

Original Article

Protective Effects of Ginger Extract on Oxidative Stress and Steroidogenesis-related Genes in The Ovary of Streptozotocin-induced Diabetic Rats

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ABSTRACT

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Keywords

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Background and Aims: Ginger has anti-oxidant and anti-diabetic properties, but its beneficial effects have not been fully understood on ovarian disorders in diabetic conditions. In the current project, the impact of ginger extract was investigated on oxidative stress and steroidogenesis-related genes in streptozotocin (STZ)-induced female rats.

Materials and Methods: The STZ-induced rats were utilized as a diabetic model and received 200 or 400 mg/kg/day ginger extract for eight weeks. The biochemical factors were measured by standard procedures in this study. Serum levels of insulin and sex hormones were assessed by the enzyme-linked immunosorbent assay (ELISA) technique, and the mRNA expression of target genes was assayed by real-time polymerase chain reaction.

Results: An increase in the levels of glucose, testosterone, and malondialdehyde (MDA) as well as a decrease in the levels of insulin, glutathione peroxidase (GPx), 3 β -hydroxysteroid dehydrogenase (3 β HSD), steroidogenic acute regulatory protein (StAR), 17 β -estradiol, and progesterone was observed in the diabetic rats. Ginger (200 mg/kg) exhibited a significant amelioration in the levels of glucose, testosterone, and MDA. Treatment with 200 mg/kg ginger enhanced the levels of GPx, StAR, and 17 β -estradiol. Administration with 400 mg/kg ginger extract also ameliorated the level of glucose, testosterone, MDA, and elevated the level of insulin, GPx, 3 β HSD, StAR, 17 β -estradiol, and progesterone in the diabetic rats.

Conclusion: The current study suggests ginger extract protects against ovarian damage in STZ-diabetic rats.

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Introduction

Diabetes mellitus is a serious public health challenge characterized by the disturbed metabolism of carbohydrates, proteins, and lipids due to inappropriate insulin secretion, insulin resistance, or both [1]. The prevalence and burden of diabetes mellitus has dramatically increased worldwide and will affect around 700 million people by 2045 [2]. Under diabetes conditions, hyperglycemia may affect female reproductive functions at several levels due to the impairment of the endocrine regulation of folliculogenesis, steroidogenesis, and oocyte maturation. Ovarian steroidogenesis is a series of enzymatic reactions through which theca and granulosa cells synthesize steroid hormones 17β -estradiol (E2), progesterone, and androgens [3]. However, decreases in the transcript level of steroidogenic genes such as steroidogenic acute regulatory protein (StAR) and 3β -hydroxysteroid dehydrogenase (3β HSD) have also been reported in a hyperglycemic state. There is growing interest in understanding the controlling of these enzymatic reactions under diabetic conditions [4, 5].

Extensive evidence has elucidated that hyperglycemia-induced oxidative stress is a potent contributor to ovarian disorders [6, 7]. Excessive production of reactive oxygen species (ROS) can trigger multiple cellular signaling pathways that serve as critical mediators in the pathogenesis of diabetes-caused reproductive disorders [8-10]. Much evidence has highlighted that excessive ROS levels can involve the apoptosis induction of granulosa cells which impairs folliculogenesis and steroidogenesis.

Therefore, counteracting excessive ROS levels might prevent diabetes-induced ovarian damage [11, 12]. Using herbal remedies effectively treats diseases, including diabetes [13, 14]. Ginger, *Zingiber Officinale* Roscoe, has been used for spice and medicine for thousands of years. Gingerols and shogaols are ginger's main phenolic compounds, accounting for various biological activities, including antioxidant, anti-inflammatory, and anti-cancer activities [15, 16]. Many studies have reported that ginger can manage multiple diseases, such as cardiovascular diseases, obesity, neurodegenerative diseases, and diabetes mellitus. The anti-diabetic effects of ginger have been proven through its anti-oxidant and anti-inflammatory properties [17, 18].

Given that the anti-diabetic activity of ginger has been confirmed, limited investigations have been carried out on the protective impact of ginger against the damage caused by hyperglycemia on the female reproductive system so far. Accordingly, the current work was designed to explore the impacts of ginger extract on reproductive hormones (progesterone, E2, testosterone) in the serum and some oxidant/anti-oxidant markers [(malondialdehyde (MDA) and glutathione peroxidase (GPx)] and steroidogenic genes (3β HSD and StAR) in the ovarian homogenate of STZ- stimulated diabetic rats.

