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Original Article

Effects of Ginger on Advanced Glycation End-Products and Inflammation in the Ovarian Tissue of Streptozotocin-Induced Diabetic Rats

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Abstract

Background and Aim: Advanced glycation end products (AGEs) and inflammation play a crucial role in the progression of diabetic complications, including ovarian disorders. The current research investigated the potential impact of hydroalcoholic extract of ginger (Zingiber Officinale) on hyperglycemia-induced AGEs and inflammatory markers.

Methods: A total of 96 female Wistar rats were randomized into four groups (n=24 in each group) as follows: 1) control, 2) diabetes, 3) diabetes + 200 mg/kg ginger, 4) diabetes + 400 mg/kg ginger. Streptozotocin (STZ) - induced diabetic rats, as our experimental model for diabetes, orally received 200 or 400 mg/kg/day ginger extract for eight weeks. At the end of the treatment period, body weight, ovarian weight, serum AGEs level, ovarian RAGE, IL-1β, and TNF-α mRNA levels were measured.

Results: At the end of the study, diabetic rats exhibited an obvious decrease in body weight ($P < 0.01$) and ovarian weight ($P < 0.01$) compared to normal rats. However, ginger supplementation (200 mg/kg) exhibited a significant increment in ovarian weight ($P < 0.05$) and body weight ($P < 0.01$). These changes were also more pronounced in the diabetic rats treated with 400 mg/kg ginger extract ($P < 0.01$). Serum AGEs ($P < 0.001$) and ovarian RAGE ($P < 0.01$), IL-1β ($P < 0.01$), and TNF-α ($P < 0.01$) mRNA levels were significantly elevated in diabetic rats compared with control group. Administration of the diabetic group with 200 mg/kg ginger extract significantly ameliorated the serum level of AGEs ($P < 0.01$) and the transcript levels of RAGE ($P < 0.05$), TNF-α ($P < 0.01$) and IL-1β ($P < 0.05$). The 400 mg/kg ginger extract dose remarkably alleviated AGEs ($P < 0.001$) in the serum and RAGE ($P < 0.01$), TNF-α ($P < 0.01$), and IL-1β ($P < 0.01$) in the ovary of diabetic rats.

Conclusion: The present study's findings revealed that daily administration of ginger extract reduces AGEs and the transcript levels of RAGE and inflammatory markers in the STZ- induced female rats.

Keywords: Diabetes; Ovary; Advanced Glycation End Products; Streptozotocin; Inflammation

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Introduction

Diabetes mellitus refers to a group of metabolic diseases described by defects in insulin secretion, insulin function, or both (1). Chronic hyperglycemia is associated with damage to the human body organs such as kidneys, eyes, heart, nerves, blood vessels, and ovary (2-4). Extensive evidence suggested that advanced glycation end products (AGEs) are one of the main causes of ovarian dysfunction in diabetic conditions (5-7). AGEs, called "glycotoxins," are byproducts of the Millard reaction in which the carbonyl group of carbohydrates interacts non- enzymatically with lipids and protein (8). Some of the most usual AGEs formed in the body include carboxymethyl lysine, carboxyethyl lysine, pentosidine, and hydroimidazolones (9, 10). AGEs can exert a spectrum of pathological effects primarily through interaction with the receptor for AGE (RAGE) on the target cell's surface (11). The RAGE gene (AGER) is located on chromosome 6 and is one of

the most frequent genetic susceptibility loci for diabetes development (12, 13). Under a diabetic state, RAGE binds to its ligands and exerts various cellular activities, including inflammation, proliferation, and autophagy (14). Increased RAGE expression leads to tissue damage in pathological conditions, immune reactions, and diabetes. In the ovary, the induction of RAGE activates nuclear transcription factor (NF-κB), followed by transcription of many pro-inflammatory genes, including tumor necrosis factor (TNF) -α and interleukin (IL-) -1β (15, 16). The imbalance between pro- and anti- inflammatory cytokines leads to delayed follicular maturation, altered steroidogenesis, and ovarian dysfunction (17, 18). Therefore, counteracting the AGE- RAGE axis might result in preventing diabetes-induced ovarian damage. The potential evidence has suggested that medicinal plants effectively manage various diseases, including diabetes (18, 19). For several hundred years, Ginger, Zingiber Officinale Roscoe has been frequently utilized as spice or for medicinal purposes. Generally, this medicinal plant exerts anti-inflammatory and antioxidant activities and modulates gene expression. In addition, several studies have revealed that ginger can delay the development of diabetic complications (20, 21).

