Accepted Manuscript

Accepted Manuscript (Uncorrected Proof)

Title: Ellagic Acid ameliorates Streptozotocin-Induced Diabetic Hyperalgesia in Rat: Involvement of Oxidative Stress

Authors: Siamak Shahidi[1], Alireza Komaki[1], Safoura Raoufi[1,*], Iraj Salehi[1], Mohammad Zarei[1], Mohamadreza Mahdian[1]

1. *Neurophysiology Research Center, Hamadan University of Medical Sciences, Hamadan, Iran.*

*Corresponding author: E-mail: safooraraufi@yahoo.com

This is a "Just Accepted" manuscript, which has been examined by the peer-review process and has been accepted for publication. A "Just Accepted" manuscript is published online shortly after its acceptance, which is prior to technical editing and formatting and author proofing. *Basic and Clinical Neuroscience* provides "Just Accepted" as an optional and free service which allows authors to make their results available to the research community as

1

soon as possible after acceptance. After a manuscript has been technically edited and formatted, it will be removed from the "Just Accepted" Web site and published as a published article. Please note that technical editing may introduce minor changes to the manuscript text and/or graphics which may affect the content, and all legal disclaimers that apply to the journal pertain.

Please cite this article as:

Abstract

Background/Aim: Hyperalgesia is one of the current complications of diabetes mellitus that Oxidative stress and inflammation have principal role in its development. Ellagic Acid (EA) as a herbal component, has some biological activities, including antioxidant and anti-inflammatory effects. This study was designed to evaluate the possible beneficial effect of EA on hyperalgesia in streptozotocin (STZ)-induced diabetic rat.

Materials and Methods: Rats were divided into control(vehicle received), diabetic, EA (25, 50 mg/kg)-treated control and EA(25, 50 mg/kg)-treated diabetic groups. Diabetes was induced by a single intraperitoneal (IP) injection of streptozotocin (STZ) (60 mg/Kg). EA was administered daily by oral gavage for 4 weeks. Hyperalgesia was assessed using tail flick (TF) and hot plate (HP) tests. Also, oxidative stress markers including malondialdehyde (MDA), total oxidant status (TOS) and total antioxidant capacity (TAC) in the serum were evaluated.

Results: Diabetic animals showed marked reductions in TF and HP latencies, elevation of serum MDA level and TOS and diminution of serum TAC compared to controls significantly. Treatment of Diabetic rats with EA ameliorated reduction of TF latency at the dose of 25 mg/kg and HP latency at the dose of 50 mg/kg. Furthermore EA significantly increased TAC and decreased MDA level at dose of 50 mg/kg and reduced TOS at both doses in the serum of diabetic animals. In EA treated normal rats we could see no significant alterations in the parameters studied.

Conclusion: These results displayed potent antinociceptive effect of EA in diabetic rats via attenuating oxidative stress. This proposes therapeutic potential of EA for damping diabetic hyperalgesia.

1. Introduction

Diabetes mellitus(DM), a serious metabolic disorder, is one of the main causes of morbidity and mortality in the world(Motamedrad, Shokouhifar et al. 2019). Diabetic neuropathy (DN) is one of the most common chronic complications of diabetes that develops in more than 60% of the diabetic patients(Cameron and Cotter 2008). Diabetic painful neuropathy (DPN) is mostly characterized by pain perception alterations, increased sensitivity to mild painful stimuli (hyperalgesia) and abnormal pain sensitivity to stimuli were not painful previously(Hasanein and Fazeli 2014). Persistent sensation of burning, aching or spontaneous pain shows significant effect on the patient quality of life, particularly by interfering with daily activities(Veves, Backonja et al. 2008). Hyperglycemia by induction of multiple changes such as fatty acid metabolism abnormalities and advanced glycation in peripheral neurons, glial and vascular cells plays a key role in the development and progression of DN(Dobretsov, Hastings et al. 2001).

Oxidative stress and inflammation are crucial pathways implicated in pathogenesis of diabetic pain neuropathy (Green, Pedersen et al. 2011, Rodrigues, Bergamaschi et al. 2011). Advanced glycation end products stimulate production of reactive oxygen species (ROS) and expression of pro inflammatory cytokines such as interleukin-1(IL-1) and tumor necrosis factor-α (TNF-α) (Kuhad, Bishnoi et al. 2009). It has been demonstrated that production of massive free radicals by the mitochondrial respiratory chain and activation of inflammatory pathway under hyperglycemic condition induce apoptosis in neuronal cells(Ahmed 2005, Yang, Jin et al. 2011). In diabetic rats, overproduction of ROS, activation of lipid peroxidation and dysfunction in the sciatic nerve have been observed (Cunha, Jolivalt et al. 2008). Also,

5

reduced pain threshold and attenuated antioxidant enzyme activity in sciatic nerve of diabetic

rats have been reported (Al-Enazi 2013). Researchers have reported that hyperglycemia

induced oxidative stress may cause spontaneous impulses in pain afferent neurons and

spinothalamic neurons by inducing hypersensitivity of these neurons in diabetic

patients(Chen and Pan 2002, Khan, Chen et al. 2002). Current medication for diabetic

neuropathic pain including tricyclic antidepressants, anticonvulsants, calcium channel

ligands, topical anesthetics and opioids have incomplete effectiveness and their various

adverse effects limit their use(Hasanein and Fazeli 2014). Therefore, new therapeutic

candidates are continuingly needed to improve DNP.

EA is a polyphenol compound found in numerous vegetables, fruits and nuts such as

tomatoes, carrots, strawberries, raspberries and pomegranates .It has been documented that

EA exhibits different pharmacological properties including antioxidant, anti-inflammatory,

anti-apoptotic, anti-atherogenic, anti-fibrosis and anti-cancer effects(Uzar, Alp et al. 2012).

