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ORIGINAL RESEARCH

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Cinnamon and ginger extracts attenuate diabetes-induced inflammatory testicular injury in rats and modulating SIRT1 expression

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Abstract

The current study aimed to evaluate the efficacy of simultaneous administration of *Zingiber officinale* (ginger) and *Cinnamomum cassia* (cinnamon) extracts in mitigating testicular changes associated with diabetes mellitus in rats and to investigate its molecular mode of action. After induction of diabetes using streptozotocin, 36 male rats were divided to six groups namely control, diabetic, metformin-treated, cinnamon-treated, ginger-treated and combined, each group having 6 rats. Fasting blood glucose, serum insulin, testosterone was measured. Expression of inflammatory mediators; tumor necrosis factor-alpha (TNF-α), Nuclear factor kappa B (NF-κB) and Sirtuin 1 (SIRT1) was assessed in the testicular tissue. Histopathological changes in the testis were observed and spermatogenesis and apoptosis were assessed immunohistochemically. The histological and biochemical studies of the untreated group confirmed structural changes in testes induced by diabetes. Oral administration of ginger and cinnamon increased insulin level significantly increased while the blood glucose level significantly decreased in diabetic rats, improving structural testicular changes considerably. Joint intake of ginger and cinnamon increased antihyperglycemic, antioxidant and anti-inflammatory effects markedly improving the testicular injury compared to the administration of either of them. SIRT1 expression in the testis significantly increased in ginger plus cinnamon-treated rats. These results indicate that when administrated together, ginger and cinnamon synergistically enhanced antioxidant, antiapoptotic and anti-inflammatory effects and induced antihyperglycemic effect comparable to metformin. The combination of ginger and cinnamon also upregulated SIRT1 in the testis.

Keywords

Diabetes mellitus; Apoptosis; Ginger; Cinnamon; NF-κB; TNF-α

1. Introduction

Diabetes mellitus (DM) is among the serious diseases which are costly, reduce life expectancy and triggers disability, and life-threatening complications [1]. Long-term hyperglycemia has numerous significant consequences, *e.g.*, increased glucose self-oxidation, protein glycosylation and oxidative stress leading to reduced glucose absorption in muscle and fat cells decreasing insulin release from beta cells [2, 3].

Erectile dysfunction due to DM, is a significant health issue [4]. Multiple mechanisms associated with pathophysiology of DM including oxidative stress and inflammation are involved in diabetes-associated testicular damage [5]. Nuclear factor kappa B (NF-κB) and tumor necrosis factor-alpha (TNF-α) are especially upregulated in DM-mediated inflammation in the testes of rats [6].

Sirtuin 1 (SIRT1), a nuclear protein plays a key role in many metabolic functions. It mediates chronic diseases pathogenesis as diabetes by deacetylating the proteins such as nuclear factor (NF)-κB and inhibiting production of reactive oxygen species (ROS) [7, 8]. Previous studies indicate that SIRT1 also regulates the development and function of the male germ cells [9, 10].

Herbal medicines are gaining popularity due to their safety and efficacy in managing various health problems. Herbs and plants possessing anti-inflammatory, antioxidant, antihyper-

glycemic and antihyperlipidemic potential have been used to target the pathogenesis of DM [11]. *Zingiber officinale* Roscoe (ginger), a Zingiberaceae plant is extensively used in Asian countries, specifically in Indian medicine, has proved anti-inflammatory, antihyperglycemic and antioxidant properties. Confirmed antidiabetic and antioxidant effects of *Cinnamomum cassia* L. (cinnamon) have also been reported [12].

Although cinnamon and ginger have been used individually to manage DM diabetes [13, 14], the efficacy of the combined administration of both is unexplored yet. Keeping in mind the synergistic effects of ginger and cinnamon in controlling DM [15, 16], this study aimed to assess the efficacy of joint administration of *Zingiber officinale* and *Cinnamomum cassia*, in preserving the testis against diabetes-induced testicular injury and illustrating the molecular mechanism of this effect.

2. Materials and methods

2.1 Drugs

Metformin purchased from "Shiguibao Medicine Co., Ltd., Shanghai, China" was used in this study. Metformin was diluted in 0.9% sodium chloride and administrated to rats *via* gastric gavage (500 mg/kg/day for 6 weeks) as reported earlier [17].

The streptozotocin (STZ) was procured from "Sigma Aldrich Chemical Company CO., St. Louis, MO, USA" for administration to the Sprague-Dawley rats. Streptozotocin was diluted in "0.01 M sodium citrate buffer, pH 4.5", just before intraperitoneal injection to 12 hours fasting rats at a dose of 60 mg/kg. Sucrose solution (5%) was given overnight to prevent the hypoglycemia enabling STZ entry into the cells [18].

Fresh roots of *Zingiber officinale* Roscoe (ginger) and bark of *Cinnamomum cassia* L (cinnamon) were purchased from Jeddah market in Saudi Arabia (voucher specimens: AQJ 84 and AQJ 15 respectively). Samples from the King Abdulaziz University (KAU) herbarium and the Saudi Arabian Flora were used to recognize ginger and cinnamon [19]. A botanist from KAU confirmed the plants' identity before depositing the voucher specimens to the herbarium.

Aqueous extract of ginger was prepared as reported earlier by Al-Amin *et al.* [20] and kept at 4 °C in the refrigerator. The extract was orally administrated *via* the gastric gavage (500 mg/kg/day for 6 weeks) [20]. Cinnamon aqueous extract was prepared according to the method used by Longe *et al.* [21]. The aqueous extract was orally administrated *via* gastric gavage (100 mg/kg for 6 weeks) [21]. Cinnamon extract at a dose less than 0.5 g/kg is considered safe as described previously [22].