Given that the anti- diabetic and anti- inflammatory effects of ginger have been proven, so far, limited studies have been conducted on the protective effect of ginger against the damage caused by hyperglycemia on the female reproductive system. According to the above, the current work was designed for the first time to explore the impacts of ginger extract on the hyperglycemia- induced AGEs- RAGE axis and inflammatory markers in the serum and ovary of female STZ- stimulated diabetic rats.

Methods

Preparation of the ginger extract

Dried ginger roots were purchased from Gol Darou Company, Isfahan, Iran. Briefly, 200 g of the ginger root was weighed and then powdered using an electrical blender. The powdered roots were soaked in 1400 ml of 70% methanol solution (420 ml of distilled water + 980 ml of methanol) for three days at room temperature and filtered using a Whatman filter. Finally, the filtered extract was evaporated using a rotary evaporator and then lyophilized, and stored at- 20°C until use (22).

Animals

All protocols were approved by the ethical committee of animal and research of Garmsar Branch, Islamic Aza University (IR.IAU.SHAHROOD.REC.1400.069). In this interventional study, 96 female Wistar rats with an average weight of 220-250 g (8-10 weeks of age) were prepared from the research center of Garmsar Branch, Islamic Azad University.

Before the experiments, all animals were housed in a 12-hour light/dark cycle and 25±2°C for one week to adapt to the laboratory environment. All rats had free access to food pellets and water during the experiments.

Induction of diabetes

The rats were weighed after the adaptation period, and 72 were randomly selected for diabetes induction. After 12 hours of fasting, 60 mg/kg streptozotocin (STZ) dissolved in 0.1 M citrate buffer solution was intraperitoneally (i.p.) injected with an insulin syringe (23). Three days after the STZ injection, fasting blood glucose was evaluated through the tail vein and a glucometer. Diabetes was confirmed based on blood glucose concentration above 220 mg/dl. Oral administration of the ginger extract was initiated two weeks after the STZ injection and continued for eight weeks.

Experimental Design

The rats were randomized into four groups of 24 animals in each group as follows:

1. Control group: Normal rats were gavaged with distilled water daily for eight weeks.
2. Diabetic group: Rats were gavaged with distilled water daily for eight weeks.
3. Diabetes + 200 mg/kg ginger: Rats were gavaged with 200 mg/kg ginger extract daily for eight weeks.
4. Diabetes + 400 mg/kg ginger: Rats were gavaged with 400 mg/kg ginger extract daily for 8 weeks.

Ginger extract was dissolved in 1.5 ml/kg of distilled water. The ginger extract doses were selected based on previous studies (22, 24). At the end of the eighth week, the rats were immediately weighted by a digital scale and then in order to humanly killing, anesthetized by i.p. injection of ketamine (60 mg/kg) and xylazine (12 mg/kg). The blood samples were directly taken from cardiac puncture, centrifuged (15 minutes, 3500 rpm), and stored at- 80°C in the freezer until future experiments. Subsequently, the ovaries were immediately isolated, weighed, and then stored at -80° C in the freezer until use.

Measurement of AGEs

Serum AGEs were measured by the Kalousova method (25). According to this method, the serum sample was diluted by phosphate buffer (pH = 7.4) at a ratio of 1 to 50, and then, the fluorescent intensity was measured at 440 nm emission wavelength and 350 nm excitation wavelength by spectrofluorimeter. The results were reported as a percentage of fluorescent emission intensity (FI %).

Quantitative Real-Time PCR

After collecting ovarian tissue samples, total RNA was isolated using a total RNA isolation kit (Yekta Tajhiz, Iran), following the provided recommendations. To synthesize cDNA from RNAs isolated from ovarian tissue, the cDNA synthesis kit was used (Yekta Tajhiz, Iran). Then, a qRT- PCR reaction was applied using 100 ng of cDNA (template), specific primers, and SYBR® Green qPCR Master Mix (Yekta Tajhiz, Iran), according to the standard protocol. The relative expression of the desired genes was measured using the $2^{(-\text{Delta Delta C (T)})}$ method. The glyceraldehyde -3-phosphate dehydrogenase (GAPDH) gene was employed as a reference gene. The sequence of primers is displayed in Table 1.

