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A COMPARATIVE STUDY OF THE EFFECT OF CRUDE AND NANOPARTICLES COSTUS SPECIOSUS ON TESTICULAR DAMAGE ASSOCIATED TO EXPERIMENTALLY INDUCED TYPE 2 DIABETES

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ABSTRACT

Background: Diabetes mellitus is linked with many macrovascular and microvascular complications including cardiovascular, neuro, eye, kidney, liver, and reproductive injuries. Both clinical and animal research disclosed deterioration of spermatogenesis, decreased sperm count, seminal fluid volume, sperm motility, and depressed testosterone concentrations during diabetes. Nanotechnology employment in the alternative medicine field is one of the rapidly rising areas. **Objective:** To investigate the therapeutic effect of crude, nanoparticles (NPs) *Costus speciosus* (*C. speciosus*), and metformin against type 2 diabetes. In addition, their role in treating diabetes-induced testicular toxicity was investigated. **Material and Methods:** Diabetes was induced by feeding the rats with a high-fat diet (HFD) for 2 weeks followed by single i.p. injection of STZ at a dose of 45 mg/kg. Two weeks after STZ injection, crude (500 mg/kg) and NPs *C. speciosus* (250 mg/kg) and metformin (200 mg/kg) were administered daily by gavage for a period of 8 weeks. **Results:** Only NPs *C. speciosus* significantly decreased serum glucose levels versus diabetic rats. The hypoglycemic effect of NPs *C. speciosus* was similar to the metformin effect and superior to crude *C. speciosus*. All treatment regimens significantly increased % body weight gain than diabetic rats. All regimens significantly increased serum insulin and testosterone levels. Concerning testosterone crude *C. speciosus* is superior to NPs *C. speciosus* an effect confirmed with the histopathological findings. **Conclusion:** A lower dose of NPs *C. speciosus* significantly ameliorated diabetes-induced hyperglycemia, body-weight loss, and testicular damage.

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Introduction

Diabetes mellitus is a complex metabolic disease characterized by an obvious hyperglycemic status accompanied by reduced insulin secretion or increased resistance of cells to insulin action [1-3]. Diabetes causes many complications on the various systems of the body, as it is accompanied by a disorder of the circulatory system, blood vessels, urinary system, eye, cardiovascular, and reproductive system [4-6]. One of the most important complications associated with high blood glucose is the male genital disorder [7]. Both clinical and animal research disclosed deterioration of spermatogenesis, decreased sperm count, seminal fluid volume, sperm motility, and depressed testosterone concentrations during diabetes [8, 9]. The decreased serum level of insulin during diabetes is associated with reduced testosterone levels and ultimately testicular damage [10].

Medicinal herbs have proven their ability to contribute to human medication for hundreds of years [11]. Currently, the demand for herbal consumption is increasing all over the world, as it is believed that it is less toxic and cheaper than manufactured medicines [12, 13]. *Costus speciosus (C. speciosus)*, family Costaceae (*Zingiberaceae*), is a popular medicinal herb that was planted in India [14]. The plant is also grown in many separate places in the Kingdom of Saudi Arabia [15]. The herb has many healing properties and is well known in India as an antiinflammatory, antihyperlipidemic, antidiabetic,

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and as a protective agent for the liver [16]. Ethyl acetate, hexane, and methyl alcohol extracts of *C. speciosus* rhizome significantly reduced the plasma glucose level in STZ- diabetes rat model [17, 18].
Nanotechnology utilization in the alternative medicine field is one of the fastest rising fields. It has many advantages that have directed to an enormous increase in the usage of natural products in the management of several chronic disease conditions. Loading of natural plants on nanoparticles (NPs) enhances their bioavailability and targeting [19].
This study aimed to explore the therapeutic effect of both crude and NPs *C. speciosus* on type 2 diabetes-induced testicular toxicity in male rats. Besides, their effect will be compared with the effect of the standard antidiabetic drug, metformin.