2.2 Experimental design

Thirty-six male Sprague-Dawley rats (average weight from 100 to 150 g with age 5 weeks ± 3 days) obtained from the animal house at King Fahd Medical Research Center, KAU, Saudi Arabia. The guidelines of ethically using animals in research adopted by this Research Center were followed in this study. Rats were kept under the conventional laboratory settings for one week to acclimatize and grouped into six groups (n = 6). Control group (CON) was administered normal saline by a gastric tube. DM induced by intraperitoneally injecting the freshly prepared STZ. Fasting blood glucose (FBG) level was assessed at the start of the experiment and after 72 hours and those with FBG <250 mg/dL were assigned to the diabetic groups [23]. Five diabetic groups were used; the untreated diabetic (STZ), metformin-treated (STZ + Met), *Zingiber officinale*-treated (STZ + Gin), *Cinnamomum cassia*-treated (STZ + Cinn), and *Zingiber officinale* plus *Cinnamomum cassia*-treated (STZ + Cinn + Gin) diabetic groups. At the end of the experiment, and after being starved for 12 hours, blood samples were obtained, centrifuged and the serum was stored at −80 °C.

2.3 Measurement of blood glucose and serum insulin levels

FBG was measured by enzymatic glucose kits (Human Gesellschaft für & Diagnostica mbH (Germany). ELISA kits (Assay Design Inc., Ann Arbour, USA) was used for insulin measuring to the manufacturer's instructions [24].

Homeostatic model assessment for insulin resistance (HOMA-IR) and homeostatic model assessment for β-cells (HOMA β-cells) were calculated based on the equations described earlier [25].

2.4 Measurement of serum testosterone

The testosterone level was measured twice by ELISA kits (Assay Design Inc., Ann Arbour, USA) as described in the manufacturer's instructions.

2.5 Measurement of total antioxidant capacity

The TAC in the serum was assessed using the Bio-diagnostic ELISA kit (Assay Design Inc., Ann Arbour, USA) method [26].

2.6 Real-time-polymerase chain reaction (PCR)

Previously reported materials and chemicals were utilized to assess the gene expression [16]. After being anesthetized with 4% isoflurane in 100% oxygen, rats underwent cervical dislocation. Cardiac perfusion (10% neutral buffered formalin) was performed, and the testes were rapidly extracted, fixed and processed to obtain paraffin blocks for histopathological examination. Other organs of the rats were also processed and studied in another piece of work.

RNA was extracted from paraffin-processed samples by a previously reported procedure [27]. Total RNA was extracted using Trizol as per the supplier's instructions. The RNA contents were quantified by a Nano Drop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). In a 20-ll reaction containing 5 ll. RNA reverse transcription was performed using oligo-dT primers. PCR Master Mix and primers were used to amplify the obtained cDNAs (Metabion International AG, Planegg, Germany) [27].

Data were analyzed using the Light Cycler 480 program (version 1.5, Roche Applied Science, Mannheim, Germany).

The comparative technique [1] was used to investigate the relative amounts of mRNA. Used primers are shown in **Supplementary Table 1**.

2.7 Histopathological assessment

Paraffin sections (4 μ thick) were prepared for histological evaluation using light microscope (Olympus, Breiningsville, PA, USA). An avidin-biotin approach was used to stain another set of slides immunohistochemically as described earlier [28]. Rabbit polyclonal primary antibodies against proliferating cell nuclear antigen (PCNA) (Santa Cruz Biotechnology), were utilized at the dilution of 1:200 to evaluate the proliferating cells in the testis. The androgen receptors (AR) were identified by a rabbit anti-AR primary antibody (Biocare Medical, Pacheco, USA; dilution 1:250). Anti-NF-κB (Dako, Missouri, USA; dilution 1:200) and anti-SIRT1 primary antibodies (Santa Cruz Biotechnology, CA, USA; dilution 1:200) were utilized. Anti-TNF-α primary antibodies (Abcam, Cambridge, UK, diluted at 1:500) were used. Negative control slides were included.

Regarding the detection of apoptosis, TACS-XL DAB *in-situ* apoptosis detection kit (Trevigen, Inc, Gaithersburg, MD, USA), was used for antigen retrieval (terminal deoxynucleotidyl transferase dUTP-mediated nick-end labeling, TUNEL assay) according to the manufacturer's guidelines [29].

Pro Plus image analysis software (version 6.0, Media Cybernetics, Inc., MD, USA) was used to assess the % area of AR, NF-κB, SIRT1 and TNF-α immunoexpression in the testes. This software was used also to evaluate the germinal epithelial thickness and cross section area of the seminiferous tubules (STs) in 5 sections/slide ($\times$100). Five slides/rat were examined and the mean was calculated, as was reported earlier [28]. TUNEL-positive and PCNA-positive cells were counted in the testes.

2.8 Statistical analysis

To perform the statistical analysis of the study variables, statistical Package for the Social Sciences (SPSS) version 16 (SPSS Inc. Chicago, IL, USA) was utilized. Kolmogorov-Smirnov test was used to check the data normality. Analysis of variance (ANOVA) (F test) was used to compare the studied groups followed by Bonferroni *post hoc* test. p value < 0.05 was considered significant. To detect the synergistic effect of the used substances, the mean difference of change in the mean FBG (the primary outcome of the study) of the treated groups was compared and the percentage of change from the mean of the untreated group was calculated.

3. Results

3.1 Biochemical assessment

3.1.1 Fasting blood glucose, serum insulin levels and HOMA

Although the FBG was in the euglycemic level at the start of the experiment in all groups, it exceeded 200 mg/dL in all rats after STZ injection. It was significantly lower in ginger plus cinnamon-treated group as compared to groups receiving ginger- ($p = 0.003$) and cinnamon- ($p = 0.01$) alone (Fig. 1A,B).

The mean difference of change in FBG was highest in the ginger plus cinnamon-treated group (218.01) versus (162.37) in ginger- and (158.26) in cinnamon-treated groups. The % change was highest in the ginger plus cinnamon-treated group (0.51) versus (0.38) in ginger and (0.37) in cinnamon-treated groups.