Table 1. The sequences of primers

Genes	Primer sequences (5'- 3')
RAGE	Forward: CCCCAATGGTTCACTCCTCC Reverse: CCCATCCAAGTGCCAGCTAA
IL-1β	Forward: GACTCGTGGGATGATGACGAC Reverse: GAGCTTTCAGCTCACATGGGT
TNF-α	Forward: ATCCGAGATGTGGAACTGGC Reverse: ACTGATGAGAGGGAGCCCAT
GAPDH	Forward: TGCCAAGTATGATGACATCAAGAAG Reverse: AGCCCAGGATGCCCTTTAGT

Statistical analysis

Statistical analyses were done using GraphPad Prism software version 8 (Inc.; San Diego, CA, USA). The findings are reported as mean ±standard deviation (SD), and their normal distribution was checked using the Kolmogorov-Smirnov test. For statistical differences among experimental groups, one-way analysis of variance (ANOVA) was utilized, followed by post- hoc test (Tukey). P-value < 0.05 was reported as statistically significant level.

Results

The effect of ginger extract on the increase or decrease of body weight and ovarian weight of diabetic rats

As depicted in Figure 1A, diabetic rats exhibited an obvious decrease in body weight compared to healthy rats ($P < 0.01$). In contrast, diabetic group recipients of ginger extract exhibited a notable improvement in body weight (Figure 1A; $P < 0.01$).

Diabetic animals showed a significant decrease in ovarian weight compared to the healthy rats (Figure 1B; ($P < 0.01$). In contrast, diabetic rats receiving 200 mg/kg ($P < 0.05$) and 400 mg/kg ($P < 0.01$) ginger exhibited a great increase in ovarian weight (Figure 1B).

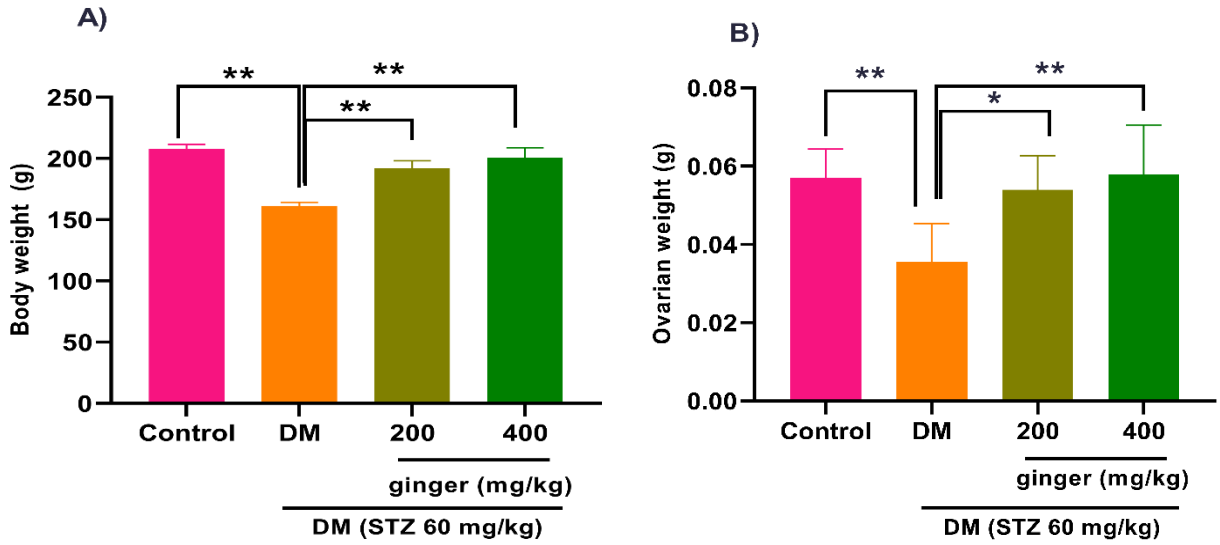

Figure 1. The ginger extract increases (A) body weight and (B) ovarian weight in the studied groups. The findings are reported as mean ± standard deviation (SD). $^{*}P < 0.05$ and $^{**}P < 0.01$ were statistically significant.

Ginger extract attenuates the AGE amount in the serum of diabetic rats

At the end of the study, STZ led to a marked increase in the AGEs amount in the diabetic group compared to the healthy group (Figure 2; $P < 0.001$). In contrast, these changes were considerably decreased in the serum of diabetic rats' recipients of 200 mg/kg ($P < 0.01$) and 400 mg/kg ($P < 0.001$) ginger extract for eight weeks (Figure 3).

