipids, proteins and DNA. The Malondialdehyde (MDA), the byproduct of lipid peroxidation, is commonly used as a marker for oxidative stress. In response to this, the body activates the enzymes with antioxidant properties like superoxide dismutase (SOD) and glutathione peroxidase (GPx) thus neutralizing ROS and preventing cellular damage. However, in I/R injury, the antioxidant defenses are often overwhelmed, leading to exacerbated oxidative damage and tissue injury².

The development of therapeutic interventions that can enhance the body's antioxidant defenses is of great importance in reducing the damaging effects of I/R injury. Several natural and synthetic compounds have been explored for their potential neuroprotective properties, including L-carnitine, melatonin and pheophytin A. These compounds have shown promise in protecting against oxidative stress, improving mitochondrial function and reducing inflammatory responses, all of which play crucial roles in cellular survival following ischemic events.

The L-carnitine is a naturally occurring substance essential for fatty acid metabolism, as it aids in the transport of long-chain fatty acids to mitochondria for oxidative reactions. Beyond its metabolic functions, L-carnitine has been shown to exhibit antioxidant properties and neuroprotective effects in various models of neurological injury. In particular, L-carnitine has demonstrated the ability to reduce oxidative stress and mitigate mitochondrial dysfunction, which are central to the pathogenesis of cerebral I/R injury. Previous research indicated

that L-carnitine supplementation might enhance antioxidant enzyme activity, improve mitochondrial function and decrease lipid peroxidation, thereby offering neuroprotection in conditions like stroke and traumatic brain injury³. The neuroprotective potential of L-carnitine, especially in the context of I/R injury, has generated significant interest and warrants further exploration.

The Melatonin, a hormone mainly produced by the pineal gland, is widely recognized for its role in regulating circadian rhythms. However, melatonin also exhibits potent antioxidant properties, acting as a free radical scavenger and modulating various cellular processes, including mitochondrial function, apoptosis and inflammation. Several studies indicated the neuroprotective actions of melatonin in cerebral ischemia, attributing these effects to its ability to neutralize ROS, restore antioxidant enzyme activity and improve mitochondrial health. Melatonin has also been shown to reduce the serum concentrations of MDA, a marker of lipid peroxidation, in I/R injury models. Given its ability to modulate oxidative stress, melatonin presents as a promising candidate for therapeutic intervention in ischemic conditions⁴.

The Pheophytin A, a chlorophyll derivative novel antioxidant compound, has recently gained attention for its antioxidant properties. Unlike other common antioxidants, pheophytin A is capable of directly scavenging ROS and stabilizing cellular membranes. Studies have suggested that pheophytin A may reduce oxidative damage in neurodegenerative diseases and brain injuries by mitigating mitochondrial dysfunction and enhancing cellular antioxidant defenses. In particular, its effects on mitochondrial protection make it a valuable compound to investigate in ischemia-reperfusion damage, where mitochondrial dysfunction is a key factor in the injury cascade. Though research on pheophytin A in ischemic conditions is still in its early stages, preliminary data suggest that it holds promise as a neuroprotective agent⁵.

The objective of this research was to examine the effects of pheophytin A, L-carnitine and melatonin on oxidative stress markers-specifically MDA, SOD and GPx in rats exposed to cerebral I/R injury. Given the established roles of L-carnitine and melatonin in modulating oxidative stress and protecting against mitochondrial dysfunction, this research aims to determine whether pre-treatment with these compounds can improve the antioxidant response and reduce oxidative damage in the brain following ischemic injury and whether the novel compound pheophytin A has a protective role in ischemic injury. The study also aims to compare the effect of the three compounds on oxidative stress markers MDA, SOD and Gpx.

MATERIALS AND METHODS

Study location: The research was conducted over the period from September, 2019 to November, 2020 at the Department of Pharmacology and Toxicology, College of Pharmacy, Qassim University, Saudi Arabia.

Drugs and chemicals: The current study used pheophytin isolated in a pure form from the halophyte plant, *Suaeda vermiculata*, using the standard chromatographic techniques⁶. The compound's structure was identified as pheophytin using NMR, IR and MS spectral analysis⁷.

The L-Carnitine capsules (2500 mg), American International Lab., USA were purchased from the Nahdi Global website. Melatonin capsules (5 mg), Pharm-RX Chemical Corporation, USA were also purchased from the Nahdi Global website. Thiopental powder was purchased from Sigma Aldrich.