The untreated diabetic rats exhibited a significantly lower ($p < 0.001$) serum insulin, while metformin, ginger, cinnamon- and ginger plus cinnamon-treated groups showed a significantly higher level compared to untreated one ($p < 0.001$, $p = 0.001$, $p = 0.003$, $p < 0.001$), respectively. Ginger plus cinnamon-treated rats had a significantly higher insulin level as compared to those receiving ginger ($p = 0.002$) or cinnamon ($p = 0.01$) alone (Fig. 1C).

The untreated group had significantly higher ($p = 0.01$) HOMA-IR as compared to the control, while it was insignificantly increased in all treated groups compared to the untreated rats. The HOMA-β cell, decreased significantly in untreated rats, while it increased significantly in groups treated with metformin and ginger plus cinnamon-treated groups ($p = 0.001$, $p < 0.001$), respectively, compared to untreated diabetic. Ginger and cinnamon together significantly increased HOMA-β cells compared to cinnamon-treated group alone ($p = 0.04$) (Fig. 1D–E).

3.1.2 Total antioxidant capacity

Although TAC level was significantly lower ($p < 0.001$) in untreated diabetic rats, it was significantly increased in all treated groups compared to untreated rat groups. Ginger plus cinnamon-treated group had significantly higher TAC level as compared to both ginger- and cinnamon-treated ($p = 0.002$, $p < 0.001$) groups respectively (Fig. 1F).

3.1.3 Serum testosterone

The untreated group had significantly lower serum testosterone level ($p < 0.001$) in untreated group, while it was significantly increased in metformin- ($p < 0.001$), ginger- ($p = 0.01$), cinnamon- ($p = 0.004$) and ginger plus cinnamon-treated ($p < 0.001$) groups. Combination of ginger and cinnamon significantly increased the serum testosterone compared with ginger- ($p = 0.03$) and cinnamon-treated ($p = 0.04$) groups (Fig. 1G).

3.2 The histological structure of the testis

Histological studies of testes of control rats showed intact tubular structure with normal interstitial Leydig cells in between. Testes of untreated diabetic rats showed shrinkage of some STs. Degenerated sheded germinal epithelial cells were observed in the lumen of some STs. The interstitial connective tissue appeared congested with dark cells. Most of STs in the testis of rats of metformin-, ginger- and cinnamon-treated groups were intact with minimal vacuolations in the germinal epithelium. The testis of rats of ginger plus cinnamon-treated had intact STs with restored germinal epithelium nearly similar to control rats (Fig. 2A–F).

Regarding the cross-sectional area of STs, it was significantly ($p < 0.001$) reduced in untreated diabetic group, while it