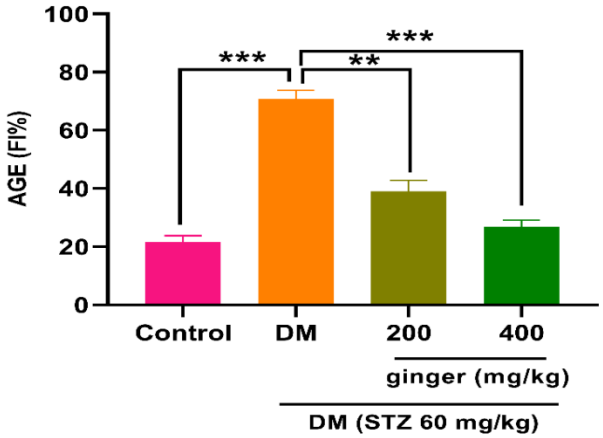

Figure 2. Effects of the ginger extract on the serum AGEs in the studied groups. The results are reported as the percentage of fluorescence intensity of AGEs (F %). $^{*}P < 0.01$ and $^{**}P < 0.001$ were statistically significant.

Ginger extract prevents RAGE mRNA levels in the ovary of diabetic rats

AGEs can bind to the RAGE on the surface of target cells and activate intracellular signaling cascades. Therefore, we evaluated the mRNA levels of RAGE in the ovary of diabetic rats. There was a notable increase in RAGE mRNA levels (Figure 3; $P < 0.001$) in diabetic rats' ovaries compared to healthy rats. These changes were markedly attenuated in the ovary of diabetic rats treated with ginger extract for eight weeks (Figure 3).

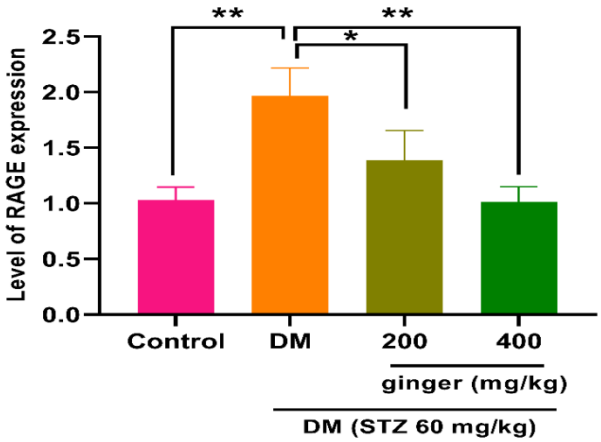

Figure 3. Ginger extract alleviates the transcript level of RAGE in the studied groups. The findings are reported as mean ± standard deviation (SD). $^{*}P < 0.05$ and $^{**}P < 0.01$ were statistically significant.

Ginger extract prevents inflammation in the ovary of diabetic rats

As illustrated in Figure 4A and Figure 4B, diabetic rats exhibited an obvious enhancement in the ovarian TNF-α and IL-1β mRNA levels compared to the healthy rats ($P < 0.01$). Administration of the diabetic group with 200 mg/kg ginger extract markedly ameliorated the transcript levels of TNF-α ($P < 0.01$) and IL-1β ($P < 0.05$). The 400 mg/kg finger extract dose remarkably alleviated TNF-α and IL-1β in the ovary of diabetic rats ($P < 0.01$; Figures 4A and 4B).