Material and Methods

Plant Material:
Rhizomes of *C. speciosus* were purchased from the local market, Jeddah, SA. NPs emulsion of *C. speciosus* was kindly gifted by Dr. Manal Mohamed Khediri, Associate Professor, Physical Chemistry, Chemistry Department, Faculty of Applied Sciences, Laith, Umm Al-Qura University, SA.

Animals:
Thirty male Wistar rats (210-290 g) were obtained from Mansour Scientific Foundation for Research and Development, Jeddah, SA. The animals were kept in standard laboratory conditions such as humidity (55 ± 5%) and temperature (23 ± 1 °C). In addition, the 12 h dark/light cycle was also controlled. Food and water were also served freely. The animals were handled according to the regulations of the animal care of King Abdulaziz University, Saudi Arabia, and the International standard and Institutional Animal Care and Use Committee [20].

Experimental Protocol:
Rats were equally divided into 5 groups (n = 6/group): (1) Control: rats were injected intraperitoneal (i.p.) with citrate buffer (0.05 M, pH 4.5); (2) Diabetics: rats were fed high-fat diet (58% fat, 25% protein and 17% carbohydrate, as a percentage of the total kcal) then they were i.p. injected with STZ (45 mg/kg) dissolved in citrate buffer (0.05 M, pH 4.5) [21]; (3) Diabetics + Metformin: diabetic rats were orally administered 200 mg/kg metformin [22]; (4) Diabetics + crude *C. speciosus*: diabetic rats were orally administered 500 mg/kg crude *C. speciosus* [23]; (5) Diabetics + NPs *C. speciosus*: diabetic rats were orally administered 250 mg/kg NPs *C. speciosus* (1/2 the dose of crude *C. speciosus*). The experiment was continued for 8 weeks.

Assessment of Body Weight (BW):
The BW of the rats was determined at the start (BW0) and at the end of the experiment (BW8). The body weight gain percentage (% BWG) was computed applying the following formula [24]:

$$\% BWG = (BW8 – BW0)/ BW0 \times 100$$

Samples Collection:
At the end of the experiment, the rats were anesthetized using ether, and then blood samples were taken from the heart and the serum was separated and kept at -80 ° C for use in the biochemistry experiments. The testis was extracted, washed with 0.9 % saline solution, then weighed and preserved in a 10% neutral solution of formalin for the histopathological examination.

Assessment of Fasting Serum Glucose:
Fasting serum glucose was assessed using the colorimetric kit of Reactivos GPL, Barcelona, Spain.

Assessment of Serum Insulin:
Insulin was assessed using the rat ELISA assay kit, Immunospec, CA.

Assessment of Serum Testosterone:
Serum testosterone was assessed using the rat ELISA assay kit (ab108666), Abcam, USA.

Assessment of Histopathological Alteration:
The formalin-preserved testes were stained with haematoxylin and eosin (H&E) and examined microscopically for any histopathological changes.

Statistics:
Results were represented as mean ± SE. One-way analysis of variance (ANOVA) followed by Tukey’s post-hoc test was conducted to investigate the difference between groups using SPSS software, version 22, Armonk, NY.

Results

Effect of Crude *C. speciosus*, NPs *C. speciosus*, and Metformin on % Body Weight Gain:

The results showed that the diabetic group had a significant ($p \leq 0.01$) decline in the % BWG versus the control group. The treatment of rats with metformin, crude *C. speciosus*, and NPs *C. speciosus* resulted in a significant ($p \leq 0.01$, 0.05, and 0.05, respectively) increase in the % BWG versus the diabetic group (Figure 1).