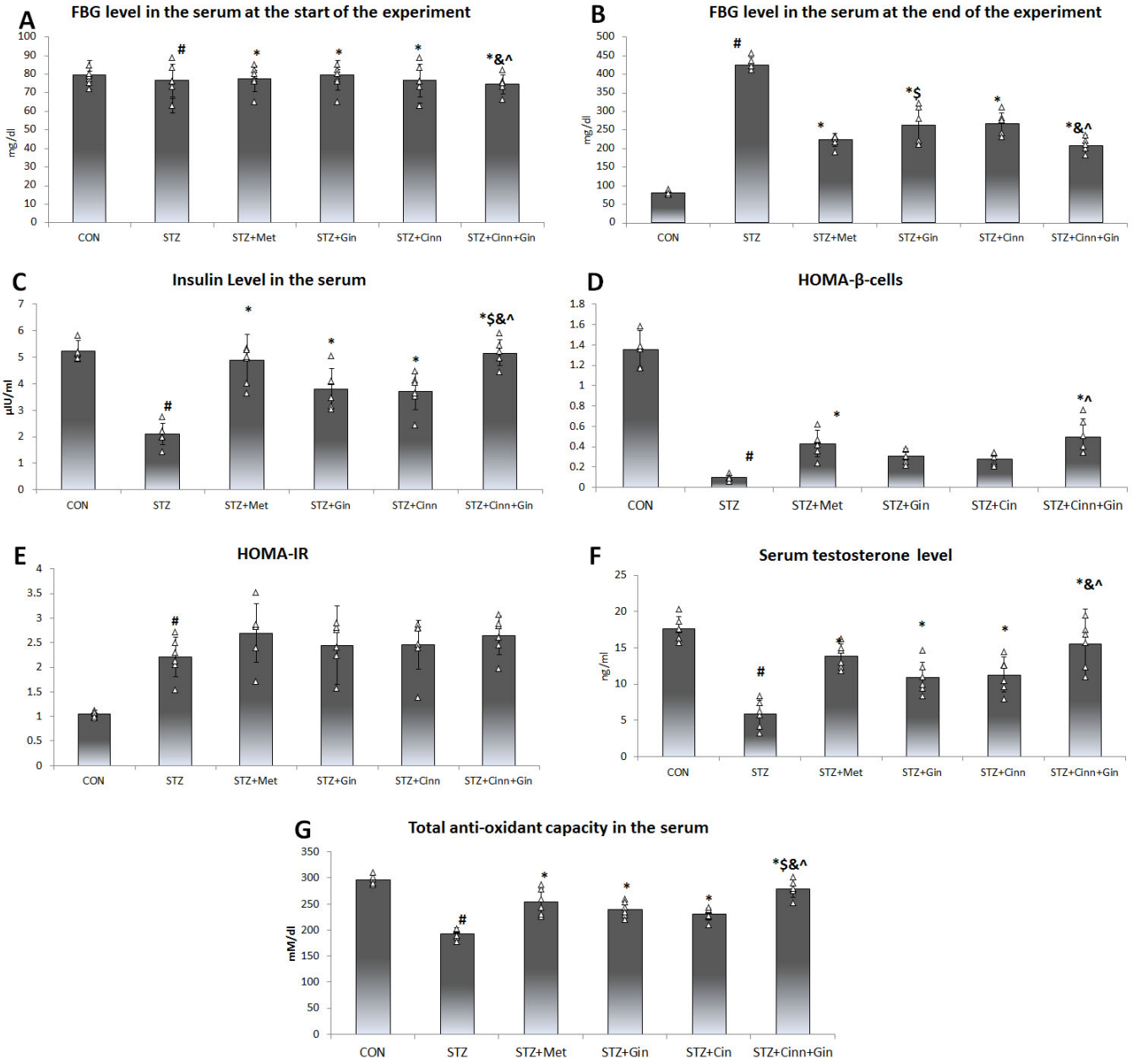

FIGURE 1. Effect of Cinnamon and ginger extracts on biochemical parameters. Fasting blood glucose (FBG) at the beginning (A) at the end of the experiment (B), serum insulin levels (C), HOMA-β (D), HOMA-IR (E), testosterone level (F) and the total anti-oxidants capacity (G) in the study groups. STZ: streptozotocin; Met: metformin; Gin: ginger; Cinn: Cinnamon. N = 6. Mean ± SD is presented. Analysis of varience test (ANOVA) was performed followed by Bonforoni *post-hoc* test. #: significant versus control; *: significant versus STZ; $: significant versus STZ + Met, &: significant versus STZ + Gin; ^: significant versus STZ + Cinn.

was significantly increased in metformin- ($p = 0.02$), ginger- ($p = 0.03$) and ginger plus cinnamon-treated ($p < 0.001$) groups. The area of STs significantly increased in the ginger plus cinnamon-treated group compared to both groups treated with ginger ($p = 0.03$) or cinnamon ($p = 0.04$) (Fig. 2G).

The height of germinal epithelial cells significantly decreased in untreated group, while it significantly increased in the metformin-, ginger- and ginger plus cinnamon-treated groups ($p = 0.001$, $p = 0.02$, $p < 0.001$). Germinal epithelial height significantly increased in the ginger plus cinnamon-treated group compared to ginger- and cinnamon-treated rats (Fig. 2H). The Leydig cells count significantly increased in metformin- and ginger plus cinnamon-treated groups ($p = 0.001$, $p < 0.001$) groups compared to untreated group, while the number of ST with germ cell loss significantly decreased in the metformin-, and ginger plus cinnamon-treated groups ($p < 0.001$) compared to untreated group (Fig. 2I,J).

TUNEL-positive cells were significantly higher ($p < 0.001$) in untreated group indicating increased apoptosis, and reduced significantly ($p < 0.001$) in all treated groups. Ginger and cinnamon administrated together significantly decreased the number of these cells when compared to both groups treated alone with ginger and cinnamon (Fig. 3).

PCNA-positive cells significantly decreased ($p < 0.001$)

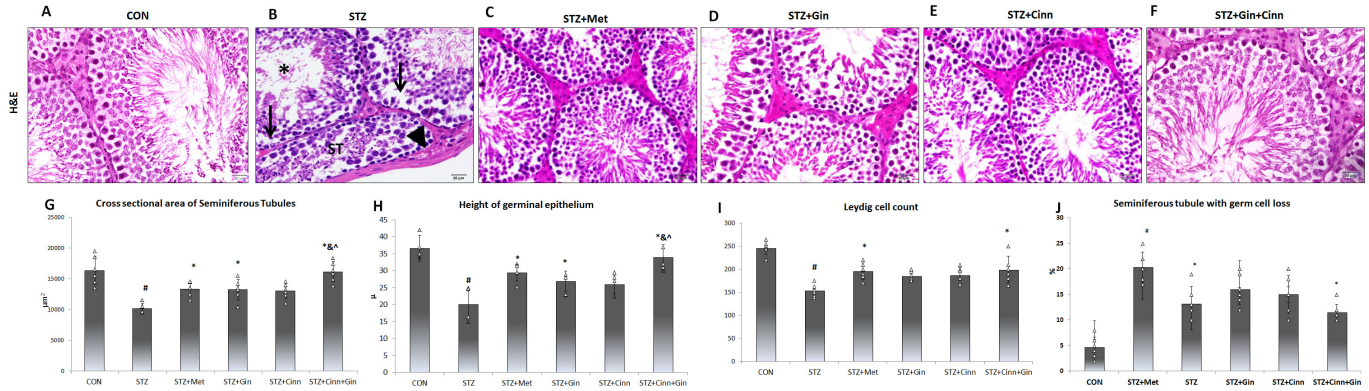

FIGURE 2. Effect of Cinnamon and ginger extracts on the histological structure of the testis. (A–F) show the histological structure of the testis of the study groups. The area of the seminiferous tubules (G) and the height of the germinal epithelium (H) is presented. Testis of STZ-treated rat (B) shows tubular deformity and shrinkage of some STs (ST). Many germinal epithelials cells were degenerated (arrow) with reduced spermatozoa number in the STs lumen (asterisk). The interstitial connective tissue appears congested (arrow head). These changes are less frequent in the treated groups (C–F). The cross sectional area of ST (G), Height of germinal epithelium (H), Leydig cell count (I) and ST with germ cell loss (J) are presented in graphs. STZ: streptozotocin; Met: metformin; Gin: ginger; Cinn: Cinnamon. N = 6. Mean ± SD is presented. Analysis of varience test was done followed by Bonforoni *post-hoc* test. #: significant versus control; *: significant versus STZ + Met; &: significant versus STZ + Gin; ^: significant versus STZ + Cinn.

in untreated group indicating reduced cell proliferation, and increased significantly in the metformin- ($p < 0.001$), ginger- ($p = 0.02$), cinnamon- ($p = 0.01$) and ginger plus cinnamon- ($p < 0.001$) treated groups. Ginger and cinnamon administrated together significantly increased the number of these cells compared to both ginger- ($p = 0.003$) and cinnamon- ($p = 0.004$) treated groups (Fig. 3).