EA is capable to reduce oxidative damage via scavenging free radicals, increasing

antioxidant enzyme activity and decreasing lipid peroxidation(Chao, Hsu et al. 2009, Uzar,

Alp et al. 2012, Kiasalari, Heydarifard et al. 2017). This compound was found to decrease

oxidative damage and the levels of MDA and TOS in diabetic rats sciatic nerve and

brain(Uzar, Alp et al. 2012). EA has been known to possess anti-inflammatory properties

such as reducing IL-6 and TNF-α, blocking NF-κB pathway, inhibiting COX-2 and

increasing IL-10 (Cornélio Favarin, Martins Teixeira et al. 2013, Kaur, Mehan et al. 2015).

Several studies have reported antinociceptive activity of EA in different animal models of

pain such as carrageenan, acid and formalin induced hyperalgesia(Mansouri, Naghizadeh et

al. 2013).

Since compounds have protective properties such as anti-oxidative and anti-inflammatory effects are good candidates for DN treatment therefore, the aim of present study was to assess the benefical effect of EA on hyperalgesia in diabetic rats.

2. Materials and Methods

2.1.Animals

Forty eight adult male Wistar rats (9-11 weeks old, 230-250 g), were obtained from the animal house of Hamadan University of Medical Sciences. The animals were maintained under the controlled conditions (50± 5% humidity and 20± 2 °C temperature) on a 12:12 light/dark cycle with water and food freely access.

Animal care and treatment procedures were approved by the Ethics committee of the Hamadan University of Medical Sciences (IR.UMSHA.REC.1395.284) and performed according to the Guide for Care and Use of laboratory animals published by the National Institute of Health, United States (NIH Publication No. 85-23, revised 1985).

2.2.Experimental design

The rats were randomly divided into six groups (n=8) including normal vehicle-treated control, EA (25, 50 mg/kg)-treated controls, diabetic and EA (25, 50 mg/kg)-treated diabetics. Diabetes was induced by a single IP injection of 60 mg/kg of STZ (Santa Cruz, USA). Three days after STZ injection, fasting blood glucose level was measured .Rats with a fasting blood glucose level over 250 mg/dl were considered as being diabetic. Then, administration of EA (Sigma Aldrich, USA) (25 and 50 mg/kg) or solvent was started once daily for a period of 4 weeks by oral gavage. The used doses of EA were selected based on a

7

previous report (Kaur, Parveen et al. 2015) and our earlier pilot study. Normal saline containing 10% DMSO was used as the solvent of EA.

Pain associated behaviors involving TF and HP tests were evaluated at the termination of the treatment as explained below.

2.3. TF Test

Nociceptive response was estimated using a tail-flick apparatus (BorjSanat, Tehran, Iran) according to method proposed by D'Amour and Smith(D'AMOUR and SMITH 1941). Briefly, the dorsal surface of the rat tail was exposed to a beam of light as a radiant heat source and the time required to flick the tail from the thermal stimulus was recorded. The cutoff time was set at 10 s in order to prevent any tail tissue injury.

2.4. HP test

Animals were put individually on a hot plate analgesia meter (BorjSanat, Tehran, Iran) with temperature adjusted to 55 ± 0.1 °C. The latency of behaviors such as jumping, hind paw licking or hind paw flicking was recorded as pain response. A 35 s cut-off time was established to avoid tissue damage(Shirafkan, Sarihi et al. 2013).

2.5. Biochemical analysis

At the end of the experiment body weight was measured then blood sample was drawn under anesthesia from inferior venacava for plasma glucose measurement using a kit (Zistshimi, Tehran, Iran) and Oxidative stress biomarkers estimations.

2.5.1. Measurement of MDA

The MDA level in the serum as the end product of lipid peroxidation was measured utilizing the thio-barbituric acid reactive substance assess as described before(Kamal, Gomaa et al.

8

1989). Briefly, trichloroacetic acid was added to serum then the mixture was heated in a boiling water bath for 45 min. following cooling on ice and mixing with n-butanol the sample was centrifuged and its absorbance was read at 532 nm. Obtained results were expressed using tetraethoxypropane standard curve.

2.5.2. Measurement of TAC

The TAC was assessed by using a measurement of the ability to decrease $Fe3+$ to $Fe2+$ via using the ferric reducing ability of plasma (FRAP) test. Here, the medium was exposed to $Fe3+$ and producing $Fe2+$ as an antioxidant activity was noticed. In short, acetate buffer and 4, 6 tripyridyl-s-trazine (TPTZ) were mixed with standards and samples at $37\circ C$ and Reagent that was not added with standard or sample was applied as a blank. Measurement of the absorbance was done at 593nm(Pohanka, Bandouchova et al. 2009).

2.5.3. Measurement of TOS

TOS was measured in the serum by using Erel method in that oxidation of $Fe2+$ to $Fe3+$ by oxidants found in the sample is determined. The color intensity that $Fe3+$ forms with xylenol orange in an acidic medium is related to the amount of sample oxidant molecules. This intensity is measured using a spectrophotometer at 560nm(Erel 2005).

2.6. Statistical analysis

Data were expressed as mean ± standard error of mean(SEM). One-way analysis of variance (ANOVA) followed by post-hoc Tukey test was used to determining the significant differences between groups. P value less than 0.05 was regarded to be significant.

3. Results

3.1. Body weight and plasma glucose level

Before diabetes induction there was not any significant difference in the body weight and plasma glucose level between experimental groups. Untreated diabetic animals had significantly lowered body weight and elevated plasma glucose level at the end of study compared to those of control group (P <0.001). EA treatment with any doses used was not effective in preventing weight loss and plasma glucose raise in diabetic rats just as did not alter the weight and plasma glucose level in control animals (Figure .1 and 2).