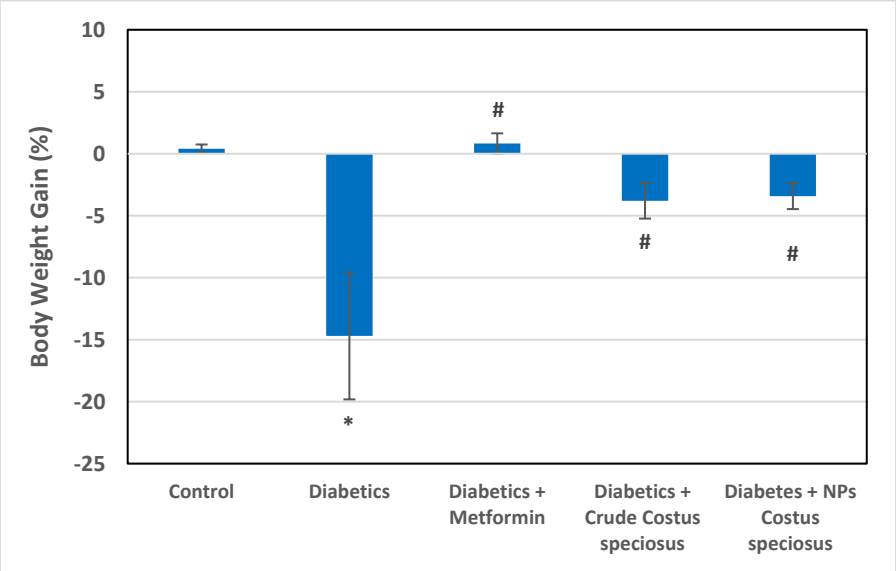

Figure 1. Effect of crude *C. speciosus*, NPs *C. speciosus*, and metformin on % body weight gain computed in high-fat diet/STZ-induced diabetes in rats. Results are expressed as mean ± SE (n = 6). *significant versus the control group; #significant versus the diabetics group. The significance was settled at $p \leq 0.01$.

Effect of Crude *C. speciosus*, NPs *C. speciosus*, and Metformin on Absolute Testis Weight:

The results showed that the diabetic group had a significant ($p \leq 0.01$) decline in the absolute testis weight versus the control group. The treatment of rats with metformin, crude *C. speciosus*, and NPs *C. speciosus* resulted in no alteration in absolute testis weight (Figure 2).

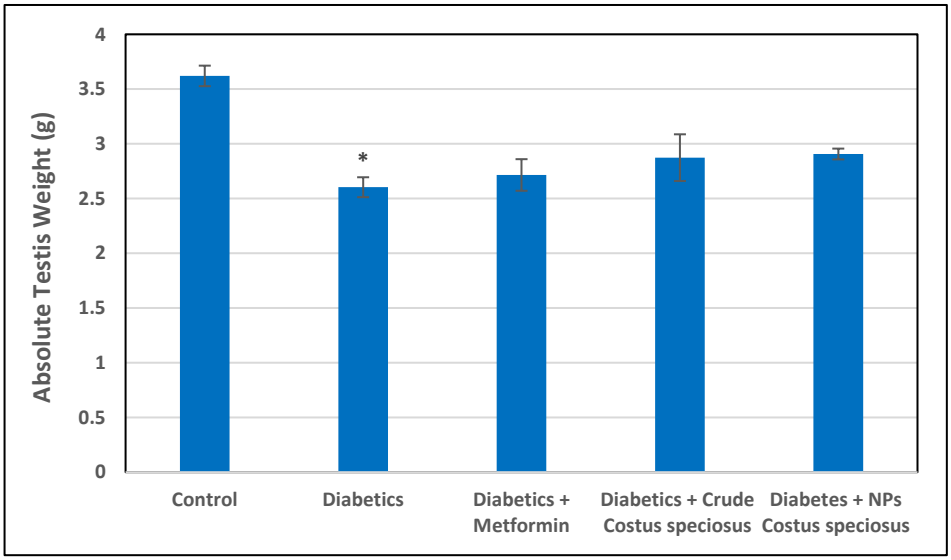

Figure 2. Effect of crude *C. speciosus*, NPs *C. speciosus*, and metformin on absolute testis weight determined in the high-fat diet/STZ-induced diabetes in rats. Results are expressed as mean ± SE (n = 6). *significant versus the control group. The significance was settled at $p \leq 0.01$.