The serum levels of superoxide dismutase, glutathione peroxidase and malonaldehyde were determined using the ELISA assay method according to the manufacturing protocols. All kits were provided by Cloud-Clone Corp., Katy, Texas, USA. The absorbance of the parameters was measured using a plate reader (Bio Tek Instruments, Santa Clara, California, USA) set to 450 nm wavelength and the concentration was calculated using the standard curve.

Experimental animals: Thirty-six adult male albino rats, weighing between 200 and 300 g, were kept in standardized conditions with a natural light-dark cycle and a temperature of $25 \pm 1^\circ\text{C}$. They had unrestricted access to food and water. The rats were randomly allocated into six experimental groups; Group 1 (Control), Group 2 (Sham-operated), Group 3 (cerebral I/R), Group 4 (I/R pretreated with pheophytin A): Received IP pheophytin A (10 mg/kg)⁸. Group 5 (I/R pretreated with L-carnitine): Received IP L-carnitine (100 mg/kg)⁹. Group 6 (I/R pretreated with melatonin): Received IP melatonin (10 mg/kg)¹⁰. Groups 1, 2 and 3 received normal saline. For pretreated groups, the drugs were administered 30 min before bilateral carotid artery occlusion. Four hours after reperfusion all the rats were sacrificed and a blood sample was collected, centrifuged and the serum levels of pro-oxidant MDA and antioxidant enzymes SOD, glutathione peroxidase were measured.

Surgical procedures: The experimental animals were anesthetized with thiopental sodium (50 mg/kg, IP) for general anesthesia and placed in a supine position on the

surgical table. Body temperature was maintained at 37°C by a heating pad. Bilateral occlusion of the common carotid arteries was performed by making a small incision on the neck to expose the arteries. The common carotid arteries are carefully isolated and identified using micro-forceps. Once exposed, vascular clamps are applied to both arteries, effectively blocking blood flow to the brain for 30 min and inducing ischemia. After the ischemic period, the clamps are removed to allow reperfusion, restoring blood flow to the brain for 4 hrs. This procedure is used to investigate the effects of ischemia and reperfusion in animal models of stroke as shown in Fig. 1. Regarding the sham group, the same surgical procedure was performed without occluding the carotid arteries, ensuring that any effects observed were due to ischemia^{11,12}.

Sample preparation: Based on manufacturer instructions, blood samples were collected and then centrifuged at 1500 rpm for 10 min to separate the serum, which was then transferred into a vial using a pipette. The serum sample required a 1000-fold dilution. Initially, 20 μL of the serum was mixed with 180 μL of Phosphate Buffered Saline (PBS) to create a 1:10 dilution. Then, 10 μL of the 1:10 diluted sample was further diluted by adding it to 990 μL of PBS, resulting in a final 1:1000 dilution.

Assay of MDA, SOD and GPx enzymatic activities: The serum level of MDA, SOD and GP were determined by the ELISA method according to the instructions of the manufacturer. Absorbance was read at 450 nm assay kits which were purchased from Cloud-Clone Corp., Katy, Texas, USA.



Fig. 1: Rat subjected to surgical procedures of bilateral occlusion of common carotid arteries

Assay procedures: The procedure begins by adding 100 μ L of the diluted standard, blank and sample solutions to the corresponding wells, followed by incubation for 1 hr at 37°C. Afterward, discard the contents of each well without washing. Then, introduce 100 μ L of Detection Reagent A working solution into each well and incubate for an additional hour at 37°C. Following the incubation, wash each well with 350 μ L of wash solution and let it stand for 1-2 min. Repeat this wash procedure three times. Next, add 100 μ L of the working solution of Detection Reagent B to each well and incubate for 30 min at 37°C. Afterward, repeat the wash step three times. Then, introduce 90 μ L substrate solution to every well and incubate for 10-20 min at 37°C, protecting the solution from light. The liquid will turn blue upon substrate addition. Finally, add 50 μ L stop solution for each well to turn the liquid yellow. Measure absorbance immediately at 450 nm with a microplate reader.

Ethics approval: All experiments in the current research were approved by the Scientific Research Unit, College of Pharmacy, Qassim University, Kingdom of Saudi Arabia (Approval ID 2018-CP-14).

Statistical analysis: Data are expressed as Mean $\pm$ Standard Deviations (SD). Data were analyzed by one-way analysis of variance and *post hoc* test using IBM SPSS version 28. A $p < 0.05$ denoted statistical significance.