3.2. TF Test

A significant reduction in the TF latency was observed in untreated diabetic rats compared with the control group (P<0.05). Diabetic rats treatment with EA at dose of 25 mg/kg afforded marked longer TF latency in comparison with untreated diabetic animals (P<0.05) although TF latency enhancement in diabetics by EA at dose of 50 mg/Kg was not significant. Administration of both doses of EA did not produce any significant change in TF latency in control rats compared with the untreated control group (Figure .3).

3.3. HP Test

Results of HP test revealed a significant decrease in HP latency response in diabetic animals compared with control group (P<0.01). HP latency deficit in diabetic rats was significantly reversed by EA treatment at dose of 50 mg/kg (P<0.05). Treatment of control animals with EA did not alter HP latency response (Figure . 4).

3.4. Oxidative stress markers

MDA levels in serum from the diabetic rats were significantly higher than those of control animals (P<0.05). Moreover, the concentration of TAC in the serum was significantly lower in the diabetic group than in the control group (P<0.05). In addition, the TOS concentration in the serum of diabetic group was significantly higher than that of the control group (P<0.001).

The EA treated diabetic group showed significant decrease in the levels of MDA (P<0.001) at dose of 50 mg/kg and TOS (P<0.001) at both doses(25 and 50 mg/kg) compared to untreated diabetic group. EA administration to diabetic animals caused significant increase in TAC when compared to untreated diabetic animals only at dose of 50 mg/kg (P<0.001). Treatment with EA did not produce any significant difference in Oxidative stress markers in control animals compared to untreated controls (Figure. 5, 6 and 7).

4. Discussion

In the present study, we evaluated the possible therapeutic effect of Ellagic acid in the rats with STZ induced *hyperalgesia* with investigating the role of oxidative stress.

The obtained results represent that four weeks administration of EA in two different doses elicited anti-hyperalgesic and antioxidant effects in a rat model of diabetic hyperalgesia although did not establish anti- hyperglycemic effect.

STZ by impelling direct injury in beta cells of pancreatic islets causes hyperglycemic condition(Solanki and Bhavsar 2015). We observed blood glucose enhancement in rats after STZ reception that is corroborated by other studies(Fatani, Al‑Rejaie et al. 2015, Ahmet, BÜYÜKŞEKERCİ et al. 2016).

11

Overstated hyperalgesic action in reaction to injurious stimuli in diabetic rats has been evidenced, therefore usage of STZ induced diabetic rats as a model of painful diabetic neuropathy has been progressingly noted(Dobretsov, Hastings et al. 2003). Thermal hyperalgesia In the form of unusual perception of heat or pain induced by heat that was indicated by the tail flick and hot-plate tests(Calcutt, Freshwater et al. 2004), was considered as manifestation of diabetic hyperalgesia in our study. In this study we saw a reduction in both tail-flick and hotplate latencies in STZ-induced diabetic rats which was compatible with another reports(Hajializadeh, Nasri et al. 2014, Ahmet, BÜYÜKŞEKERCİ et al. 2016).

The pathophysiology of the diabetic neuropathy is indistinct and abstruse, nevertheless possible factors include hyperglycemia, oxidative stress, inflammation and apoptosis(Ahmed 2005, Green, Pedersen et al. 2011, Rodrigues, Bergamaschi et al. 2011, Yang, Jin et al. 2011). Hyperglycemia induces increased free radicals that affect peripheral and central nervous system(Coppey, Gellett et al. 2002). Excess formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) causes mitochondrial damage in diabetic neurons under hyperglycemic condition(Pacher, Obrosova et al. 2005). Altogether toxicity mediated via enhanced free radicals has been well documented in STZ-diabetic animals(Sen 1995). In addition STZ-diabetes is specified by endogenous antioxidant enzymes disturbance(Toleikis and Godin 1995), so excitation of antioxidant enzymes and reduction of free radicals production may provide protection against some diabetes complication. Reduction of TAC and Increased concentrations of MDA and TOS were observed in diabetic animals in the present study. These data are in agreement with the previous findings that have reported increased level of MDA and TOS and decreased TAC and activity of catalase (CAT) and

12

superoxide dismutase (SOD) In STZ diabetic rats(Çolak, Geyikoğlu et al. 2014, Mehanna, El Askary et al. 2017).

Several antioxidant compounds have been reported to significantly inhibit the induction of painful diabetic neuropathy. Similarly, in our study oral administration of EA for 4 weeks could attenuate the reduction in latencies of TF (at dose of 25 mg/kg) and HP(at dose of 50 mg/kg) in diabetic rats although did not alter thermal pain threshold of control animals.

Antioxidant effect of EA is one of the main mechanisms that might involve the effective analgesic property of this substance. Potent antioxidant properties of EA such as scavenging free radicals, increasing antioxidant enzyme activity, decreasing lipid peroxidation and reducing ROS formation have been demonstrated(Chao, Hsu et al. 2009, Uzar, Alp et al. 2012, Kiasalari, Heydarifard et al. 2017). Accordingly, in the present study biochemical analysis of the serum showed that treatment of diabetic rats with EA decreased the MDA level(at dose of 50 mg/kg) and TOS (at doses of 25 and 50 mg/kg) and increased TAC (at dose of 50 mg/kg).In the same way EA have been shown to Ameliorate learning and memory deficits and mitigate oxidative stress by decreasing MDA and increasing GSH and activity of catalase in a rat model of Alzheimer disease(Kiasalari, Heydarifard et al. 2017). Moreover EA has suppressed oxidative damage of sciatic nerve and brain and reduced the levels of malondialdehyde (MDA) and total oxidant status (TOS) of these tissues in diabetic rats(Uzar, Alp et al. 2012).