Effect of Crude *C. speciosus*, NPs *C. speciosus*, and Metformin on Fasting Serum Glucose Levels:

The results showed that the diabetic group had a significant ($p \leq 0.01$) increase in the fasting serum glucose level versus the control group. The treatment of rats with metformin, crude *C. speciosus*, and NPs *C. speciosus* resulted in a significant ($p \leq$

0.01) decline in the fasting serum glucose level versus the diabetic group. The NPs *C. speciosus*-effect was equivalent to metformin and more effective than crude *C. speciosus* (Figure 3).

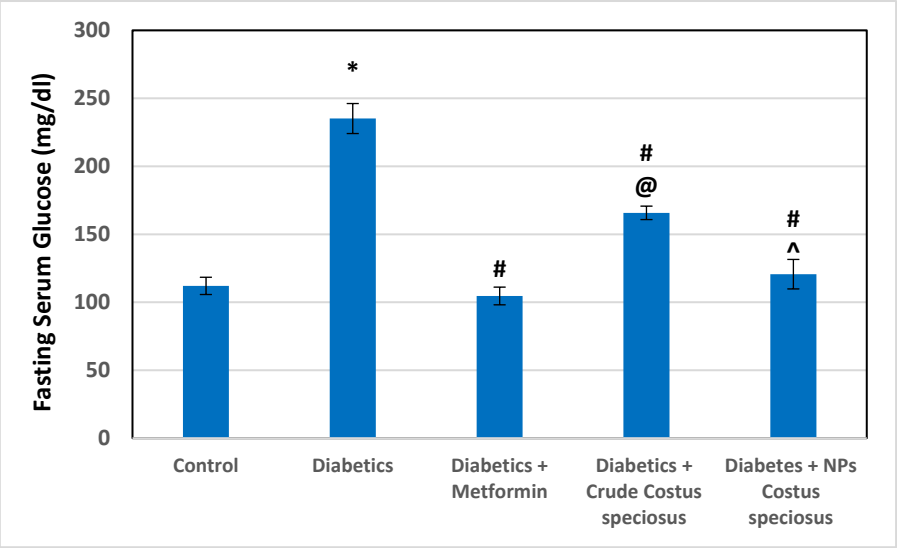

Figure 3. Effect of crude *C. speciosus*, NPs *C. speciosus*, and metformin on fasting serum glucose levels measured in high-fat diet/STZ-induced diabetes in rats. Results are expressed as mean ± SE (n = 6). *significant versus the control group; #significant versus the diabetics group; @significant versus the diabetics + metformin group; ^significant versus the diabetics + crude *C. speciosus* group. The significance was settled at $p \leq 0.01$.

Effect of Crude *C. speciosus*, NPs *C. speciosus*, and Metformin on Fasting Serum Insulin Levels:

The results showed that the diabetic group had a significant ($p \leq 0.05$) decline in the level of fasting insulin versus the control group. The treatment of rats with metformin, crude *C. speciosus*, and NPs *C. speciosus* resulted in a significant ($p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.01$, respectively) elevate in the level of fasting serum insulin versus the diabetic group (Figure 4).