Materials and Methods

Plant preparation and extraction

Dried rhizomes of ginger have concurred from Gol Darou Company (Isfahan, Iran). For extraction, 200 g ginger rhizomes were

firstly ground to powder in an electric blender. Then, the rhizome powder was soaked in 70% methanol solution (v/v) at 25 °C for three days. The obtained extract was filtered, evaporated, freeze-dried, and then stored at -20 °C until later experiments [19].

Animals

The current research was an interventional study, and all procedures were confirmed by the animal care committee of the Garmsar Branch, Islamic Azad University (IR.IAU.SHAHROOD.REC.1400.069). In brief, a total of 96 female Wistar rats (220–250 g) were prepared from Garmsar Branch, Islamic Azad University., All animals were maintained at 25 ± 2 °C and a 12 h light/dark cycle for seven days before experimentations.

Experimental design

Diabetes was induced in overnight fasted rats by intraperitoneal injection of 60 mg/kg streptozotocin (STZ; Sigma) dissolved in 0.1 M citrate buffer (pH 4.5) [20]. After 72 hours, the fasting blood glucose levels were monitored, and the rats. Diabetes was confirmed according to blood glucose levels above 220 mg/ dl. All rats were randomly distributed into four groups (24 animals per each group), as follows:

- 1- Control: Healthy control animals were gavaged with distilled water daily for eight weeks.
- 2- Diabetes: Diabetic animals were gavaged with distilled water daily for eight weeks.
- 3- Diabetes +200 mg/kg ginger extract:
Diabetic animals were gavaged 200 mg/kg ginger extract daily for eight weeks.

- 4- Diabetes+400 mg/kg ginger extract:

Diabetic animals were gavaged 400 mg/kg ginger extract daily for eight weeks.

The ginger extract doses were selected based on prior studies [21, 22].

Samples collection and preparation

After eight weeks of treatment, the whole blood samples were directly taken by cardiac puncture after anesthetizing animals. The whole blood samples were immediately centrifuged (3500 rpm for 15 min), and the serum was kept at -80 °C for biochemical assays. Subsequently, all rats were then sacrificed, and the ovaries were removed. Ovarian homogenates were kept at -80 °C for later assays.

Biochemical assays

Determination of serum glucose and insulin

The serum glucose level was determined using a colorimetric assay kit from Pars Azmoon Company (Tehran, Iran) following instructions and an automatic biochemical analyzer. Following the manufacturer's instructions, blood insulin level was assayed using a commercial rat enzyme-linked immunosorbent assay (ELISA) kit (Monobind, USA).

Determination of MDA and GPx

The amount of MDA and activity of GPx were investigated in the ovarian homogenate using suitable kits obtained by Navand Health Company (Iran) following the manufacturer's instructions.

Determination of hormones

Progesterone, 17β-estradiol, and testosterone levels in the serum of all groups were assayed

by ELISA kits purchased from the Monobind Company (USA), following the manufacturer's recommendations.

Gene expression

RNA isolation was performed in the ovarian homogenate for gene expression analyses using the commercially available RNA isolation kit (Yekta Tajhiz, Iran). The quantity and integrity of total RNA were assessed using the nanodrop spectrophotometer (Thermo Scientific NanoDrop™ 1000) and 1.5% agarose gel electrophoresis. Trace to moderate amounts of genomic DNA contamination during the total RNA isolation is a frequent cause of false-positive signals in RT-qPCR-based analysis. Therefore, we performed an additional genomic DNA removal by DNase during total RNA extraction. Subsequently, about one µg of total RNA was subjected to cDNA synthesis using the reverse transcriptase kit (Yekta Tajhiz, Iran). Quantitative real-time PCR was done on a quantitative PCR system (ABI7500, USA) using SYBR® Green Master Mix (Yekta Tajhiz, Iran). The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was applied as a reference gene. Relative gene expression was analyzed using the $2^{-\Delta\Delta CT}$

formula. The sequences of desired primers are provided in Table 1.

Statistical analysis

GraphPad Prism 8 software (San Diego, California, USA) analyzed data and drew graphs. All data are displayed as mean \pm standard deviation (SD), and the difference among experimental groups was analyzed using one-way ANOVA and Tukey's posthoc. $p < 0.05$ were set as significant levels.