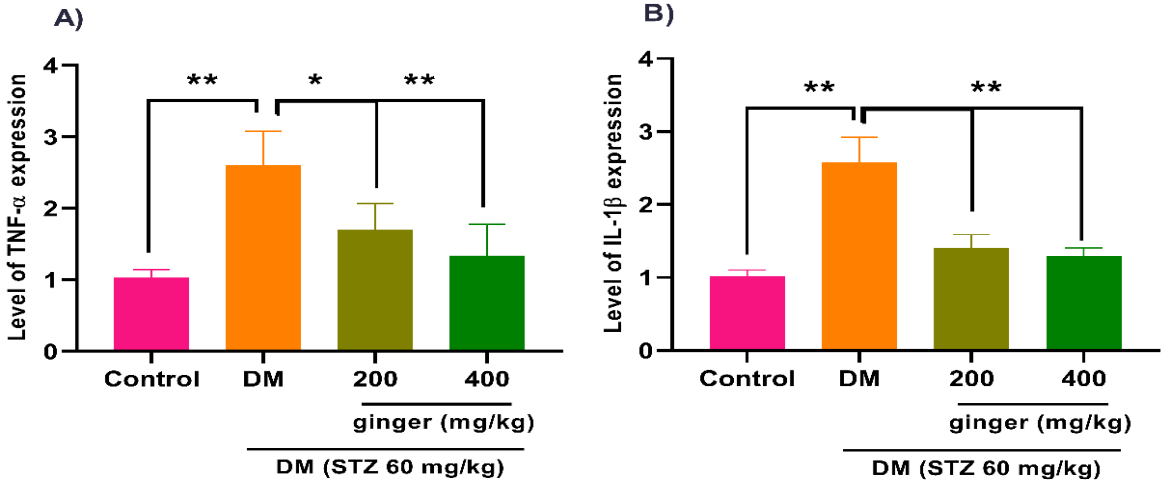

Figure 4. Effects of the ginger extract on the transcript level of (A) TNF-α and (B) IL-1β in the studied groups. The findings are reported as mean ± standard deviation (SD). $^{*}P < 0.05$ and $^{**}P < 0.01$ were statistically significant.

Discussion

Diabetes is linked to various diseases, including reproductive disorders such as abnormal folliculogenesis and steroidogenesis, anovulation, and miscarriage (26-28). Several studies have investigated the potential role of ginger extract in the prevention and management of diabetes and its complications (29, 30). The current research investigated the potential impact of ginger supplementation on AGEs and inflammatory markers in STZ diabetic rats.

STZ is an alkylating compound that causes DNA damage and especially destroys pancreatic β-cells (31). Evidence has shown that a single dose of STZ in a rat will cause β-cell loss of the islets followed by chronic hyperglycemia (32, 33). In the present work, our findings manifested that a single dose of STZ (60 mg/kg) considerably increased fasting glucose concentration in the blood of rats. Chronic hyperglycemia was observed until the end of the study. A significant increase in fasting blood glucose in the diabetic group may be due to damage to pancreatic β cells by STZ (34, 35).

Dehydration and weight loss have been reported in the diabetic state (36). In the current study, we observed significant weight loss in the diabetic rat group. This finding was consistent with the data from previous reports. According to the results presented by Dekel et al., the diabetic mice exhibited approximately 40% body weight loss within 14 days of the STZ injection. This finding could be due to muscle loss due to insufficient carbohydrates as an energy source (37). However, Al Hroob et al. reported that treatment with 400 mg/kg and 800 mg/kg ginger greatly improved the body weight of diabetic rats (38). Our results were also similar to the findings of Al Hroob and his co-workers. Herein, we also indicated that the ginger supplementation caused a notable increase in the body weight of diabetic rats, which could be a direct result of improving blood glucose levels. The protective impact of ginger extract on body weight loss in diabetic rats may reveal the ability of ginger to reverse gluconeogenesis and glycogenolysis via mimicry of insulin action (39-41).

It has also been reported that STZ- induced diabetes diminishes the number of antral follicles and enhances atretic follicles, leading to destructive effects on ovarian tissue (42, 43). One of the important indicators for evaluating ovarian function in diabetic conditions is ovarian weight alteration, resulting from changes in ovarian structure and follicle numbers. Mehrabianfar et al. observed the loss of ovarian weight in an STZ-induced diabetic rat model. Their results also showed that ovarian tissue was damaged by diabetes, and metformin partially improved this injury (44). In line with the results of Mehrabianfar et al., in our study, ovarian weight loss was seen in diabetic rats. On the other hand, the diabetic group treated with ginger showed significant weight gain.

Diamanti- Kandarakis and her research group have indicated that the AGE-RAGE axis may be responsible for ovulation failure, characteristic of polycystic ovary syndrome (PCOS) (45). In ovarian tissues of PCOS women, the expression of AGE and RAGE was increased in the theca and granulosa cell layers (46, 47). The binding of AGEs to their receptors on the surface of ovarian cells causes cellular oxidative stress and increases inflammation. Chronic inflammation found in the cytoplasm of ovarian cells is a characteristic of cellular necrosis. However, AGE restriction in humans is associated with a remarkable reduction in inflammatory biomarkers, such as plasma CRP and TNF-α (48). Many polyphenols such as catechins, proanthocyanidins, anthocyanins, acetylbenoids, and flavonols have been identified to prevent the formation of AGEs (49). Nonaka et al. demonstrated that the levels of RAGE, IL-6, and p65 were increased in human gingival fibroblast cells (HGFs) treated with AGEs. Their results also suggested that 6- shogaol inhibits inflammatory responses caused by AGEs and may have protective effects on periodontitis induced by diabetes mellitus (50). We showed that the level of AGEs and the mRNA level related to RAGE, TNF-α, and IL-1β genes were significantly raised in STZ- induced rats, but the administration of ginger extract significantly alleviated the level of these compounds.