In our study EA in the doses and duration that used here did not ameliorate hyperglycemia in diabetic rats therefore obtained anti-hyperalgesic effect is not correlated to blood glucose reduction.

An association between the development of diabetic neuropathy and elevated levels of proinflammatory cytokines on the condition of hyperglycemia in the diabetic rats has been indicated(Fatani, Al‑Rejaie et al. 2015). Furthermore, inflammatory biomarkers over-expression is known to arouse dysfunction and death of neural cells(Li, Wei et al. 2013). Previous studies have displayed that EA exerts protective effects against inflammation and apoptosis.

Rizk reported that EA treatment relieved the neuronal injury induced by toxic agent via ameliorating proinflammatory cytokines as TNF-α, iNOS and attenuating markers of oxidative stress as MDA and exerting antiapoptotic property as reducing caspase-3(Rizk, Masoud et al. 2017).Similarly, it has been shown that treatment by EA protects rats from ischemic brain injury via inhibiting inflammatory reactions and apoptosis(Chen, Zheng et al. 2016). Other study reported neuroprotective effect of EA that could improve nociception and cognition deficiencies in a rat model of Parkinson's disease(Dolatshahi, Farbood et al. 2015).Therefore, such anti-inflammatory and anti-apoptotic mechanisms may have occurred in our study by EA to protect against DN. In the present study EA did not alter pain perception and oxidative stress markers including MDA, TOS and TAC in control rats probably to maintain their physiological balance. This finding is confirmed by this fact that several antioxidant compounds that had no effects on pain threshold and oxidative stress criteria in intact rats indicated considerable effects on these parameters in STZ diabetic rats(Prince and Kamalakkannan 2006, Mirshekar, Roghani et al. 2010).Elevated ROS production has crucial role in pathogenesis of diabetes complications such as hyperalgesia, so EA could improve oxidative status and pain perception in diabetic condition by its antioxidant properties. In

control group there was not imbalance in oxidative status therefore anti-oxidant properties of EA could not alter these parameters.

Finally, one of the limitations of this investigation is induction of diabetes type I through STZ injection, while it could be more appropriate to use transgenic rat but it was impossible for us. Furthermore we studied only antioxidant mechanism while evaluation of additional mechanisms can fortify the utility of EA to ameliorate diabetic hyperalgesia.

5. Conclusion

Taken together, our findings indicated that EA could mitigate hyperalgesic state in diabetic rats and antioxidant properties of EA may be involved in this protective effect although more researches are required to explain other underlined mechanisms.

Acknowledgment

Conflict of interest

The authors declare that there are no conflicts of interest for this research.

References

1. Ahmed, N. (2005). "Advanced glycation endproducts—role in pathology of diabetic complications." Diabetes research and clinical practice 67(1): 3-21.
2. Ahmet, İ., M. BÜYÜKŞEKERCİ and H. B. Ulusoy (2016). "Antinociceptive effect of mirtazapine in rats with diabetic neuropathy." Nöro Psikiyatri Arşivi 53(1): 12.
3. Al-Enazi, M. M. (2013). "Neuroprotective effect of silymarin by modulation of endogenous biomarkers in streptozotocin induced painful diabetic neuropathy." British Journal of Pharmacology and Toxicology 4(3): 110-120.
4. Calcutt, N., J. Freshwater and A. Mizisin (2004). "Prevention of sensory disorders in diabetic Sprague-Dawley rats by aldose reductase inhibition or treatment with ciliary neurotrophic factor." Diabetologia 47(4): 718-724.
5. Cameron, N. E. and M. A. Cotter (2008). "Pro-inflammatory mechanisms in diabetic neuropathy: focus on the nuclear factor kappa B pathway." Current drug targets 9(1): 60-67.
6. Chao, P.-c., C.-c. Hsu and M.-c. Yin (2009). "Anti-inflammatory and anti-coagulatory activities of caffeic acid and ellagic acid in cardiac tissue of diabetic mice." Nutrition & metabolism 6(1): 33.
7. Chen, S.-R. and H.-L. Pan (2002). "Hypersensitivity of spinothalamic tract neurons associated with diabetic neuropathic pain in rats." Journal of neurophysiology 87(6): 2726-2733.
8. Chen, S.-Y., K. Zheng and Z.-q. Wang (2016). "Neuroprotective effects of ellagic acid on neonatal hypoxic brain injury via inhibition of inflammatory mediators and down-regulation of JNK/p38 MAPK activation." Tropical Journal of Pharmaceutical Research 15(2): 241-251.
9. Çolak, S., F. Geyikoğlu, A. Aslan and G. Y. Deniz (2014). "Effects of lichen extracts on haematological parameters of rats with experimental insulin-dependent diabetes mellitus." Toxicology and industrial health 30(10): 878-887.
10. Coppey, L. J., J. S. Gellett, E. P. Davidson, J. A. Dunlap and M. A. Yorek (2002). "Effect of treating streptozotocin-induced diabetic rats with sorbinil, myo-inositol or aminoguanidine on endoneurial blood flow, motor nerve conduction velocity and vascular function of epineurial arterioles of the sciatic nerve." Experimental Diabetes Research 3(1): 21-36.
11. Cornélio Favarin, D., M. Martins Teixeira, E. Lemos de Andrade, C. de Freitas Alves, J. E. Lazo Chica, C. Artério Sorgi, L. H. Faccioli and A. Paula Rogerio (2013). "Anti-inflammatory effects of ellagic acid on acute lung injury induced by acid in mice." Mediators of inflammation 2013.
12. Cunha, J. M., C. G. Jolivalt, K. M. Ramos, J. A. Gregory, N. A. Calcutt and A. P. Mizisin (2008). "Elevated lipid peroxidation and DNA oxidation in nerve from diabetic rats: effects of aldose reductase inhibition, insulin, and neurotrophic factors." Metabolism-Clinical and Experimental 57(7): 873-881.
13. D'AMOUR, F. E. and D. L. SMITH (1941). "A method for determining loss of pain sensation." Journal of Pharmacology and Experimental Therapeutics 72(1): 74-79.