Immunoexpression of AR, expressed in the interstitial cells of Leydig, was decreased significantly ($p < 0.001$) in untreated rats, and increased significantly in metformin- ($p = 0.01$) and ginger plus cinnamon- ($p < 0.001$) treated rats compared to the untreated rats. Administrating ginger and cinnamon simulantinously significantly increased the AR expression when compared to the groups receiving them alone ($p = 0.03$, $p = 0.02$) (Fig. 3).

3.2.1 TNF-α and NF-κB expression in the testis

The testicular immunoexpression of TNF-α and NF-κB was very low in the control group, whereas it was significantly ($p < 0.001$) higher in the untreated group. Immunoexpression of TNF-α and NF-κB were significantly lower in metformin- ($p = 0.01$, $p < 0.001$), ginger- ($p = 0.01$, $p = 0.004$), cinnamon- ($p = 0.04$, $p < 0.001$) and ginger plus cinnamon-treated groups, respectively. Joint administration of ginger and cinnamon significantly reduced immunoexpression of TNF-α and NF-κB when compared to the groups receiving them alone (Fig. 4).

Testicular gene expression of both TNF-α and NF-κB increased significantly ($p < 0.001$) in the untreated diabetic group, and decreased significantly in metformin- ($p = 0.01$, $p < 0.001$), ginger- ($p = 0.02$, $p < 0.001$), cinnamon- ($p = 0.01$, $p < 0.001$) and ginger plus cinnamon-treated ($p < 0.001$) groups, respectively. Joint adminstration of ginger and cinnamon significantly decreased gene expression of both TNF-α and NF-κB compared to ginger- ($p = 0.01$, $p = 0.02$) and cinnamon-treated ($p = 0.01$, $p = 0.03$) groups (Fig. 5).

3.2.2 SIRT1expression in the testis

Immuno- and gene expression of SIRT1 in testes decreased significantly ($p < 0.001$) in untreated group, and increased significantly in metformin- ($p < 0.001$, $p = 0.002$) and ginger plus cinnamon-treated ($p < 0.001$) groups, respectively. Ginger and cinnamon together significantly reduced the immuno- and gene expression of TNF-α compared to ginger- ($p = 0.04$, $p = 0.01$) and cinnamon-treated ($p = 0.03$, $p = 0.04$) groups individually (Fig. 6).

4. Discussion

Being one of the common metabolic disorders affecting various body organs and the leading cause of death globally, DM attracts researcher interest and curiosity [30]. Diabetes and testicular injury are interlinked disorders affecting male fertility [31].

The current study used STZ to develop animal model for DM, confirmed by the significant increase in FBG level above 200 mg/dL in all STZ-treated rats compared to controls. Induced diabetes in STZ-treated rats affected the pancreatic cell function, as revealed by the significantly reduced insulin level (insulin output) and HOMA–β cells and a significant increase in the HOMA-IR indicating insulin resistance. These results are in accordance with those of previous studies [32, 33]. The STZ can induce diabetes by changing the expression of Glucose transporter 2 (GLUT2), leading to a decreased sensitivity of peripheral insulin receptors due to decreased insulin secretion [34].

The diabetic impact on testicular function was recorded in this study as there was a significantly reduced testosterone levels in diabetic rats and this is consistent with earlier studies in both animal models and human [35, 36]. Decreased testosterone level in diabetic rats is due to reduced luteinizing hormone (LH) production from the pituitary gland as a result

