Acta Biochimica Indonesiana

https://doi.org/10.32889/actabioina.79

RESEARCH ARTICLE

Open Access

Comparing the effect of *Centella asiatica* L. and *Acalypha indica* L. treatment to carbonyl and glutathione level in the old brain rats

Ninik Mudjihartini[1*], Reni Paramita[1], Astrid Mariam Khairani Siregar[2], Estiana Filzadiyanti[2], Pungguri Ayu Nega Sarsanti[3], Erni Purwaningsih[4]

[1]Department of Biochemistry and Molecular Biology, Faculty of Medicine, Universitas Indonesia. Jakarta. 10440. Indonesia
[2]Undergraduate Programme, Faculty of Medicine, Universitas Indonesia. Jakarta. 10440. Indonesia
[3]Neuroscience and Brain Development Cluster, Indonesian Medical Education and Research Institute. Jakarta. 10440. Indonesia
[4]Departement of Pharmacy, Faculty of Medicine, Universitas Indonesia. Jakarta. 10440. Indonesia
[*]Correspoding author: Jl. Salemba Raya No. 6, Central Jakarta 10430, phone (021) 31930373. Email: ninikbiokim@gmail.com

ABSTRACT

Background: Free radicals in excessive concentrations damages cells and accelerate the aging process. Antioxidants found in *Centella asiatica* (CA) and *Acalypha indica* (AI) have the potential to prevent oxidative cellular damage.

Objective: This study aimed to compare the effect of CA and AI on carbonyl and glutathione levels in the brain of older rats.

Methods: 18-month age rats were treated using either AI, CA, or vitamin E. In addition, 18-month age and 2-month age untreated rats were used as a negative control. The brain carbonyl and glutathione levels were measured by Agustyanak and the Elmann method, respectively.

Results: Treatment with CA significantly decreased brain carbonyl levels (2.87 nmol/mL) than the control rats (4.54 nmol/mL). Furthermore, treating AI did not reduce the brain carbonyl and GSH levels in aged brain rats.

Conclusion: *Centella asiatica* can reduce the protein destruction that occurs with increasing age.

Keywords: *Acalypha indica*, *Centella asiatica,* carbonyl, GSH, old rat brain

Introduction

Herbs have been used as medicines for many centuries. In China, these treatments have been used for 2000 years, while in India, they have been used for 3000 years. Various herbal plants have been demonstrated to have antioxidant activity. *Centella asiatica* (CA), widely known as "pegagan," is believed to have high antioxidant activity due to their ability to scavenge free radicals [1–3]. Similarly, *Acalypha indica* (AI) is reported to have antioxidant activity depending on the dose of extract used [4]. Furthermore, the two plants have been shown to protect against liver damage in post-hypoxic conditions [5].

The main chemical components of *Centella asiatica* that play a role in its pharmacological activity are triterpenes, mostly asiaticoside, asiatic acid, madecassoside, and madecassic acid. Ethanol, methanol, and water extracts of *Centella asiatica* increase nervous system function [6]. CA and its triterpenoids have been shown in animal studies to increase SOD and GPX activity, activate Nrf-2, ameliorate cognitive impairment, and, as a consequence, reduce symptoms of linked disorders [7–9].

Aging is the progressive loss of function of tissues and organs over time [10]. Aging is a biological process in which biochemical macromolecules such as DNA, proteins, and cell organelles are broken down [11]. Under normal conditions, the body's quantities of oxidants, antioxidants, and biomolecules are kept in a

balanced state. Free radical exposure at normal amounts does not harm cells. However, a high concentration of free radicals causes cellular damage and accelerates the aging process [12]. Free radicals can damage proteins, and when they are oxidized, they undergo carbonylation [13]. Protein carbonylation is one of the most harmful oxidative protein modifications because it produces toxic carbonyl groups, which can cause a range of negative effect when they accumulate [14]. This process is also employed as the most common indicator of oxidative stress-related problems. Protein carbonyl measurements are frequently used to detect levels of oxidative stress in the context of cell damage, aging, and some age-related disorders [15].

The brain is a vital organ with high metabolic activity that consumes about 20% of the body's total oxygen and glucose. A high metabolic rate makes brain more vulnerable to oxidative stress and allow free radicals to attack proteins in the brain [16]. Moreover, as people age, the oxygen supply and partial oxygen pressure in the tissues diminish, increasing the risk of hypoxia [17]. Therefore, the brain is a suitable organ for monitoring carbonyl levels as a result of protein damage induced by free radicals during the aging process.