RESULTS

The mean values of SOD, GPx serum levels in the ischemia/reperfusion group (1553 ± 124.8 and 561.5 ± 20.74 , respectively) were significantly higher than the control group (2087 ± 261.1 and 616.9 ± 61.12 , respectively). Meanwhile, the level of the pro-oxidant MDA was significantly increased (361.3 ± 80.17) in the ischemia-reperfusion group about the control group (234.3 ± 40.33). Moreover, the mean values of the serum levels of SOD, GPx in pheophytin A (2325 ± 272.8 and 683.1 ± 28.28), L-carnitine (2117 ± 407.0 and 621.5 ± 111.3) and melatonin (2325 ± 272.8 , 671.3 ± 41.49) pretreated groups were significantly increased compared to the values of the control group. However, the mean values of the serum

levels of pro-oxidant MDA were significantly reduced in pheophytin A, L-carnitine and melatonin pretreated groups (257.0 ± 15.06 , 262.0 ± 23.17 and 255.0 ± 19.17 , respectively) about the control group. The mean values of serum levels of the pro-oxidant MDA were insignificantly different in L-carnitine, melatonin and pheophytin A pretreated groups. Also, the mean values of the serum levels of SOD and GPx were insignificantly different in the three treated groups (Table 1).

DISCUSSION

The current study investigates the effects of three compounds-pheophytin A, L-carnitine and melatonin on the serum levels of MDA, SOD and GPx in rats subjected to cerebral I/R injury. The results demonstrate increased oxidative stress in the I/R Group. The rats in the I/R group exhibited significantly higher levels of MDA (a marker of lipid peroxidation and oxidative damage) compared to the control group. Moreover, the serum levels of the antioxidant enzymes SOD and GPx were found to be significantly lower in the I/R group compared to the control group. This finding supports the idea that ischemia/reperfusion injury exceeds the capacity of the body's natural antioxidant defenses¹³⁻¹⁵.

In contrast to the I/R group, pre-treatment with pheophytin A, L-carnitine and melatonin resulted in a significant increase in the levels of SOD and GPx in serum. This indicates that these compounds have a protective effect, enhancing the antioxidant defense system against the oxidative damage caused by I/R injury. Specifically, levels of these enzymes were restored to values higher than those of the control group, which further supports the hypothesis that these compounds have antioxidant potential. Moreover, pre-treatment with pheophytin A, L-carnitine and melatonin significantly reduced the MDA levels compared to the I/R group. This suggested that all three compounds effectively mitigate the oxidative damage associated with I/R injury. Interestingly, the serum MDA levels in the pretreated groups (pheophytin A, L-carnitine and melatonin) were not significantly different, implying that these compounds may offer a similar degree of protection against oxidative stress.

Table 1: Effect of pheophytin A, L-carnitine and melatonin on MDA, SOD, GPx serum levels in rats subjected to cerebral ischemia/reperfusion injury

Parameter	Mean $\pm$ SD (ng/mL)					
	Control group	Sham-operated group	Cerebral I/R group	Pheophytin A group	L-carnitine group	Melatonin group
Superoxide dismutase	2087 ± 261.1^A	2020 ± 209.6^A	1553 ± 124.8^B	2253 ± 238.4^A	2117 ± 407.0^A	2325 ± 272.8^A
Glutathione peroxidase	616.9 ± 61.12^A	566.9 ± 35.82^A	561.5 ± 20.7^B	683.1 ± 28.28^A	621.5 ± 111.3^A	671.3 ± 41.49^A
Malondialdehyde	234.3 ± 40.33^A	245.0 ± 51.38^A	361.3 ± 80.17^B	257.0 ± 15.06^A	262.0 ± 23.17^A	255.0 ± 19.17^A

SOD: Superoxide dismutase, GP: Glutathione peroxidase, MDA: Malondialdehyde, values do not share the subscript capital letters considered significantly different and values are presented as (Mean $\pm$ SD)

The Pheophytin A, L-carnitine and melatonin demonstrated promising antioxidant properties. Their ability to increase antioxidant enzyme activity (SOD and GPx) and decrease MDA levels supports their potential as adjunctive treatments for oxidative stress-related conditions, such as stroke and other forms of ischemic brain injury. The findings of the current research were supported by other studies which showed that pheophytin A, L-carnitine and melatonin act by enhancing mitochondrial function, reducing inflammation or directly scavenging free radicals^{5,16,17}.

Current findings support the results of Hsu *et al.*¹⁸, who reported antioxidant and free radical scavenging activities of chlorophylls and pheophytins. Furthermore, the work of Kim *et al.*¹⁹, aligns with our observations, as they reported the neuroprotective effect of extract from *Agarum clathratum* extract against experimentally induced transient cerebral ischemic, one of the compounds of the extract is pheophytin²⁰. Additionally, the research by Huang *et al.*²¹, further corroborates current findings, highlighting the role of pheophytin A in attenuating I/R injury by reducing oxidative stress.