14. Dobretsov, M., S. L. Hastings, D. Romanovsky, J. R. Stimers and J.-M. Zhang (2003). "Mechanical hyperalgesia in rat models of systemic and local hyperglycemia." Brain research 960(1-2): 174-183.

15. Dobretsov, M., S. L. Hastings, J. Stimers and J.-M. Zhang (2001). "Mechanical hyperalgesia in rats with chronic perfusion of lumbar dorsal root ganglion with hyperglycemic solution." Journal of neuroscience methods 110(1-2): 9-15.

16. Dolatshahi, M., Y. Farbood, A. Sarkaki, S. M. T. Mansouri and A. Khodadadi (2015). "Ellagic acid improves hyperalgesia and cognitive deficiency in 6-hydroxidopamine induced rat model of Parkinson's disease." Iranian journal of basic medical sciences 18(1): 38.

17. Erel, O. (2005). "A new automated colorimetric method for measuring total oxidant status." Clinical biochemistry 38(12): 1103-1111.

18. Fatani, A. J., S. S. Al‑Rejaie, H. M. Abuohashish, A. Al‑Assaf, M. Y. Parmar, M. S. Ola and M. M. Ahmed (2015). "Neuroprotective effects of Gymnema sylvestre on streptozotocin-induced diabetic neuropathy in rats." Experimental and therapeutic medicine 9(5): 1670-1678.

19. Green, C. J., M. Pedersen, B. K. Pedersen and C. Scheele (2011). "Elevated NF-κB activation is conserved in human myocytes cultured from obese type 2 diabetic patients and attenuated by AMP-activated protein kinase." Diabetes 60(11): 2810-2819.

20. Hajializadeh, Z., S. Nasri, A. Kaeidi, V. Sheibani, B. Rasoulian and S. Esmaeili-Mahani (2014). "Inhibitory effect of Thymus caramanicus Jalas on hyperglycemia-induced apoptosis in in vitro and in vivo models of diabetic neuropathic pain." Journal of ethnopharmacology 153(3): 596-603.

21. Hasanein, P. and F. Fazeli (2014). "Role of naringenin in protection against diabetic hyperalgesia and tactile allodynia in male Wistar rats." Journal of physiology and biochemistry 70(4): 997-1006.

22. Kamal, A.-A. M., A. Gomaa, M. El Khafif and A. S. Hammad (1989). "Plasma lipid peroxides among workers exposed to silica or asbestos dusts." Environmental Research 49(2): 173-180.

23. Kaur, R., S. Mehan, D. Khanna and S. Kalra (2015). "Polyphenol Ellagic Acid–Targeting To Brain: A Hidden Treasure." International Journal of Neurology Research 1(3): 141-152.

24. Kaur, R., S. Parveen, S. Mehan, D. Khanna and S. Kalra (2015). "Neuroprotective effect of ellagic acid against chronically scopolamine induced Alzheimer's type memory and cognitive dysfunctions: possible behavioural and biochemical evidences." Int. J. Preven. Med. Res 1: 45-64.

25. Khan, G., S.-R. Chen and H.-L. Pan (2002). "Role of primary afferent nerves in allodynia caused by diabetic neuropathy in rats." Neuroscience 114(2): 291-299.

26. Kiasalari, Z., R. Heydarifard, M. Khalili, S. Afshin-Majd, T. Baluchnejadmojarad, E. Zahedi, A. Sanaierad and M. Roghani (2017). "Ellagic acid ameliorates learning and memory deficits in a rat model of Alzheimer's disease: an exploration of underlying mechanisms." Psychopharmacology 234(12): 1841-1852.

27. Kuhad, A., M. Bishnoi, V. Tiwari and K. Chopra (2009). "Suppression of NF-κβ signaling pathway by tocotrienol can prevent diabetes associated cognitive deficits." Pharmacology Biochemistry and Behavior 92(2): 251-259.

28. Li, J., G.-H. Wei, H. Huang, Y.-P. Lan, B. Liu, H. Liu, W. Zhang and Y.-X. Zuo (2013). "Nerve injury-related autoimmunity activation leads to chronic inflammation and chronic neuropathic pain." Anesthesiology: The Journal of the American Society of Anesthesiologists 118(2): 416-429.

29. Mansouri, M. T., B. Naghizadeh, B. Ghorbanzadeh and Y. Farbood (2013). "Central and peripheral antinociceptive effects of ellagic acid in different animal models of pain." European journal of pharmacology 707(1-3): 46-53.

30. Mehanna, O. M., A. El Askary, S. Al-Shehri and B. El-Esawy (2017). "Effect of phosphodiesterase inhibitors on renal functions and oxidant/antioxidant parameters in streptozocin-induced diabetic rats." Archives of physiology and biochemistry: 1-6.

31. Mirshekar, M., M. Roghani, M. Khalili, T. Baluchnejadmojarad and S. A. Moazzen (2010). "Chronic oral pelargonidin alleviates streptozotocin-induced diabetic neuropathic hyperalgesia in rat: involvement of oxidative stress." Iranian biomedical journal 14(1-2): 33.