Natural antioxidants, both enzymatic and non-enzymatic, exist in the human body and can prevent the accumulation of free radicals. One of the factors that contribute to aging is free radicals. As a result, antioxidants are critical in the detoxification of free radical toxicity. They can protect cells from oxidative stress-induced cell damage [18]. One of these antioxidants is glutathione (GSH), which belongs to the non-enzymatic endogenous antioxidant group.

GSH is required for cell protection against oxidative stress, xenobiotic electrophile toxicity, and redox reaction homeostasis [19]. The brain shrinks in size and undergoes chemical changes as it ages. Because vascularization declines with age, oxygen transport into tissues decreases, resulting in hypoxia [17]. This hypoxia causes oxidative stress which is very susceptible to the brain. Furthermore, as antioxidant production declines in the aged brain, the risk of the brain being harmed by oxidative stress effects, such as nerve cell injury, increases [18].

Although *Centella asiatica* and *Acalypha indica* have the potential to be developed as antioxidants, no research has been done on their effect on the aging process, particularly on carbonyl and glutathione levels. Therefore, this study aims to compare the effect of *Centella asiatica* and *Acalypha indica* treatment to carbonyl and glutathione level in the old brain rats.

Methods

Sample

We examined brain homogenates from male Spraque Dawley rats aged 18 and 2 months. Brain samples from 24 Sprague Dawley rats were were derived from the previous study [20]. As biological material, the homogenate of the rat brain was stored at -80°C in the Department of Biochemistry and Molecular Biology, Faculty of Medicine, Universitas Indonesia, until the parameters were measured. This study has a Certificate of Ethical Review from the Health Research Ethics Committee, Faculty of Medicine, University of Indonesia Cipto Mangunkusumo Hospital with the number:118/UN 2. F1/ETIK/II/2018.

Treatment

In this study, we used stored brain tissues derived from 24 male Sprague Dawley rats, consist of five groups. Four groups of 18-month-old rats were studied, one of which was left untreated as a control group. Three groups of 18-month-old rats were given the following treatments: 7 rats received 150 mg/kg body weight of AI, 6 rats received 300 mg/kg body weight of CA, and 4 rats received 15 UI/kg body weight of vitamin E. In addition, six 2-month-old rats in the fifth group were not given any treatment. The AI, CA, and vitamin E (as a positive control) were administered orally for 29 days in a row as described in previous study [20].

The carbonyl levels measurement

The test sample was treated with 0.8 mL DNPH 10 mM, while the blank was treated with 0.8 mL HCl 2.5 M. The tests and blanks were kept at room temperature and protected of direct sunlight. To precipitate proteins, 20% TCA (trichloroacetic acid) and 10% TCA (0.8 mL) were added. At a rate of 10,000 x g, this mixture were centrifuged. After that, the supernatant was discarded. After washing the pellet three times with 0.8 mL ethanol-ethyl acetate, 0.4 mL urea in NaOH 0.4 N was added. Finally, the absorbance of the supernatant was read on a spectrophotometer with a wavelength of 390 nm. Carbonyl content was calculated using the molar absorption coefficient, which is 22,000 M^{-1} cm^{-1} relative to protein concentration [21].

The measurement of glutathione (GSH)

The GSH levels was determined using the Ellman method [22]. Dithiobis nitro benzoic acid (DNTB) reacts with compounds with -SH groups to form compounds with -S-S- bonds. DNTB was reduced by compounds containing -SH groups, which then breaks down into trinitrobenzene. This alkaline environment produced a yellow solution that absorbed maximum light at a wavelength of 412 nm. GSH levels in brain tissue were measured in two stages: the GSH standard curve, which includes six variations of standard glutathione concentration, and the sample measurement.

Phosphate buffer (0.1 M, pH 8.0, 1.78 mL) was added to 50 µL brain homogenates and 50 µL standard with different concentrations. After that, 0.2 mL of 5% TCA was added and mixed thoroughly. The mixture was centrifuged at 3000 x rpm for 5 minutes at 4°C, and the supernatant was separated. 0.001 mL of DNTB was added to tubes containing 0.8 mL supernatant. After one hour of incubation, the absorbance was measured using a spectrophotometer at a wavelength of 412 nm. The remaining supernatant solution was used as a blank.

Data analysis

Data were shown as average±SD. When the data was normally distributed, one-way ANOVA was used, while Kruskal-Wallis was used when the data distribution was not normal. The significance level was set at $p<0.05$.