Results

The ginger extract improves glycemic indices

As depicted in Figure 1A, STZ induction in rats greatly increased fasting blood glucose concentration compared to the healthy group ($p < 0.001$). However, the administration of diabetic rats with ginger extract (200 and 400 mg/kg) remarkably attenuated fasting blood glucose levels ($p < 0.01$). As expected, the serum level of insulin was markedly reduced in the diabetic animals compared to the control animals ($p < 0.001$). However, diabetic rats administrated with 400 mg/kg ginger extract exhibited an obvious improvement in the insulin level (Fig. 1B; $p < 0.05$).

Table 1. The primer sequences

Genes	Primer sequences (5'- 3')	Product length	Tm	Ref.
3βHSD	Forward: CCCTGCTCTACTGGCTTGC	189	60.45	[23]
	Reverse: TCTGCTTGGCTTCCTCCC		58.92	
StAR	Forward: CCCAAATGTCAAGGAAATCA	187	53.73	[24]
	Reverse: AGGCATCTCCCCAAAGTG		56.49	
GAPDH	Forward: TGCCAAGTATGATGACATCAAGAAG	71	59.41	[25]
	Reverse: AGCCCAGGATGCCCTTTAGT		60.92	

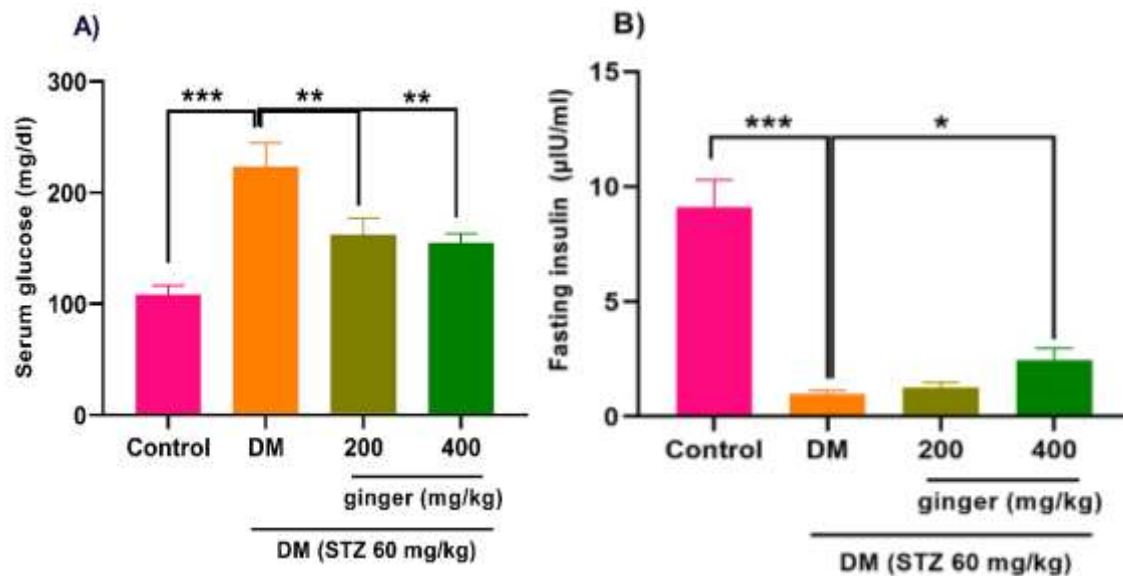


Fig. 1. Effect of ginger extract on (A) glucose level and (B) insulin level in the serum of studied groups. All data are expressed as mean \pm standard deviation. Twenty-four animals in each group were employed for this experiment. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. DM= Diabetes mellitus; STZ= Streptozotocin.

Ginger extract ameliorates oxidative stress

The diabetic animals indicated a considerable increase in ovarian MDA compared to the healthy animals ($p < 0.001$). However, MDA levels in the ovary of diabetic rats were markedly mitigated after the treatment of diabetic rats with ginger extract ($p < 0.01$; $p < 0.001$; Fig. 2A).