32. Motamedrad, M., A. Shokouhifar, M. Hemmati and M. Moossavi (2019). "The regulatory effect of saffron stigma on the gene expression of the glucose metabolism key enzymes and stress proteins in streptozotocin-induced diabetic rats." Research in pharmaceutical sciences 14(3): 255.

33. Pacher, P., I. G. Obrosova, J. G. Mabley and C. Szabó (2005). "Role of nitrosative stress and peroxynitrite in the pathogenesis of diabetic complications. Emerging new therapeutical strategies." Current medicinal chemistry 12(3): 267-275.

34. Pohanka, M., H. Bandouchova, J. Sobotka, J. Sedlackova, I. Soukupova and J. Pikula (2009). "Ferric reducing antioxidant power and square wave voltammetry for assay of low molecular weight antioxidants in blood plasma: Performance and comparison of methods." Sensors 9(11): 9094-9103.

35. Prince, P. and N. Kamalakkannan (2006). "Rutin improves glucose homeostasis in streptozotocin diabetic tissues by altering glycolytic and gluconeogenic enzymes." Journal of biochemical and molecular toxicology 20(2): 96-102.

36. Rizk, H. A., M. A. Masoud and O. W. Maher (2017). "Prophylactic effects of ellagic acid and rosmarinic acid on doxorubicin-induced neurotoxicity in rats." Journal of biochemical and molecular toxicology 31(12).

37. Rodrigues, A. M., C. T. Bergamaschi, R. C. Araújo, M. G. Mouro, T. S. Rosa and E. M. Higa (2011). "Effects of training and nitric oxide on diabetic nephropathy progression in type I diabetic rats." Experimental Biology and Medicine 236(10): 1180-1187.

38. Sen, C. K. (1995). "Oxygen toxicity and antioxidants: state of the art." Indian journal of physiology and pharmacology 39(3): 177-196.

39. Shirafkan, T., A. Sarihi and A. Komaki (2013). "Role of capsaicin receptors in periaqueductal gray on pain modulation in diabetic rats." Feyz Journal of Kashan University of Medical Sciences 17(4).

40. Solanki, N. D. and S. K. Bhavsar (2015). "An evaluation of the protective role of Ficus racemosa Linn. in streptozotocin-induced diabetic neuropathy with neurodegeneration." Indian journal of pharmacology 47(6): 610.