Results

Brains carbonyl level

With age, oxidative stress rises. In this condition, the endogenous antioxidants are unable to control the increasing production of free radicals, resulting in free radical cell damage. In the context of cell damage, aging, and some age-related disorders, protein carbonyl assays are frequently used to detect levels of oxidative stress.

The brain carbonyl levels in untreated 18-month-old rats (control), treated with AI, CA, vitamin E, and untreated 2-month-old rats were: 4.54 nmol/mL; 4.03 nmol/mL; 2.87 nmol/mL; 2.64 nmol/mL; and 2.55 nmol/mL, respectively. The brain carbonyl levels of the older rats aged 18 months that were treated with CA 300 mg/kg body weight, vitamin E 15 IU/kg body weight, and the untreated group of young rats aged 2 months were significantly lower than the brain carbonyl levels of the aged rats (Figure 1).

These findings imply that giving rats 18 months old with CA extracts inhibited the age-related increase in carbonyl levels. The CA-treated rats exhibited similar brain carbonyl levels as the 18-month-old group of rats that had received vitamin E and the control young 2-month-old rats. In contrast, 150 mg/kg body weight AI treatment did not affect the brain carbonyl levels of the 18-month-old group of rats.

Brain glutathione level

GSH is required for cell protection against oxidative stress, xenobiotic electrophile toxicity, and redox reaction homeostasis. The brain GSH levels of older rats aged 18 months (control) and that were given AI, CA, vitamin E, and control young rats aged 2 months were 0.905 µg/mL;

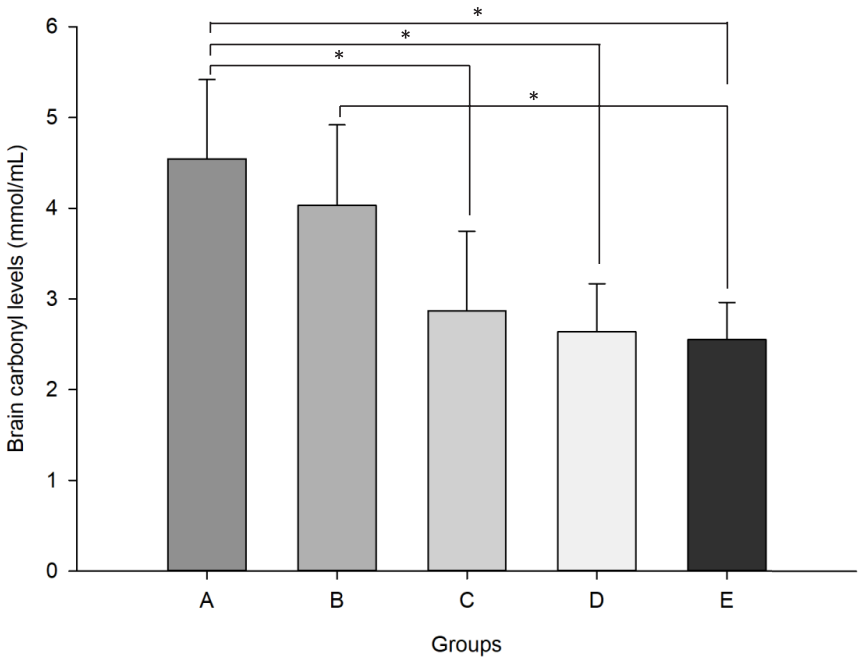

Figure 1. Carbonyl levels in rat brains. A) Brain tissues of five old rats without treatment (control group), B) Brain tissues of five old rats given *Acalypha indica* (AI) for 29 days, C) Brain tissues of five old rats treated *Centella asiatica* (CA) for 29 days, D) Brain tissues of four old rats treated vitamin E for 29 days, E) Brain tissues of five young rats without treatment. *p < 0.05 (Anova)

0.915 µg/mL; 0.338 µg/mL; 0.359 µg/mL; 0.37 µg/mL. The statistical test analysis revealed that brain GSH levels in the CA, vitamin E, and control young rats decreased significantly than in the aged control group. CA or vitamin E treatment was demonstrated to have antioxidant effects, resulting in GSH levels similar to those found in the brains of young control rats. On the other hand, the brain GSH levels of the AI-treated group did not differ significantly from those the control group (Figure 2).

Discussion

This study aimed to compare the effect of CA and AI on carbonyl and glutathione level in the brains of aged rats. Our findings suggest that CA extract decreased brain GSH level while inhibiting the increase in carbonyl levels that occurs with age. The reduction in brain carbonyl and GSH levels in this group was comparable to that seen in the vitamin E-treated rats and the 2-month-old control group. The aged rats' brain carbonyl and GSH levels, on the other hand, were unaffected by AI treatment.