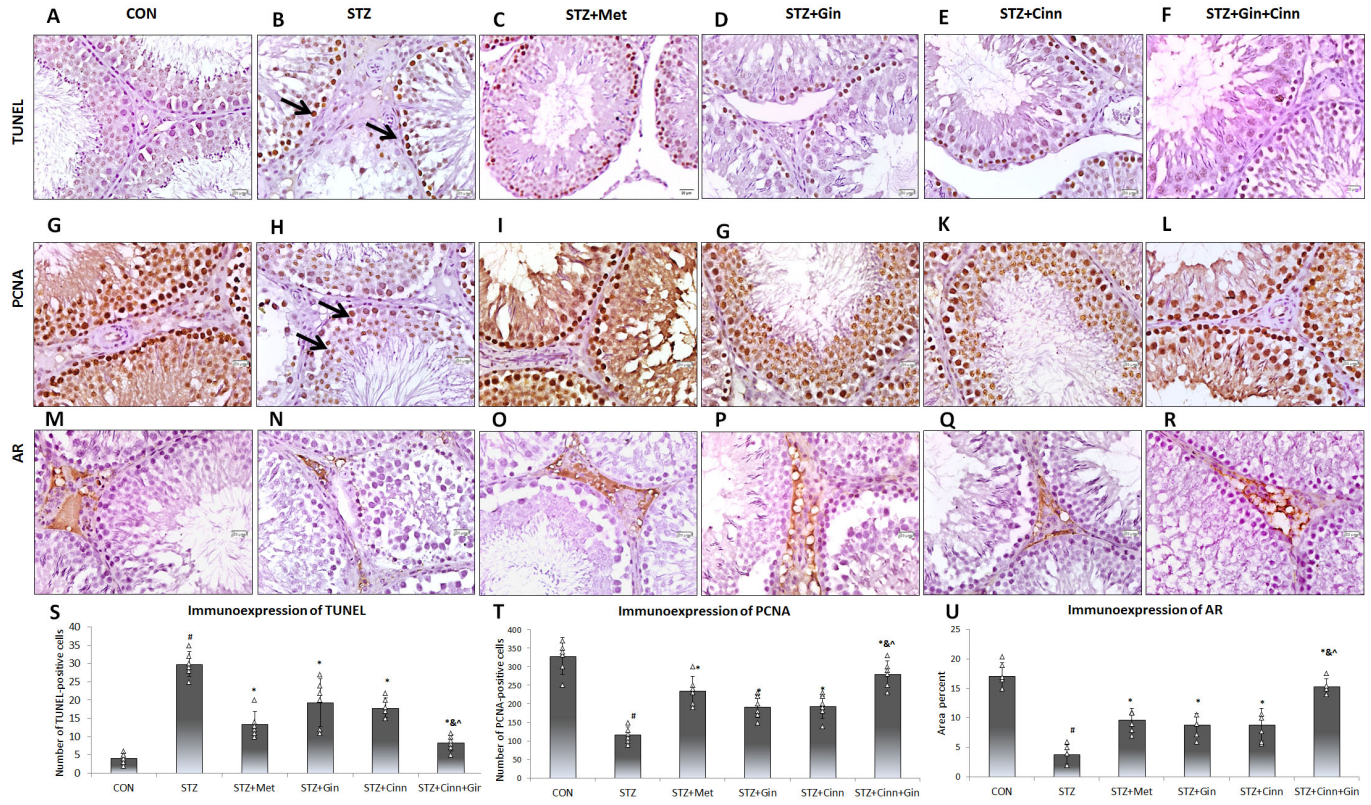

FIGURE 3. Effect of Cinnamon and ginger extracts on TUNEL, PCNA, AR testicular immunoexpression. Immunoexpression of TUNEL (A–F), PCNA (G–L), AR (M–R) in the testis of the study groups is presented. TUNEL + ve cells (arrow) are more prevalent while the PCNA + ve cells (arrow) are less prevalent in STZ group. Semi-quantitative assessment of this expression is presented (S–U). TUNEL: terminal deoxynucleotidyl transferase dUTP-mediated nick-end labeling, TUNEL assay. PCNA: proliferating cell nuclear antigen; AR: androgen receptor; STZ: streptozotocin; Met: metformin; Gin: ginger; Cinn: Cinnamon. N = 6. Mean ± SD is presented. ANOVA test followed by Bonforoni *post-hoc* test was used. #: significant versus control; *: significant versus STZ + Met; &: significant versus STZ + Gin; ^: significant versus STZ + Cinn.

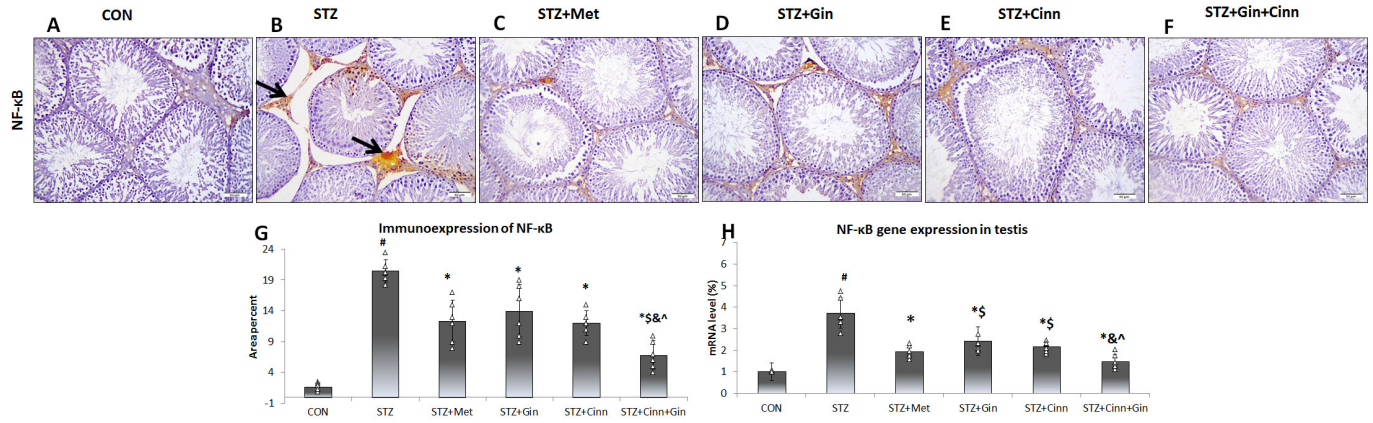

FIGURE 4. Effect of Cinnamon and ginger extracts on immunoexpression of NF-κB in the testis. Immunoexpression of testicular NF-κB (A–F) in the study groups is presented. NF-κB + ve cells (arrow) frequently appear in the interstitial cells of STZ group (B). Semi-quantitative assessment of NF-κB immunoexpression is presented (G). Gene expression analysis of NF-κB using qRT-PCR is presented (H). NF-κB: nuclear factor kappa B; STZ: streptozotocin; Met: metformin; Gin: ginger; Cinn: Cinnamon. N = 6. Mean ± SD is presented. ANOVA test followed by Bonforoni *post-hoc* test was used. #: significant versus control; *: significant versus STZ, $: significant versus STZ + Met; &: significant versus STZ + Gin; ^: significant versus STZ + Cinn.