As depicted in Figure 2B, the activity of GPx was diminished in the ovarian homogenate of the diabetic group compared to the healthy group ($p < 0.01$). Diabetic group recipients of 200 mg/kg ginger exhibited a remarkable improvement in ovarian GPx activity (Fig. 2B; $p < 0.05$). However, the higher ginger concentration greatly enhanced GPx activity ($p < 0.01$) in the ovarian homogenate of diabetic animals.

Ginger extract ameliorates 3β HSD and StAR mRNA expression

The q-RT-PCR analysis indicated that the StAR mRNA expression was markedly

attenuated in the ovary of the diabetic group relative to the control group ($p < 0.01$). At the same time, a remarkable up-regulation was seen in the StAR mRNA expression in diabetic animals recipients of ginger extract compared to the untreated diabetic animals ($p < 0.05$; $p < 0.01$; Fig. 3A). Similarly, a significant down-regulation was seen in the 3β HSD mRNA levels in the diabetic group relative to the control group ($p < 0.01$). Diabetic rats' recipients of 400 mg/kg ($p < 0.01$) of ginger extract for eight weeks exhibited a significant increment in the 3β HSD mRNA levels. However, no statistical change in the 3β HSD mRNA levels was observed among the 200 mg/kg ginger-treated diabetic group and the untreated diabetic group (Fig. 3B).

Ginger extract ameliorates steroid hormones production

As illustrated in Figures 4A-C, the level of testosterone hormone ($p < 0.001$) was increased, whereas the levels of E2 ($p < 0.001$) and

progesterone ($p < 0.001$) hormones were diminished in the serum of the diabetic group compared to the healthy group. After treatment of diabetic rats with 200 mg/kg of ginger extract for eight weeks, the serum testosterone levels ($p < 0.05$) were significantly attenuated, whereas the serum E2 greatly increased ($p < 0.01$). No statistical difference was detected in the progesterone level between the

200 mg/kg ginger-treated diabetic group and the untreated diabetic group (Fig. 4B). However, the administration of diabetic rats with 400 mg/kg of ginger extract exhibited a substantial decrement in testosterone ($p < 0.01$) and a remarkable increment in the E2 ($p < 0.001$) and progesterone ($p < 0.01$) levels relative to the untreated diabetic rats.

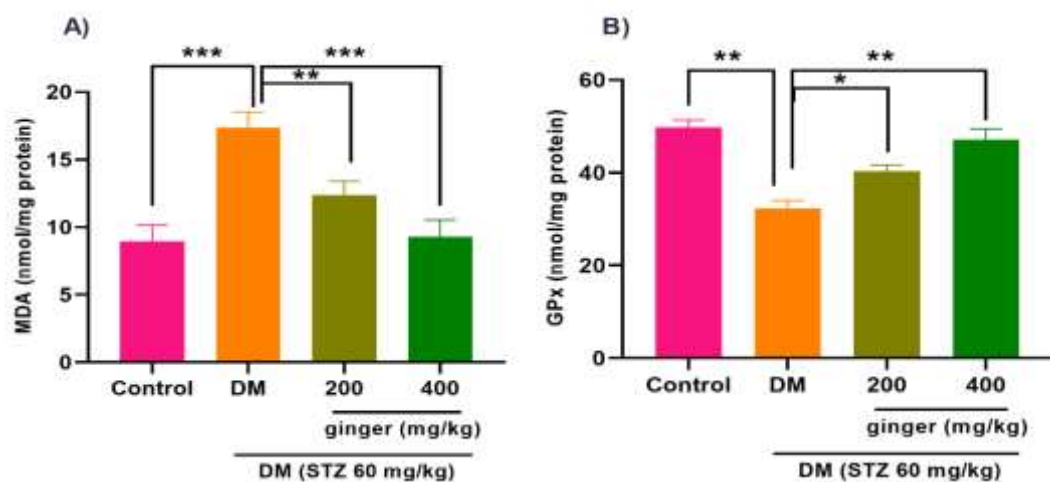


Fig. 2. Effect of ginger extract on (A) malondialdehyde (MDA) level and (B) glutathione peroxidase (GPX) level in the ovarian of studied groups

All results are demonstrated as mean \pm standard deviation. Twenty-four animals in each group were employed for this experiment. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. DM= Diabetes mellitus; STZ= Streptozotocin.