During the aging process, a decrease in vascularization can increase the diffusion of tissues and increases tissue hypoxia [17]. Hypoxia increases brain tissue activation in response to oxidative stress as people age, resulting in nerve cell damage [18]. Protein carbonyl in the bone marrow and lymphatic cells of mice in old age increased than those of young mice. The production of this protein carbonyl is linked to oxidative stress due to aging, which causes changes in cell proteostasis [23]. CA extract contains asiatic acid, a natural aglycone of pentacyclic triterpenoids [24]. The antioxidant effect of asiatic acid can reduce the free radical changes that form under hypoxic conditions with age. CA reduces oxidative stress in the cells and inhibits the protein cell damage caused by free radicals as a protein carbonyl.

The levels of carbonyl and GSH in the brains of rats given AI were similar to those in aged control group. These results are in line with Liu et al., who found that quercetin can increase the synthesis of antioxidant enzymes such glutathione peroxidase (GPx), glutathione reductase (GR), superoxide dismutase (SOD), and catalase. Giving

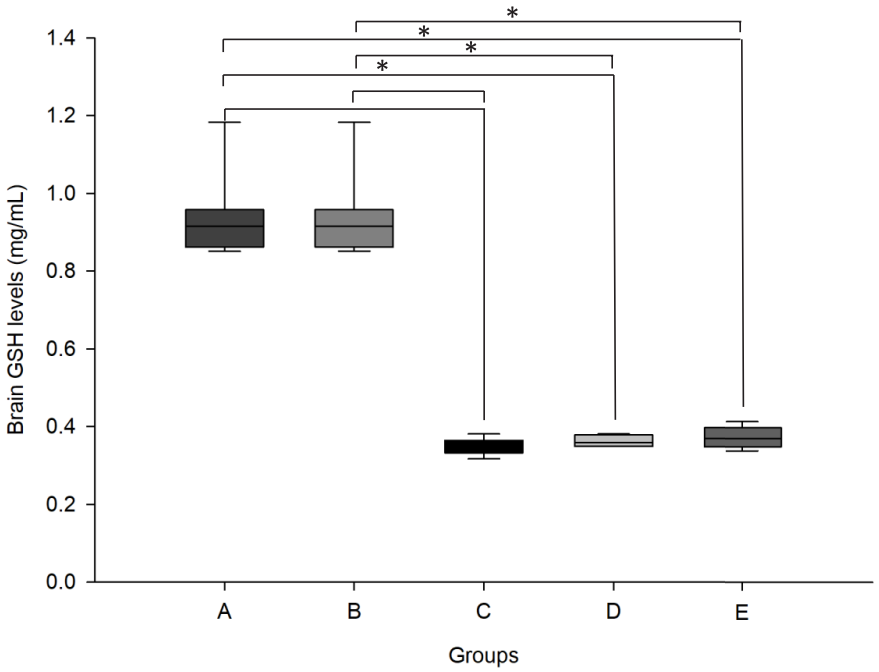

Figure 2. GSH levels in rat brain tissue. A) Brain tissue of five old rats without treatment (control group), B) Brain tissue of five old rats treated *Acalypha indica* (AI) for 29 days, C) Brain tissue of five old rats treated *Centella asiatica* (CA) for 29 days, D) Brain tissue of four old rats treated vitamin E for 29 days, E) Brain tissue of five young rats with no treatment. *p < 0.05 (Kruskal Wallis, Mann Whitney)

quercetin to rats is considered to have increased GPx activity, causing in a need for GSH as a substrate for the increased GPx enzyme [25].

The higher levels of GSH in the brains of the older rat control group align with the Gladyshev et al. finding. They proposed that the aging process is caused by an accumulation of free radicals that antioxidants are unable to absorb into the body, resulting in increased oxidative stress. As a result, antioxidants including GSH, are produced in the body as a form of compensatory [12]. Under stressful or mildly diseased conditions, Zhu et al. found a compensatory increase in GSH levels in the target organ [26].