41. Toleikis, P. M. and D. V. Godin (1995). "Alteration of antioxidant status in diabetic rats by chronic exposure to psychological stressors." Pharmacology Biochemistry and Behavior 52(2): 355-366.
42. Uzar, E., H. Alp, M. U. Cevik, U. Fırat, O. Evliyaoglu, A. Tufek and Y. Altun (2012). "Ellagic acid attenuates oxidative stress on brain and sciatic nerve and improves histopathology of brain in streptozotocin-induced diabetic rats." Neurological Sciences 33(3): 567-574.
43. Veves, A., M. Backonja and R. A. Malik (2008). "Painful diabetic neuropathy: epidemiology, natural history, early diagnosis, and treatment options." Pain medicine 9(6): 660-674.
44. Yang, H., X. Jin, C. W. K. Lam and S.-K. Yan (2011). "Oxidative stress and diabetes mellitus." Clinical chemistry and laboratory medicine 49(11): 1773-1782.
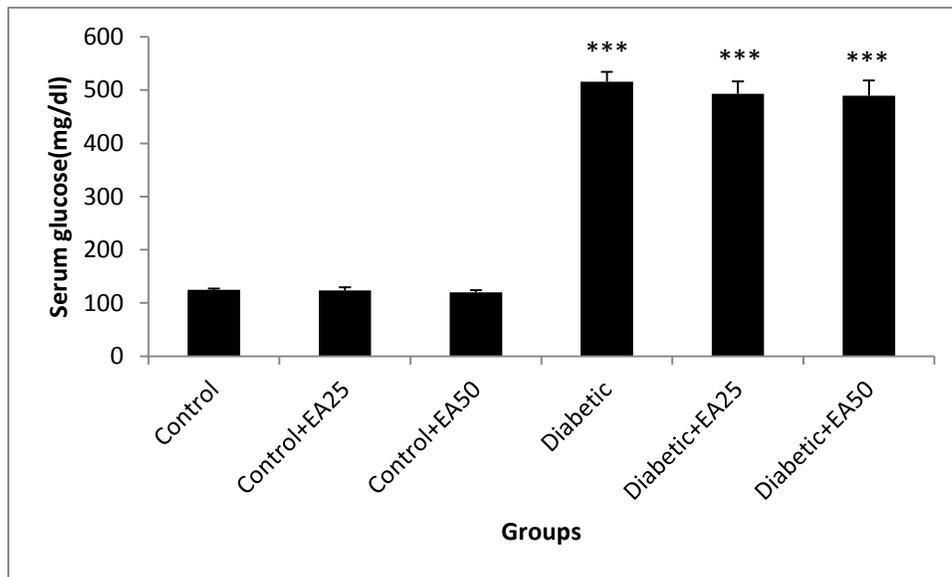
Figure. 1.Effect of EA treatment on Body weight. All data represent mean ± SEM. ***: p < 0.001 (as compared with control).
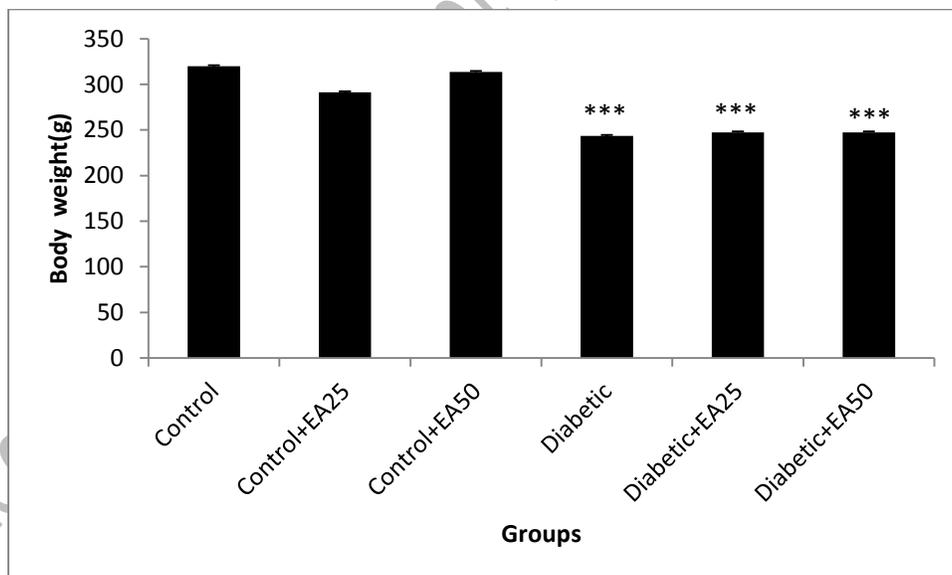
Figure. 2.Effect of EA treatment on Serum glucose. All data represent mean ± SEM. ***: p < 0.001 (as compared with control).
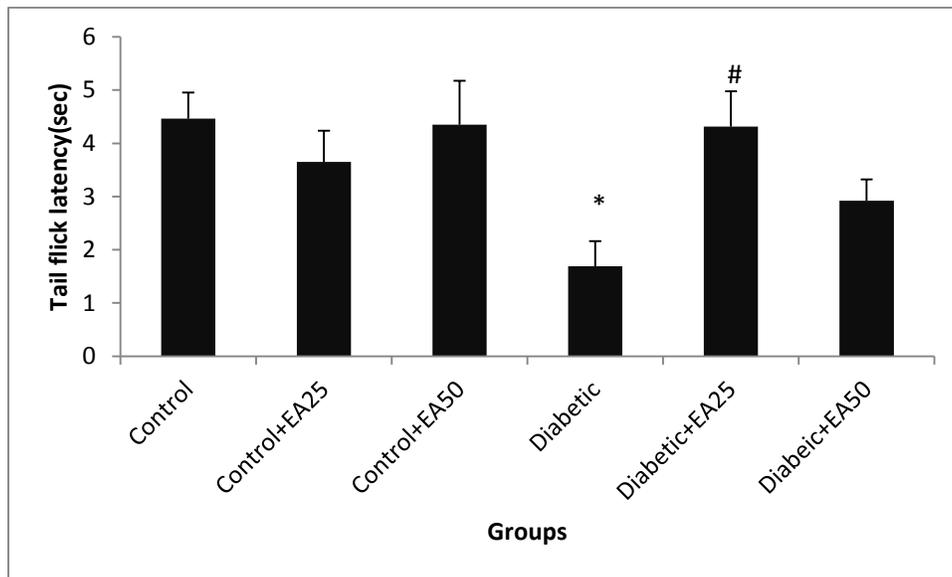
Figure. 3.Effect of EA treatment on hyperalgesia in tail flick test. All data represent mean ±
SEM. *: p < 0.05 (as compared with control); #: p < 0.05 (as compared with diabetic).
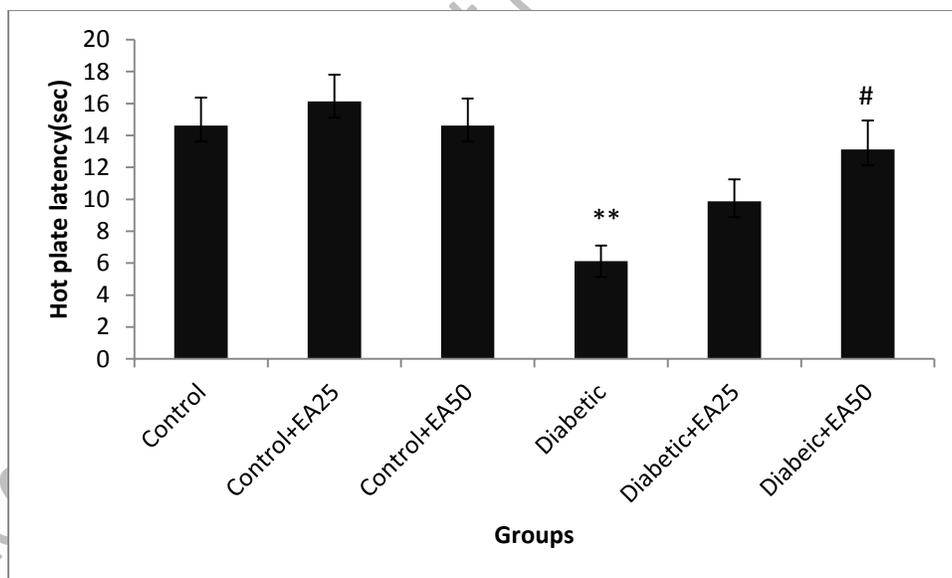
Figure. 4.Effect of EA treatment on hyperalgesia in hot plate test. All data represent mean ±
SEM. **: p < 0.01 (as compared with control); #: p < 0.05 (as compared with diabetic).
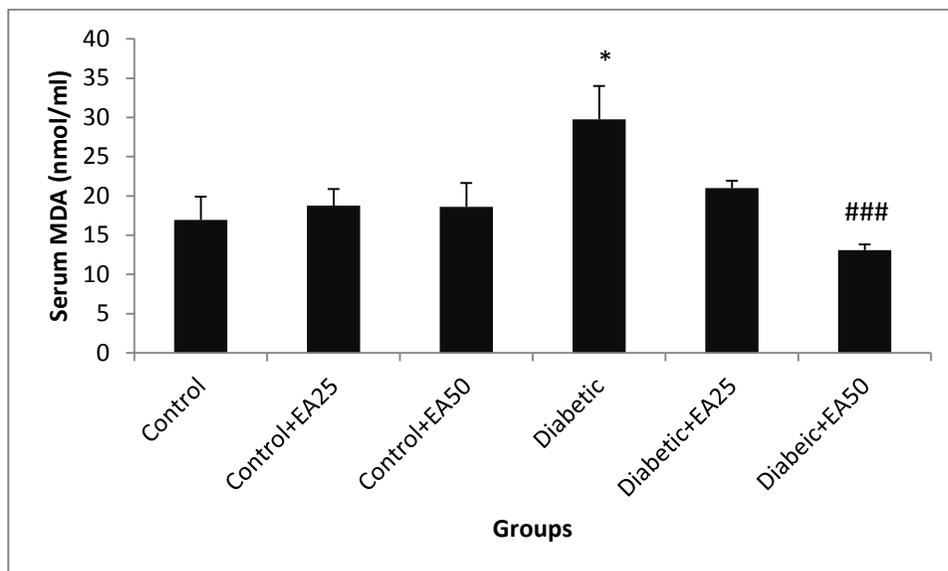
Figure. 5.Effect of EA treatment on Serum MDA. All data represent mean ± SEM. *: $p <$ 0.05 (as compared with control); ###: $p < 0.001$ (as compared with diabetic).
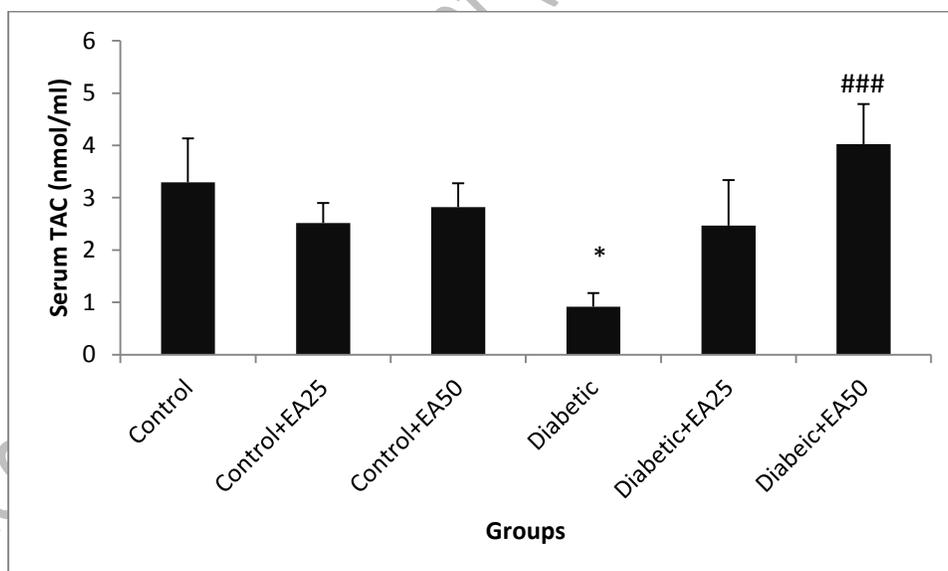