One of the limitations of this study is the lack of evaluation of RAGE and inflammatory markers at the protein levels. Also, AGE levels were not evaluated in ovarian tissue samples.

Conclusion

The findings of this study exhibited that daily administration of ginger extract ameliorated the serum AGEs and the ovarian transcript levels of RAGE and inflammatory markers in the STZ- induced female rats, which are the potential risk factors for ovarian complications. It is suggested that in future studies, the effect of ginger on AGE- RAGE- related signaling pathways in the ovaries of diabetic rats be investigated.

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Conflict of Interest

The authors declare that there is no conflict of interests

associated with this work.

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Ethics

All protocols were approved by the ethical committee of animal and research of Garmsar Branch, Islamic Azad University.

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References

1. Association AD. Introduction: standards of medical care in diabetes—2020. Am Diabetes Assoc; 2020. p. S1-S2.

2. Forouhi NG, Wareham NJ. Epidemiology of diabetes. Medicine. 2019;47(1):22-7.

3. Eckel RH, Bornfeldt KE, Goldberg IJ. Cardiovascular disease in diabetes, beyond glucose. Cell Metabolism. 2021;33(8):1519-45.

4. Hill-Briggs F, Adler NE, Berkowitz SA, Chin MH, Gary-Webb TL, Navas-Acien A, et al. Social determinants of health and diabetes: a scientific review. Diabetes Care. 2021;44(1):258-79.

5. Lin P-H, Chang C-C, Wu K-H, Shih C-K, Chiang W, Chen H-Y, et al. Dietary glycotoxins, advanced glycation end products, inhibit cell proliferation and progesterone secretion in ovarian granulosa cells and mimic PCOS-like symptoms. Biomolecules. 2019;9(8):327.

6. Zhu J-l, Cai Y-q, Long S-l, Chen Z, Mo Z-c. The role of advanced glycation end products in human infertility. Life Sciences. 2020;255:117830.

7. Fattah A, Morovati A, Niknam Z, Mashouri L, Asadi A, Rizi ST, et al. The Synergistic Combination of Cisplatin and Piperine Induces Apoptosis in MCF-7 Cell Line. Iranian Journal of Public Health. 2021;50(5):1037-47.

8. Perrone A, Giovino A, Benny J, Martinelli F. Advanced glycation end products (AGEs): biochemistry, signaling, analytical methods, and epigenetic effects. Oxidative medicine and cellular longevity. 2020;2020.

9. Nowotny K, Schröter D, Schreiner M, Grune T. Dietary advanced glycation end products and their relevance for human health. Aging research reviews. 2018;47:55-66.

10. Maleki N, Ravesh RK, Salehiyeh S, Faiz AF, Ebrahimi M, Sharbati A, et al. Comparative effects of estrogen and silibinin on cardiovascular risk biomarkers in ovariectomized rats. Gene. 2022;823:146365.

11. Zhang Q, Wang Y, Fu L. Dietary advanced glycation end-products: Perspectives linking food processing with health implications. Comprehensive Reviews in Food Science and Food Safety. 2020;19(5):2559-87.

12. Le Bagge S, Fotheringham AK, Leung SS, Forbes JM. Targeting the receptor for advanced glycation end products (RAGE) in type 1 diabetes. Medicinal research reviews. 2020;40(4):1200-19.

13. Musavi H, Abazari O, Safaee MS, Variji A, Koohshekan B, Kalaki-Jouybari F, et al. Mechanisms of COVID-19 Entry into the Cell: Potential Therapeutic Approaches Based on Virus Entry Inhibition in COVID-19 Patients with Underlying Diseases. Iranian journal of allergy, asthma, and immunology. 2021;20(1):11-23.

14. Cepas V, Collino M, Mayo JC, Sainz RM. Redox signaling and advanced glycation endproducts (AGEs) in diet-related diseases. Antioxidants. 2020;9(2):142.

15. Parwani K, Mandal P. Role of advanced glycation end products and insulin resistance in diabetic nephropathy. Archives of physiology and biochemistry. 2020:1-13.

16. Sharifat N, Jafari-Hafshejani F, Dayati P, Lorestanpoor P, Paydar A, Babaahmadi Rezaei H. Inhibitory effect of Curcumin on phosphorylation NFκB-p65 induced by hydrogen peroxide in Bovine Endothelial Cells. Journal of Fasa University of Medical Sciences. 2017;7(2):283-90.