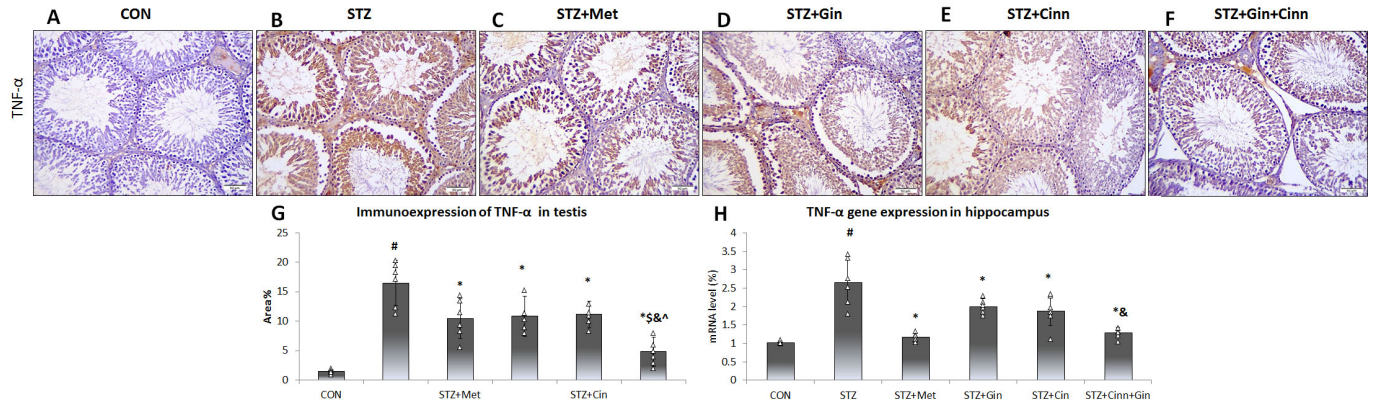

FIGURE 5. Effect of Cinnamon and ginger extracts on immunoexpression of TNF-α in the testis. Testicular immunoexpression of TNF-α (A–F) of the studied group is presented. TNF-α + ve cells (arrow) frequently appear in STZ group (B). Semi-quantitative assessment of TNF-α immunoexpression is presented (G). Gene expression analysis of TNF-α using qRT-PCR is presented (H). TNF-α: tumor necrosis factor-alpha; STZ: streptozotocin; Met: metformin; Gin: ginger; Cinn: Cinnamon. N = 6. Mean ± SD is presented. ANOVA test followed by Bonforoni *post-hoc* test was used. #: significant versus control; *: significant versus STZ + Met; &: significant versus STZ + Gin; ^: significant versus STZ + Cinn; $: significant versus STZ + Met.

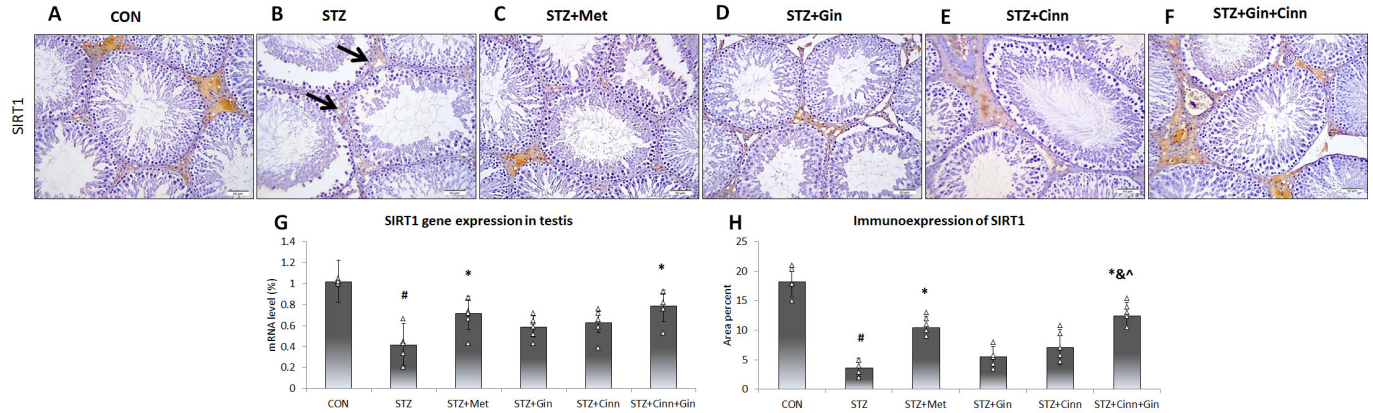

FIGURE 6. Effect of Cinnamon and ginger extracts on immunoexpression of SIRT1 in the testis. Testicular immunoexpression of SIRT1 (A–F) in testis of the study groups is presented. SIRT1 + ve cells (arrow) less frequently appear in the interstitial cells of STZ group (B). Semi-quantitative assessment of SIRT1 immunoexpression is presented (G). Gene expression analysis of SIRT1 using qRT-PCR is presented (H). SIRT1: Sirtuin 1; STZ: streptozotocin; Met: metformin; Gin: ginger; Cinn: Cinnamon. N = 6. Mean ± SD is presented. ANOVA test followed by Bonforoni *post-hoc* test was used. #: significant versus control; *: significant versus STZ + Met; &: significant versus STZ + Gin; ^: significant versus STZ + Cinn.

of elevated estradiol produced by aromatase enzyme in hypertrophied adipose tissue during testosterone metabolism [37]. Steroidogenesis in Leydig cells decreased in insulin-resistant conditions due to Leydig cell resistance to insulin action [38].

The effect of DM on the testicular structure was confirmed histopathologically, by shrinkage and disruption of STs, reduced number of spermatozoa in the lumen, and degeneration and shedding of germinal epithelium with a subsequent decreased height. Similar disturbed testicular morphology and reduced diameter of the STs and germinal epithelium height were observed previously [38–40]. Reduced height of germinal epithelium might be attributed to apoptosis of the spermatogenic cells as confirmed immunohistochemically in this study. Immunohistochemically studies confirmed increased apoptosis in testes of diabetic rats as reported earlier [41, 42]. Spermatogenesis was affected in diabetic rats in the current study and many previous studies. The PCNA expression was assessed in this study to detect the impact of diabetes on spermatogenesis as it is considered a definitive marker of germinal proliferation [39]. Previous studies also report significantly reduced AR expression in diabetic rats indicating a defect in spermatogenesis, as reduced AR expression are usually associated with spermatogenic disorders [40, 43, 44].

The oxidative stress and inflammation are the main reasons of diabetes-induced reproductive dysfunction [45, 46], hence, serum TAC and inflammatory cytokines were assessed in this study. We also assessed testicular expression of NF-κB, a key factor sensitive to oxidative stress. A marked reduction in TAC level and upregulation of NF-κB testicular expression was observed in diabetic rats. Increased NF-κB expression has been reported in several organs of diabetic rats, including the testes, kidney, nerves and heart [47, 48].