Current results are also consistent with Scafidi *et al.*²² who demonstrated that L-carnitine has protective action against ischemic injury. Additionally, the results were consistent with the study by Siktir *et al.*²³, which highlighted the protective role of L-carnitine supplementation against oxidative stress induced by exhaustive exercise in rats.

The results of the current study were in line with the study by Romero *et al.*²⁴, who demonstrated the synergistic neuroprotective effect of combined low concentrations of galantamine and melatonin against oxidative stress in SH-SY5Y neuroblastoma cells. Additionally, the work of Yang *et al.*²⁵ supports current results, as they found that melatonin reduced inflammation and apoptosis in neuronal cells exposed to ischemic conditions in a rat stroke model. Moreover, the results of the current work were consistent with those of Bocheva *et al.*²⁶, who reported the neuroprotection and retardation of brain aging with melatonin use.

CONCLUSION

The current study highlights the protective effects of pheophytin A, L-carnitine and melatonin against oxidative stress induced by brain I/R injury in rats. These compounds effectively reduced oxidative damage, as proven by a significant reduction in serum MDA levels and enhanced the antioxidant defense system, as reflected by the increase in serum SOD and GPx activity. Current findings support the

potential therapeutic applications of pheophytin A, L-carnitine and melatonin in preventing or mitigating oxidative stress-related conditions such as stroke and ischemic brain injury. These results indicated that these compounds exert their beneficial effects through mechanisms such as mitochondrial protection, reduction of inflammation and direct free radical scavenging.

SIGNIFICANCE STATEMENT

The study demonstrates the protective effects of pheophytin A, L-carnitine and melatonin against oxidative stress caused by brain I/R injury, highlighting their potential as therapeutic agents for conditions like stroke and ischemic brain injury. These compounds significantly reduced oxidative damage and enhanced antioxidant defenses, aligning with mechanisms such as mitochondrial protection and free radical scavenging. Future research should explore their clinical applications, optimal dosages and long-term effects to better understand their efficacy and safety in human populations.

REFERENCES

1. Li, M., H. Tang, Z. Li and W. Tang, 2022. Emerging treatment strategies for cerebral ischemia-reperfusion injury. *Neuroscience*, 507: 112-124.
2. Finkel, T. and N.J. Holbrook, 2000. Oxidants, oxidative stress and the biology of ageing. *Nature*, 408: 239-247.
3. Virmani, M.A. and M. Cirulli, 2022. The role of L-carnitine in mitochondria, prevention of metabolic inflexibility and disease initiation. *Int. J. Mol. Sci.*, Vol. 23. 10.3390/ijms23052717.
4. Reiter, R.J., J.C. Mayo, D.X. Tan, R.M. Sainz, M. Alatorre-Jimenez and L. Qin, 2016. Melatonin as an antioxidant: Under promises but over delivers. *J. Pineal Res.*, 61: 253-278.
5. Lanfer-Marquez, U.M., R.M.C. Barros and P. Sinnecker, 2005. Antioxidant activity of chlorophylls and their derivatives. *Food Res. Int.*, 38: 885-891.
6. Mohammed, H.A., M.S. Al-Omar, M.Z. El-Readi, A.H. Alhowail, M.A. Aldubayan and A.A.H. Abdellatif, 2019. Formulation of ethyl cellulose microparticles incorporated pheophytin A isolated from *Suaeda vermiculata* for antioxidant and cytotoxic activities. *Molecules*, Vol. 24. 10.3390/molecules24081501.
7. Mohammed, S.A.A., H.M. Ali, H.A. Mohammed, M.S. Al-Omar and S.A. Almahmoud *et al.*, 2021. Roles of *Suaeda vermiculata* aqueous-ethanolic extract, its subsequent fractions, and the isolated compounds in hepatoprotection against paracetamol-induced toxicity as compared to silymarin. *Oxid. Med. Cell. Longevity*, Vol. 2021. 10.1155/2021/6174897.