17. Artimani T, Karimi J, Mehdizadeh M, Yavangi M, Khanlarzadeh E, Ghorbani M, et al. Evaluation of pro-oxidant-antioxidant balance (PAB) and its association with inflammatory cytokines in polycystic ovary syndrome (PCOS). Gynecological endocrinology. 2018;34(2):148-52.

18. Panji M, Behmard V, Zare Z, Malekpour M, Nejadbiglari H, Yavari S, et al. Synergistic effects of green tea extract and paclitaxel in the induction of mitochondrial apoptosis in ovarian cancer cell lines. Gene. 2021;787:145638.

19. Jacob B, Narendhirakannan R. Role of medicinal plants in the management of diabetes mellitus: a review. 3 Biotech. 2019;9(1):1-17.

20. Zhu J, Chen H, Song Z, Wang X, Sun Z. Effects of ginger (Zingiber officinale Roscoe) on type 2 diabetes mellitus and components of the metabolic syndrome: A systematic review and meta-analysis of randomized controlled trials. Evidence-based complementary and alternative medicine. 2018;2018.

21. Shahidi M, Moradi A, Dayati P. Zingerone attenuates zearalenone-induced steroidogenesis impairment and apoptosis in TM3 Leydig cell line. Toxicon. 2022;211:50-60.

22. Lamuchi-Deli N, Aberomand M, Babaahmadi-Rezaei H, Mohammadzadeh G. Effects of the hydroalcoholic extract of Zingiber officinale on arginase i activity and expression in the retina of streptozotocin-induced diabetic rats. International Journal of Endocrinology and Metabolism. 2017;15(2).

23. Tzeng T-F, Liou S-S, Chang CJ, Liu I-M. Zerumbone, a tropical ginger sesquiterpene, ameliorates streptozotocin-induced diabetic nephropathy in rats by reducing the hyperglycemia-induced inflammatory response. Nutrition & metabolism. 2013;10(1):1-12.

24. Abdi T, Mahmoudabady M, Marzouni HZ, Niazmand S, Khazaei M. Ginger (Zingiber Officinale Roscoe)

extract protects the heart against inflammation and fibrosis in diabetic rats. Canadian Journal of Diabetes. 2021;45(3):220-7.

25. Kalousova M, Skrha J, Zima T. Advanced glycation end-products and advanced oxidation protein products in patients with diabetes mellitus. Physiological research. 2002;51(6):597-604.

26. Kim C, Miller R, Braffett B, Pan Y, Arends V, Saenger A, et al. Ovarian markers and irregular menses among women with type 1 diabetes in the Epidemiology of Diabetes Interventions and Complications study. Clinical endocrinology. 2018;88(3):453-9.

27. Thong EP, Codner E, Laven JS, Teede H. Diabetes: a metabolic and reproductive disorder in women. The Lancet Diabetes & Endocrinology. 2020;8(2):134-49.

28. Panji M, Behmard V, Zare Z, Malekpour M, Nejadbiglari H, Yavari S, et al. Suppressing effects of Green tea extract and Epigallocatechin-3-gallate (EGCG) on TGF-β-induced Epithelial-to-mesenchymal transition via ROS/Smad signaling in human cervical cancer cells. Gene. 2021:145774.

29. Arablou T, Aryaeian N, Valizadeh M, Sharifi F, Hosseini A, Djalali M. The effect of ginger consumption on glycemic status, lipid profile and some inflammatory markers in patients with type 2 diabetes mellitus. International journal of food sciences and nutrition. 2014;65(4):515-20.

30. Shidfar F, Rajab A, Rahideh T, Khandouzi N, Hosseini S, Shidfar S. The effect of ginger (Zingiber officinale) on glycemic markers in patients with type 2 diabetes. Journal of Complementary and Integrative Medicine. 2015;12(2):165-70.

31. Wang-Fischer Y, Garyantes T. Improving the reliability and utility of streptozotocin-induced rat diabetic model. Journal of diabetes research. 2018;2018.

32. Wu J, Yan L-J. Streptozotocin-induced type 1 diabetes in rodents as a model for studying mitochondrial mechanisms of diabetic β cell glucotoxicity. Diabetes, metabolic syndrome and obesity: targets and therapy. 2015;8:181.

33. Pourgholi M, Abazari O, Pourgholi L, Ghasemi-Kasman M, Boroumand M. Association between rs3088440 (G> A) polymorphism at 9p21. 3 locus with the occurrence and severity of coronary artery disease in an Iranian population. Molecular Biology Reports. 2021;48(8):5905-12.