Figure. 6.Effect of EA treatment on Serum TAC. All data represent mean ± SEM. *: $p <$ 0.05 (as compared with control); ###: $p < 0.001$ (as compared with diabetic).
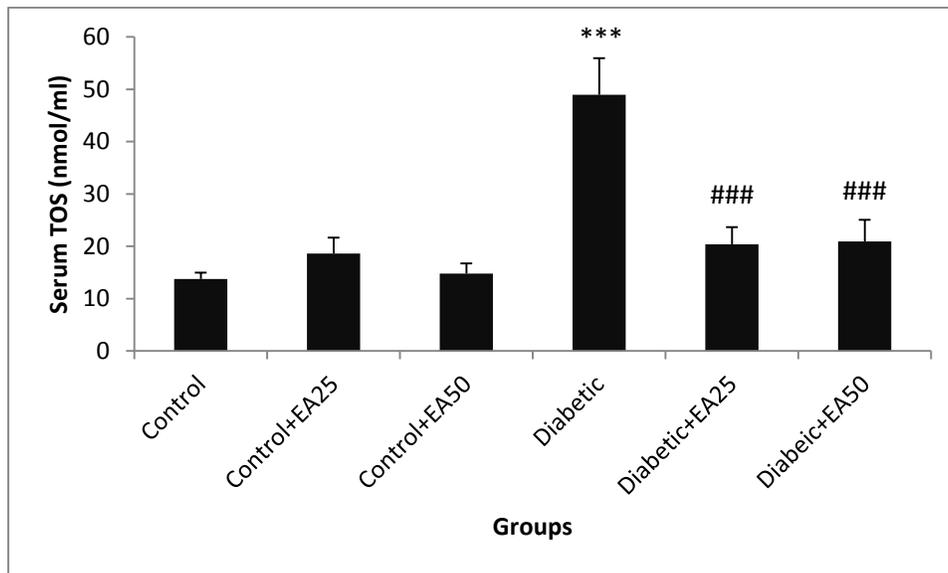
Figure. 7.Effect of EA treatment on Serum TOS. All data represent mean ± SEM. ***: p < 0.001 (as compared with control); ###: p < 0.001 (as compared with diabetic).