Other research has found that AI extracts contain antioxidants, which contradicts this finding. The administration of AI at a dose of 150 mg/kg body weight, on the other hand, did not reduce the increase in free radical levels in the brain caused by the aging process' hypoxia. The administration of AI was unable to suppress the increase in free radicals. The synthesis of endogenous antioxidants, such as GSH, is triggered by an increase in free radicals. Because 18 months in rats is equivalent to 45 years in humans, the same mechanism is likely to apply to aging humans [27]. Oxidative stress that has not yet been neutralized will induce antioxidant formation through the Nrf2 signaling pathway. The study by Zang et al. shows that ROS can induce Nrf2, which stimulates antioxidant formation [6]. The GSH expression relates to Nrf2, which can upregulate antioxidant response element (ARE) activity, promoting the translation of antioxidant proteins.

As we become older, all of the cells in our bodies will begin to age. In the present study, CA can decrease brain protein carbonyl levels in the elderly rat. The ability of CA to slow down the aging of nerve cells can be exploited, particularly the CA compounds that are primarily involved in neuroprotective and neurodegenerative qualities, as well as their mechanisms of action.

Conclusion

CA can reduce the protein destruction that occurs with increasing age. AI did not reduce the GSH that occurs in the brain with increasing age.

Acknowledgment

None.

Funding

This study was financially supported by the Directorate of Research and Community Service, Universitas Indonesia through HIBAH PDUPT 2019 (Grant No 331/UN2.R3.1/HKP.05.00 Perjanjian/2018).

Author contributions

Conceptualization, NM, RP, and EP; Metodology, NM and RP; Investigation, RP, PANS; Writing – Original Draft, NM and PANS, Writing – Review & Editing, NM; Funding Acquisition, NM and RP; Resources, AMKS and EF; Supervision, NM and RP

Declaration of interest

The authors declare that they have no conflict of interest in this study.

Received: 21 March 2022
Revised: 17 May 2022
Accepted: 1 June 2022
Published online: 8 June 2022

References

Jatayu D, Nursyam H, Maizar Suryanto Hertika A. Antioxidant Effect of Centella asiatica Ethanolic Extract to Superoxide Dismutase (SOD) Level on Cyprinus carpio Liver. RJLS. 2018;5: 163-172. https://doi.org/10.21776/ub.rjls.2018.005.03.4

2. Zofia N-Ł, Martyna Z-D, Aleksandra Z, Tomasz B. Comparison of the Antiaging and Protective Properties of Plants from the Apiaceae Family. Oxid Med Cell Longev. 2020;2020: 5307614. https://doi.org/10.1155/2020/5307614
3. Buranasudja V, Rani D, Malla A, Kobtrakul K, Vimolmangkang S. Insights into antioxidant activities and anti-skin-aging potential of callus extract from Centella asiatica (L.). Sci Rep. 2021;11: 13459. https://doi.org/10.1038/s41598-021-92958-7
4. Sahukari R, Punabaka J, Bhasha S, Ganjikunta VS, Kondeti Ramudu S, Kesireddy SR, et al. Phytochemical Profile, Free Radical Scavenging and Anti-Inflammatory Properties of Acalypha Indica Root Extract: Evidence from In Vitro and In Vivo Studies. Molecules. 2021;26. https://doi.org/10.3390/molecules26206251
5. Dwijayanti A, Frethernety A, Hardiany NS, Purwaningsih EH. Hepatoprotective effects of acalypha indica and centella asiatica in rat's liver against hypoxia. Procedia Chem. 2015;14: 11-14. https://doi.org/10.1016/j.proche.2015.03.003
6. Sun B, Wu L, Wu Y, Zhang C, Qin L, Hayashi M, et al. Therapeutic Potential of Centella asiatica and Its Triterpenes: A Review. Front Pharmacol. 2020;11: 568032. https://doi.org/10.3389/fphar.2020.568032
7. Gray NE, Zweig JA, Matthews DG, Caruso M, Quinn JF, Soumyanath A. Centella asiatica Attenuates Mitochondrial Dysfunction and Oxidative Stress in Aβ-Exposed Hippocampal Neurons. Oxid Med Cell Longev. 2017;2017: 7023091. https://doi.org/10.1155/2017/7023091
8. Chintapanti S, Pratap Reddy K, Sreenivasula Reddy P. Behavioral and neurochemical consequences of perinatal exposure to lead in adult male Wistar rats: protective effect by Centella asiatica. Environ Sci Pollut Res Int. 2018;25: 13173-13185. https://doi.org/10.1007/s11356-018-1500-x
9. Welbat JU, Chaisawang P, Pannangrong W, Wigmore P. Neuroprotective Properties of Asiatic Acid against 5-Fluorouracil Chemotherapy in the Hippocampus in an Adult Rat Model. Nutrients. 2018;10. https://doi.org/10.3390/nu10081053
10. Liguori I, Russo G, Curcio F, Bulli G, Aran L, Della-Morte D, et al. Oxidative stress, aging, and diseases. Clin Interv Aging. 2018;13: 757-772. https://doi.org/10.2147/CIA.S158513
11. Pyo IS, Yun S, Yoon YE, Choi J-W, Lee S-J. Mechanisms of Aging and the Preventive Effects of Resveratrol on Age-Related Diseases. Molecules. 2020;25. https://doi.org/10.3390/molecules25204649
12. Gladyshev VN. The free radical theory of aging is dead. Long live the damage theory! Antioxid Redox Signal. 2014;20: 727-731. https://doi.org/10.1089/ars.2013.5228
13. Reeg S, Grune T. Protein oxidation in aging: does it play a role in aging progression? Antioxid Redox Signal. 2015;23: 239-255. https://doi.org/10.1089/ars.2014.6062
14. Gonos ES, Kapetanou M, Sereikaite J, Bartosz G, Naparło K, Grzesik M, et al. Origin and pathophysiology of protein carbonylation, nitration and chlorination in age-related brain diseases and aging. Aging (Albany, NY). 2018;10: 868-901. https://doi.org/10.18632/aging.101450
15. Sies H. Oxidative stress: a concept in redox biology and medicine. Redox Biol. 2015;4: 180-183. https://doi.org/10.1016/j.redox.2015.01.002
16. Garbarino VR, Orr ME, Rodriguez KA, Buffenstein R. Mechanisms of oxidative stress resistance in the brain: Lessons learned from hypoxia tolerant extremophilic vertebrates. Arch Biochem Biophys. 2015;576: 8-16. https://doi.org/10.1016/j.abb.2015.01.029
17. Valli A, Harris AL, Kessler BM. Hypoxia metabolism in