34. Zhang Y, Mei H, Shan W, Shi L, Chang X, Zhu Y, et al. Lentinan protects pancreatic β cells from STZ-induced damage. Journal of cellular and molecular medicine. 2016;20(10):1803-12.

35. Subash-Babu P, Alshatwi AA, Ignacimuthu S. Beneficial antioxidative and antiperoxidative effect of cinnamaldehyde protect streptozotocin-induced pancreatic β-cells damage in wistar rats. Biomolecules & therapeutics. 2014;22(1):47.

36. Hall R, Keeble L, Sünram-Lea S-I, To M. A review of risk factors associated with insulin omission for weight loss in type 1 diabetes. Clinical Child Psychology and Psychiatry. 2021;26(3):606-16.

37. Dekel Y, Glucksam Y, Elron-Gross I, Margalit R. Insights into modeling streptozotocin-induced diabetes in ICR mice. Lab animal. 2009;38(2):55-60.

38. Al Hroob AM, Abukhalil MH, Alghonmeen RD, Mahmoud AM. Ginger alleviates hyperglycemia-induced oxidative stress, inflammation and apoptosis and protects rats against diabetic nephropathy. Biomedicine & Pharmacotherapy. 2018;106:381-9.

39. Bi X, Lim J, Henry CJ. Spices in the management of diabetes mellitus. Food chemistry. 2017;217:281-93.

40. Adeniyi P, Sanusi R, Obatolu V. Dietary Ginger extracts enhanced glucose uptake by muscle and adipose of normal and diabetic rats via mimicry of insulin action. American Journal of Biomedical Research. 2017;5(3):46-56.

41. Zare Z, Dizaj TN, Lohrasbi A, Sheikhalishahi ZS, Asadi A, Zakeri M, et al. Silibinin inhibits TGF-β-induced MMP-2 and MMP-9 through Smad Signaling pathway in colorectal cancer HT-29 cells. Basic and Clinical Cancer Research. 2020;12(2):81-90.

42. Majd NE, Azizian H, Tabandeh MR, Shahriari A. Effect of Abelmoschus esculentus powder on ovarian histology, expression of apoptotic genes and oxidative stress in diabetic rats fed with high fat diet. Iranian journal of pharmaceutical research: IJPR. 2019;18(1):369.

43. Maleki N, Yavari N, Ebrahimi M, Faiz AF, Ravesh RK, Sharbati A, et al. Silibinin exerts anti-cancer activity on human ovarian cancer cells by increasing apoptosis and inhibiting epithelial-mesenchymal transition (EMT). Gene. 2022;823:146275.

44. Mehrabianfar P, Dehghani F, Karbalaei N, Mesbah F. The effects of metformin on stereological and ultrastructural features of the ovary in streptozotocin-induced diabetes adult rats: An experimental study. International Journal of Reproductive Biomedicine. 2020;18(8):651.

45. Diamanti-Kandarakis E, Piperi C, Patsouris E, Korkolopoulou P, Panidis D, Pawelczyk L, et al. Immunohistochemical localization of advanced glycation end-products (AGEs) and their receptor (RAGE) in polycystic and normal ovaries. Histochemistry and cell biology. 2007;127(6):581-9.

46. Garg D, Merhi Z. Relationship between advanced glycation end products and steroidogenesis in PCOS. Reproductive Biology and Endocrinology. 2016;14(1):1-13.

47. Zare Z, Dizaj TN, Lohrasbi A, Sheikhalishahi ZS, Panji M, Hosseinabadi F, et al. The Effect of Piperine on MMP-9, VEGF, and E-cadherin Expression in Breast Cancer MCF-7 Cell Line. Basic & Clinical Cancer Research. 2020;12(3).

48. Garg D, Merhi Z. Advanced glycation end products: link between diet and ovulatory dysfunction in PCOS? Nutrients. 2015;7(12):10129-44.

49. Spagnuolo L, Della Posta S, Fanali C, Dugo L, De Gara L. Antioxidant and antiglycation effects of polyphenol compounds extracted from hazelnut skin on advanced glycation end-products (ages) formation. Antioxidants. 2021;10(3):424.

50. Nonaka K, Bando M, Sakamoto E, Inagaki Y, Naruishi K, Yumoto H, et al. 6-Shogaol inhibits advanced glycation end-products-induced IL-6 and ICAM-1 expression by regulating oxidative responses in human gingival fibroblasts. Molecules. 2019;24(20):3705.