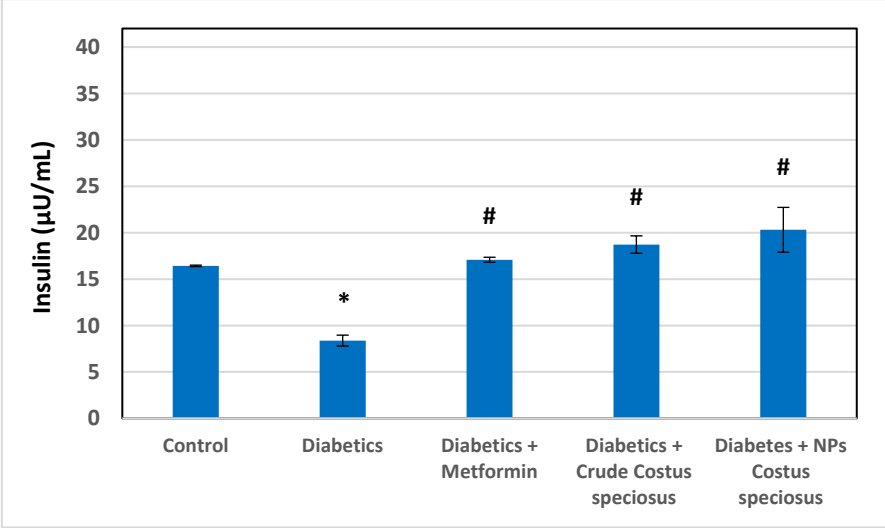

Figure 4. Effect of crude *C. speciosus*, NPs *C. speciosus*, and metformin on fasting serum insulin levels measured in high-fat diet/STZ-induced diabetes in rats. Results are expressed as mean ± SE (n = 6). *significant versus the control group ($p \leq 0.05$); #significant versus the diabetics group ($p \leq 0.05$). The significance was settled at $p \leq 0.01$.

Effect of Crude *C. speciosus*, NPs *C. speciosus*, and Metformin on Serum Testosterone Levels:

The results showed that the diabetic group had a significant ($p \leq 0.01$) decline in the level of serum testosterone versus the control group. The treatment of rats with metformin, crude *C. speciosus*, and NPs *C. speciosus* resulted in a significant ($p \leq 0.01$) elevate in the level of serum testosterone versus the diabetic group. The crude *C. speciosus*-effect was equivalent to metformin and more effective than NPs *C. speciosus* (Figure 5).

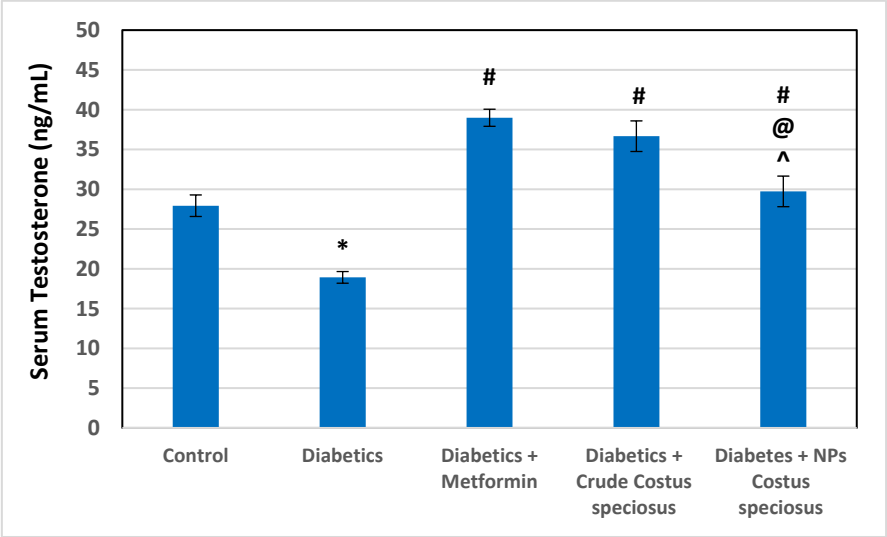

Figure 5. Effect of crude *C. speciosus*, NPs *C. speciosus*, and metformin on serum testosterone levels measured in high-fat diet/STZ-induced diabetes in rats. Results are expressed as mean ± SE (n = 6). *significant versus the control group; #significant versus the diabetics group; @significant versus the diabetics + metformin group; ^significant versus the diabetics + crude *C. speciosus* group. The significance was settled at $p \leq 0.01$.