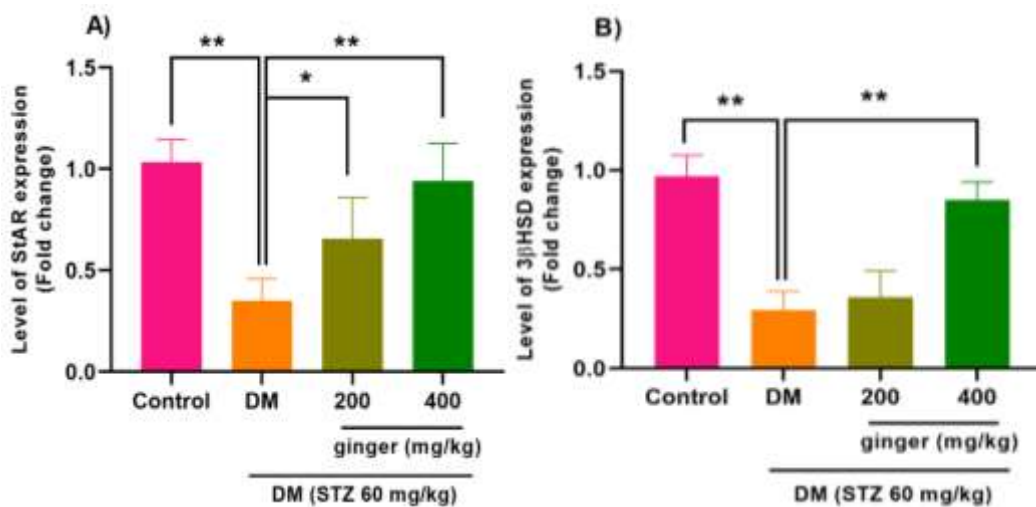


Fig. 3. Effect of ginger extract on (A) steroidogenic acute regulatory (StAR) transcript level and (B) 3β-hydroxysteroid dehydrogenase (3βHSD), transcript level in the ovarian of studied groups. All results are indicated as mean \pm standard deviation. Twenty-four animals in each group were employed for this experiment. * $P < 0.05$ and ** $P < 0.01$. DM= Diabetes mellitus; STZ= Streptozotocin.

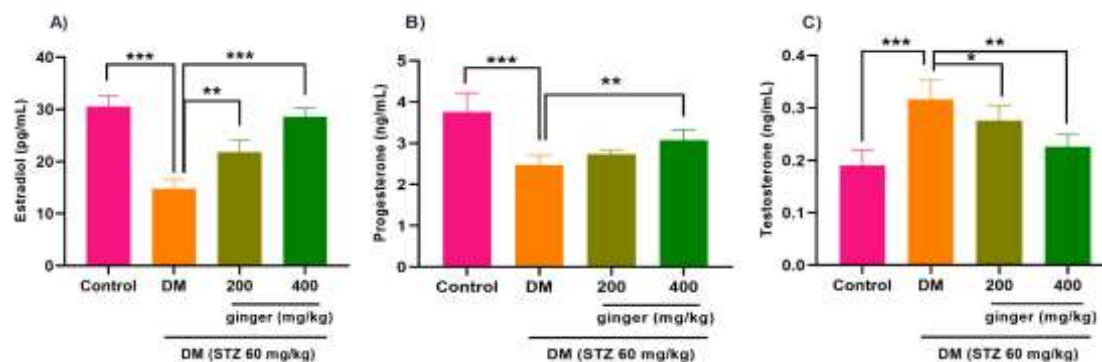


Fig. 4. Effect of ginger extract on (A) estradiol, (B) progesterone, and (C) testosterone level in the serum of studied groups

All results are indicated as mean \pm standard deviation. Twenty-four animals in each group were employed for this experiment. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. DM= Diabetes mellitus; STZ= Streptozotocin.

Discussion

Previous evidence has elucidated that hyperglycemia-provoked oxidative stress can promote the downstream events implicated in ovarian disorders [11, 12, 26]. There is increasing evidence of ginger extract's favorable effect in managing hyperglycemia [27]. Herein, we studied the beneficial impacts of ginger extract against oxidative stress and its related complications in diabetic rats.