Regarding the proinflammatory cytokines in this study, a significant upregulation in TNF-α gene and immunoexpression was observed in testes of untreated diabetic rats indicating testicular inflammation. Similar findings are reported previously [49]. The proinflammatory cytokines cause testicular inflammation, leading to testicular damage, germ cell depletion, reduced testicular size and apoptosis [50]. The TNF-α is primarily linked with excessive production of ROS and nitric oxide, that aggravates and prolongs inflammatory feedback, including "the activation of the NF-κB pathway and cell apoptosis" with sbsequently damaged sperms [51]. In line with these observations, several studies reported a crosslink between any drug metabolites or diseases causing inflammation, oxidative stress and decrease in sperm cell production and testicular damage [52].

The significant downregulation of testicular SIRT1 expression in untreated diabetic rats observed in this study is augmented by some previous studies [53]. Reduced SIRT1 expression has been observed in cases with reduced spermatogenesis, germ cell function, defective spermatogenic stem cell differentiation and lower sperm counts [54]. The reduced SIRT1 expression could be related to insulin resistance in diabetic rats [55]. The regulation mechanism of SIRT1 in chronic diseases including diabetes was attributed to deacetylate proteins, such as NF-κB and other inflammatory cytokines and its efficacy in inhibiting ROS formation [7, 8], and both of these changes were observed in current study.

To alleviate the pathological effects of diabetes on the testicular structure, extracts of two medicinal plants with evident hypoglycemic and antioxidant activity were administrated simultaneously to regulate blood glucose and stop diabetic complications. Metformin has a good antidiabetic effect due to its regulatory effect on blood glucose and insulin sensitivity. It is an excellent drug for pharmacological validation of antidiabetic effects of medicinal plants extracts due to its protective effect against testicular structure and male infertility associated with DM [56, 57]. Metformin helped apoptosis [58], attenuated cellular apoptosis and senescence [59, 60] and decreased apoptotic cells in testes of diabetic rats [53].

In the current study, hypoglycemic effect of ginger, cinnamon and the combination of both was observed by the significant reduction of FBG level, increased insulin level, and HOMA-β-cells with greatest significance observed in the combination group. No significant difference was observed between the antidiabetic effect induced by metformin and that induced by ginger and cinnamon when administrated together. Ginger stimulates insulin release, enhances glucose clearance, and protects from oxidative stress by reducing lipid peroxidation. Cinnamon increases the glycogen stores making them available for energy production by stimulating glucose transporter (GLUT4) for glycogenesis and adipocytes [40].

Joint administration of ginger and cinnamon induced an effect comparable to metformin and better than the separate administration of ginger and cinnamon regarding preserving the testicular structure, germinal cell height, reducing spermatocytes apoptosis, maintaining spermatogenesis and AR immunoexpression and TAC serum level. This could be attributed to the fact that since the herbs effectively reduce free radicals preventing tissue damage, therefore, they are used as natural antioxidants [61, 62]. Cinnamon recovers the reproductive changes in diabetic male rats by increasing the concentrations of testosterone, Luteinizing hormone (LH) and Follicular stimulating hormone (FSH) [63]. Additionally, bioactive compounds of cinnamon and ginger alter the hypothalamus-pituitary axis leading to increased hormone levels [62].

Interestingly ginger and cinnamon, when administrated separately, did not significantly increase SIRT1 gene or immunoexpression, while the combined administration of both significantly increased SIRT1 expression in the testis. This is in partial agreement with an earlier study [65] about the effect of cinnamon on SIRT1 levels in the serum of diabetic patients. Ginger extract and 6-Gingerol, one of the key bioactive compounds of ginger extract upregulated SIRT1 expression, in high-fat diet-induced liver injury and in Arsenic trioxide As_2O_3-induced cardiotoxicity model, respectively [65, 66]. Together administration of cinnamon and ginger in current study induced superior antiapoptotic, anti-inflammatory and antioxidant effects compared to the administration of either of them and to metformin. These findings are in line with the recorded capacity of both ginger and cinnamon in reducing diabetes-induced oxidative stress, apoptosis and inflammation [11]. From these findings, it is postulated that the synergism between ginger and cinnamon is mainly through SIRT1 upregulation by reducing the inflammatory process mediated by NF-κB and TNF-α besides enhancing the antioxidant status.

One of the main limitations of this study, that will be tackled in a future study, was the inability to assess the effect of joint administration of ginger and cinnamon with the other parts of the male reproductive system like epididymis and seminal vesicles.

5. Conclusions

Concludingly joint administration of ginger and cinnamon extracts had a synergistic antidiabetic, antioxidant, antiapoptotic and anti-inflammatory effects that were comparable to those of metformin. These synergistic effects seemed to be mediated through the upregulation of SIRT1 with subsequent inhibition of NF-κB-mediated inflammation and might be the reason of the preserved testicular structure in diabetic rats in this study. Further studies are encouraged to confirm the molecular basis of this synergistic effect by clinical trials in diabetic patients.

AVAILABILITY OF DATA AND MATERIALS

The data of this work will be made available from the corresponding author on reasonable request.

AUTHOR CONTRIBUTIONS

GIA, SAA, HMA, NA, MSA, FMA, NSA, ZMM, FMS—methodology designed the research study and methodology. SAA, GIA, NAS, HMA, NA—conducted the research investigation. SAA, NA, NAS, MSA, HMA, GIA, FMA—wrote the original draft and approved the final version of the manuscript. All authors read and agreed to the published version of the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the biomedical research ethics committee at the Faculty of Medicine, King Abdulaziz University (KAU), Saudi Arabia (Reference No 55-21). Consent to Participate is not applicable in this study.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

SUPPLEMENTARY MATERIAL

Supplementary material associated with this article can be found, in the online version, at https://oss.jomh.org/files/article/1696768202730553344/attachment/Supplementary%20material.docx.

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