Effect of Crude *C. speciosus*, NPs *C. speciosus*, and Metformin on Testicular Tissue Histopathology:

Figure 6 showed all the experiment groups' H & E photos. Photos a and b show control testis with normal testicular structure. Photos c and d show diabetics testes with decreased thickness germ layer and degenerated Leydig cells. Photos e and f show that metformin treatment preserved most of testes histology except some degenerated Leydig cells. Photos g and h show that crude *C. speciosus* treatment preserved all of testes histology. Photos i and j show that NPs *C. speciosus* treatment preserved most of testes histology except a decreased number of Leydig cells.

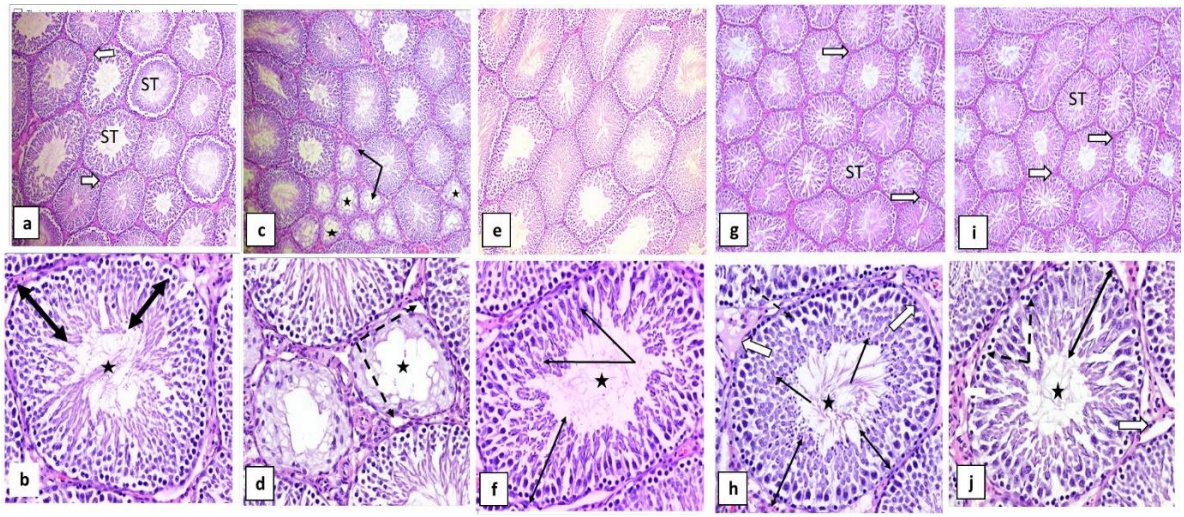

Figure 6. Control testis: Photo (a) shows seminiferous tubules (ST) with regular outlines and full-thickness germ layers. It also shows narrow interstitial spaces that contain Leydig cells. Photo (b) shows a seminiferous tubule with full-thickness germ layers, narrow lumen, and sperm tails. The interstitial tissue contains a normal population of Leydig cells.

Diabetics' testes: Photo (c) shows shrunken tubules with irregular outlines, wide lumen, and decreased thickness of germ cell layers. Numerous tubules exhibited loss of most germ cell layers thus appeared empty except for the basal spermatogenic stem cells. Photo (d) shows degenerated Leydig cells between the tubules.

Diabetics + Metformin testis: Photo (e) shows seminiferous tubules with regular outlines and full-thickness germ layers. Photo (f) shows narrow interstitial spaces containing the normal population of Leydig cells, however, some Leydig cells showed a slight degenerated nuclei near congested blood vessels.

Diabetics + crude *C. speciosus* testis: Photo (g) showed preservation of normal seminiferous tubules structure. Photo (h) showed full-thickness of germ layers with narrow interstitial spaces that contain Leydig cells. The lumen also contains mature sperm heads.

Diabetics + NPs *C. speciosus* testis: Photo (i) shows the preservation of normal seminiferous tubules' structure. Photo (J) shows seminiferous tubule with full-thickness germ layers, narrow lumen, and sperm tails. The interstitial tissue contains the normal population of Leydig cells. However, the interstitial spaces are slightly widened and contain congested blood vessels and few Leydig cells.