8. Pyo, M.Y., B.K. Park, J.J. Choi, M. Yang and H.O. Yang *et al.*, 2013. Pheophytin *a* and chlorophyll *a* identified from environmentally friendly cultivation of green pepper enhance interleukin-2 and interferon- γ in peyer's patches *ex vivo*. *Biol. Pharm. Bull.*, 36: 1747-1753.
9. Zhang, R., H. Zhang, Z. Zhang, T. Wang, J. Niu, D. Cui and S. Xu, 2012. Neuroprotective effects of pre-treatment with L-carnitine and acetyl-L-carnitine on ischemic injury *in vivo* and *in vitro*. *Int. J. Mol. Sci.*, 13: 2078-2090.
10. Lee, C.H., J.H. Park, J.H. Ahn and M.H. Won, 2016. Effects of melatonin on cognitive impairment and hippocampal neuronal damage in a rat model of chronic cerebral hypoperfusion. *Exp. Ther. Med.*, 11: 2240-2246.
11. Wang, H., R. Jiang, Q. He, Y. Zhang and Y. Zhang *et al.*, 2012. Expression pattern of peroxisome proliferator-activated receptors in rat hippocampus following cerebral ischemia and reperfusion injury. *PPAR Res.*, Vol. 2012. 10.1155/2012/596394.
12. Traystman, R.J., 2003. Animal models of focal and global cerebral ischemia. *ILAR J.*, 44: 85-95.
13. Sies, H., 2015. Oxidative stress: A concept in redox biology and medicine. *Redox Biol.*, 4: 180-183.
14. Jurcau, A. and A.I. Ardelean, 2022. Oxidative stress in ischemia/reperfusion injuries following acute ischemic stroke. *Biomedicines*, Vol. 10. 10.3390/biomedicines10030574.
15. Lee, K.H., M. Cha and B.H. Lee, 2020. Neuroprotective effect of antioxidants in the brain. *Int. J. Mol. Sci.*, Vol. 21. 10.3390/ijms21197152.
16. Moghaddas, A. and S. Dashti-Khavidaki, 2016. Potential protective effects of L-carnitine against neuromuscular ischemia-reperfusion injury: From experimental data to potential clinical applications. *Clin. Nutr.*, 35: 783-790.
17. Hacışevki, A. and B. Baba, 2018. An Overview of Melatonin as an Antioxidant Molecule: A Biochemical Approach. In: *Melatonin-Molecular Biology, Clinical and Pharmaceutical Approaches*, Drăgoi, C.M. and A.C. Nicolae (Eds.), IntechOpen, London, United Kingdom, ISBN: 978-1-78984-505-1, pp: 59-85.
18. Hsu, C.Y., P.Y. Chao, S.P. Hu and C.M. Yang, 2013. The antioxidant and free radical scavenging activities of chlorophylls and pheophytins. *Food Nutr. Sci.*, 4: 1-8.
19. Kim, I.H., K.Y. Yoo, J.H. Park, B.C. Yan and J.H. Ahn *et al.*, 2014. Comparison of neuroprotective effects of extract and fractions from *Agarum clathratum* against experimentally induced transient cerebral ischemic damage. *Pharm. Biol.*, 52: 335-343.
20. Ina, A. and Y. Kamei, 2006. Vitamin B₁₂, a chlorophyll-related analog to pheophytin a from marine brown algae, promotes neurite outgrowth and stimulates differentiation in PC12 cells. *Cytotechnology*, 52: 181-187.
21. Huang, R., J. Shu, X. Dai, Y. Liu, F. Yu and G. Shi, 2020. The protective effect of polyphyllin I on myocardial ischemia/reperfusion injury in rats. *Ann. Transl. Med.*, Vol. 8. 10.21037/atm-20-3371.
22. Scafidi, S., J. Racz, J. Hazelton, M.C. McKenna and G. Fiskum, 2011. Neuroprotection by acetyl-L-carnitine after traumatic injury to the immature rat brain. *Dev. Neurosci.*, 32: 480-487.
23. Şıktar, E., D. Ekinçi, E. Şıktar, Ş. Beydemir, İ. Gülçin and M. Günay, 2011. Protective role of L-carnitine supplementation against exhaustive exercise-induced oxidative stress in rats. *Eur. J. Pharmacol.*, 668: 407-413.
24. Romero, A., J. Egea, A.G. García and M.G. López, 2010. Synergistic neuroprotective effect of combined low concentrations of galantamine and melatonin against oxidative stress in SH-SY5Y neuroblastoma cells. *J. Pineal Res.*, 49: 141-148.
25. Yang, B., L.E. Zang, J.W. Cui, M.Y. Zhang, X. Ma and L.L. Wei, 2020. Melatonin plays a protective role by regulating miR-26a-5p-NRSF and JAK2-STAT3 pathway to improve autophagy, inflammation and oxidative stress of cerebral ischemia-reperfusion injury. *Drug Des. SDev. Ther.*, 14: 3177-3188.
26. Bocheva, G., D. Bakalov, P. Iliev and R. Tafradjiiska-Hadjiolova, 2024. The vital role of melatonin and its metabolites in the neuroprotection and retardation of brain aging. *Int. J. Mol. Sci.*, Vol. 25. 10.3390/ijms25105122.