ageing. Aging (Albany, NY). 2015;7: 465-466. https://doi.org/10.18632/aging.100782

18. Conti V, Izzo V, Corbi G, Russomanno G, Manzo V, De Lise F, et al. Antioxidant Supplementation in the Treatment of Aging-Associated Diseases. Front Pharmacol. 2016;7: 24. https://doi.org/10.3389/fphar.2016.00024
19. Aquilano K, Baldelli S, Ciriolo MR. Glutathione: new roles in redox signaling for an old antioxidant. Front Pharmacol. 2014;5: 196. https://doi.org/10.3389/fphar.2014.00196
20. Dwijayanti A, Farida S, Purwaningsih EH. Comparing Anti Aging Potential Between Centella asiatica and Acalypha indica : Focus on Forelimb Muscle Strength. Adv Sci Lett. 2018;24: 6058-6060. https://doi.org/10.1166/asl.2018.12621
21. Augustyniak E, Adam A, Wojdyla K, Rogowska-Wrzesinska A, Willetts R, Korkmaz A, et al. Validation of protein carbonyl measurement: a multi-centre study. Redox Biol. 2015;4: 149-157. https://doi.org/10.1016/j.redox.2014.12.014
22. Ellman GL, Courtney KD, Andres V, Feather-Stone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961;7: 88-95. https://doi.org/10.1016/0006-2952(61)90145-9
23. Tanase M, Urbanska AM, Zolla V, Clement CC, Huang L, Morozova K, et al. Role of Carbonyl Modifications on Aging-Associated Protein Aggregation. Sci Rep. 2016;6: 19311. https://doi.org/10.1038/srep19311
24. Meeran MFN, Goyal SN, Suchal K, Sharma C, Patil CR, Ojha SK. Pharmacological properties, molecular mechanisms, and pharmaceutical development of asiatic acid: A pentacyclic triterpenoid of therapeutic promise. Front Pharmacol. 2018;9: 892. https://doi.org/10.3389/fphar.2018.00892
25. Liu H, Guo X, Chu Y, Lu S. Heart protective effects and mechanism of quercetin preconditioning on anti-myocardial ischemia reperfusion (IR) injuries in rats. Gene. 2014;545(1):149-55. https://doi.org/10.1016/j.gene.2014.04.043
26. Lushchak VI. Glutathione homeostasis and functions: potential targets for medical interventions. J Amino Acids. 2012;2012: 736837. https://doi.org/10.1155/2012/736837
27. Andreollo NA, Santos EF dos, Araújo MR, Lopes LR. Idade dos ratos versus idade humana: qual é a relação? ABCD, arq bras cir dig. 2012;25: 49-51. https://doi.org/10.1590/S0102-67202012000100011