Discussion

Type 2 diabetes is a chronic disease accompanied by many complications comprising organ damage caused by injury of the micro blood vessels, including the eye, nerves, and testis [25]. This study was based on the induction of a model of type 2 diabetes using a high-fat diet with a single small dose of STZ [22]. The success of the model confirmed a noticeable increase in the level of fasting serum sugar in rats [25]. It was also observed that the bodyweight of the rats was lower than that of the control group. Weight loss is one of the signs of high blood sugar as it causes loss of calories, muscles, and fatty tissues due to protein depletion [10]. Moreover, the study found a significant decline in testicular weight in diabetic rats as an indication of its vulnerability to the high serum sugar level [25]. The high serum glucose of rats was accompanied by a significant decline in the level of serum insulin an indicator of insulin resistance that is commonly associated with type 2 diabetes [26].
The obtained results showed an impairment of the testicular function in rats with type 2 diabetes, which was supported by a decline in testicular weight and a decrease in the level of testosterone in the serum. These results were consistent with many recent studies [27, 28]. Several scientific works have documented a correlation between low blood testosterone and type 2 diabetes [29, 30]. Previous studies have shown that the reason for low testicular weight is due to the low level of testosterone, which is the hormone that stimulates the growth of the organs of the reproductive system [31]. Testosterone deficiency occurred due to the low number of Leydig cells that accompanied diabetes. In addition, previous studies have shown that insulin deficiency causes a decrease in Follicle-stimulating hormone (FSH) production and this negatively affects testosterone production and fertility [32]. The histopathologic study done in this work confirmed these results as it revealed the occurrence of degenerated Leydig cells between the tubules in diabetic rats [28].
The results of this study showed that the consumption of both crude *C. speciosus* and NPs *C. speciosus* in diabetic rats increases the level of testosterone in the serum. The results also showed the superiority of the nano-formulation compared to both metformin and the crude costus in increasing the male hormone. In support of this, testicular histopathological examination showed that crude and nano costus were more effective in preserving the Leydig cells than metformin. It is possible to explain the improvement in the concentration of the male hormone in different treatment groups to the decreased blood glucose level, where studies confirmed the existence of an inverse relationship between the level of sugar and testosterone [33]. Therefore, the increase in testosterone may be linked to the observed antidiabetic effect confirmed by improved blood sugar levels, improvement in body weight gain, as well as an increase in the level of insulin in the crude costus, nano costus, and metformin treatment groups. The studies confirmed the results of this scientific paper, where it was reported about a hypoglycemic effect of costus, and attributed this to the ability of the plant to stimulate pancreatic beta cells to produce insulin and also increases the sensitivity of the body tissues to the work of insulin [18, 34, 35]. Furthermore, a previous study demonstrated the effect of costus in inhibiting the action of α-glucosidase activity and that it may be the mechanism behind its antidiabetic effect [36]. The superiority of the nanostructure over the crud extract of costus concerning the antidiabetic effect, as well as the protective effect against diabetes-induced testicular damage, may be attributed to improved bioavailability and absorption of the nano formula [37].
With regard to the effect of costus on repairing testicular dysfunction observed in type 2 diabetes mellitus developed in rats with a high-fat diet and STZ in this research, we did not find previous studies confirming our results. Instead, there was a previous study that reported the inability of diosgenin extract from costus in fixing sperm defects associated with type 2 diabetes mellitus induced by alloxan in mice [38]. The latest study did not measure the level of testosterone as an indicator of testicular functions as approved by the current study. Therefore, this study recommends that other studies must be carried out to ensure the effectiveness of costas in treating the testicular disorders associated with type 2 diabetes.

Conclusion

The results of this study showed that both crude and nanoparticles *Costus speciosus* exerted antidiabetic effects against high-fat diet/STZ-induced type 2 diabetes in rats. In addition, both formulas preserved the testicular function and histology. The nanoparticles *Costus speciosus* was superior in action and more potent.

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