STZ is an alkylating agent involved in DNA alkylation and the β cell death, leading to hyperglycemia [28]. This work observed a significant increment in the glucose levels of STZ-treated rats following prior reports [29, 30]. Hyperglycemia is associated with chronic injury to various organs, including ovaries [31, 32]. Therefore, achieving an optimal glycemic state is necessary to control or delay hyperglycemia complications. Previously published articles described the favorable impacts of medicinal plants on hyperglycemia in STZ-induced diabetic rats. Karimi et al. demonstrated that silymarin successfully alleviated retinal microvascular damage in STZ-provoked diabetic rats [14]. Al Hroob et al. reported that ginger

protects rats against diabetic nephropathy by alleviating hyperglycemia [27]. Yi et al. evaluated the effects of 6-shogaol, a polyphenolic compound of ginger, on glucose and insulin levels in the serum of STZ-treated mice. The mice treated with 6-Shogaol exhibited a considerable improvement in glucose and insulin [33]. In the present work, diabetic rats treated with ginger extract exhibited a remarkable amelioration in serum glucose and a great elevation in insulin levels. Ginger was found to provoke liver glycolytic enzymes' cellular activity, which contained pyruvate kinase, phosphofructokinase, and glucokinase [27]. Growing evidence elucidated that hyperglycemia-induced oxidative stress primarily contributes to diabetes complications [8]. For instance, Almatroodi et al. reported that STZ-induced diabetes caused the generation of oxidative stress and renal damage. Their results exhibited that 6-gingerol decreased oxidative stress in diabetic rats, thereby reducing renal injury [34]. Oxidative stress in different organs arose due to excessive cellular ROS production and reduced anti-oxidant agents' activity [35].

Excessive ROS levels can cause extensive damage to cellular biomolecules, including lipids, proteins, and nucleic acids [36]. Lipid peroxidation is the most common indicator of cell membrane destruction. GPx is one of the major members of the anti-oxidant defense system, and the increased activity of this enzyme is an important therapeutic strategy in oxidative stress conditions [37]. Herein, diabetic induction caused a substantial enhancement in the MDA amount and declined GPx activity in the ovary of rats. The oral administration of ginger extract ameliorated MDA levels and boosted GPx activity in the ovary of diabetic rats. Diabetes in women is related to amenorrhea and disturbance in ovarian hormone secretion [38]. Previous studies have revealed that hyperglycemia-provoked oxidative stress was associated with altered folliculogenesis and steroidogenesis in diabetic female rats [12, 39]. Chabrolle et al. highlighted that the serum concentrations of progesterone and E2 hormones were lowered in STZ-treated rats than in controls, indicating that ovarian function was changed. They also assayed the impact of hyperglycemia on the levels of steroid hormones and the enzymes involved in the steroidogenesis pathway in rat granulosa cells. Their findings indicated that in the hyperglycemic conditions, the transcript levels of StAR, p450scc, p450 aromatase, 3 β HSD, and the production of E2 and progesterone were reduced [40]. Therefore, an imbalance in the oxidant and anti-oxidant activities due to diabetes negatively affects steroidogenic function and ovarian hormone levels. Diabetes-related ovarian hormonal disturbance can lead to various health consequences and reduce

folliculogenesis [38, 41, 42]. In 2017, Atashpour et al. evaluated the impacts of ginger extract on the concentrations of sex hormones in the ovaries of rats with polycystic ovary syndrome. Their data demonstrated that ginger extract (350 mg/ kg) has beneficial impacts on improving polycystic ovary syndrome [43]. In the current project, our findings revealed a decrease in the mRNA expression of 3 β HSD and StAR steroidogenic genes and the levels of E2 and progesterone and an increase in the testosterone level in diabetic rats. However, our results indicated that the mRNA levels of 3 β HSD and StAR steroidogenic genes increased in the ovary of the diabetic rats compared to the healthy rats. The ginger extract also improved E2 and progesterone levels and caused a significant reduction in testosterone levels. One of the limitations of this study is the lack of assessment of StAR and 3 β HSD markers at the protein levels. Also, other steroidogenic genes were not investigated in ovarian tissue samples of diabetic rats.

Conclusion

Our findings indicated that ginger extract exerts a protective impact in STZ-treated rats. Ginger attenuated diabetes-provoked oxidative stress and improved the steroidogenic function in the ovary of diabetic rats. Further research is recommended to elucidate the underlying molecular mechanisms associated with improving ovarian dysfunctions by ginger under diabetic conditions.

Conflict of Interest

The authors declare that there is no conflict of interest associated with this work.

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