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Silent Information Regulator 1 Mediates Quercetin Protective Effect on Cadmium-induced Testicular Damage in Adult Male Rats

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- SIRT1
- Quercetin
- Cadmium
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Abstract

Male infertility can occur due to environmental factors such as exposure to heavy metals. Cadmium is one of the heavy metals that leads to infertility on prolonged exposure. Quercetin is a naturally occurring dietary flavonoid with antioxidant, anti-inflammatory, hepatoprotective and neuroprotective effects. We aim in this study to evaluate the role of silent information regulator 1 (SIRT1) in the protective effect of quercetin on cadmium-induced testicular damage in rats. In the current study, testicular injury was induced by cadmium. The study was carried out on forty adult male albino rats divided into four groups. Group (I): Normal control group. Group (II): Quercetin group. Group (III): Cadmium group. Group (IV): Cadmium + Quercetin group. At the end of the experiment, we measured reproductive hormones, epididymal sperm functional parameters, testicular reduced glutathione, superoxide dismutase activity and malondialdehyde. Immunoassays of Bcl-2 & tumor necrosis factor alpha (TNF-α), testicular apoptotic marker caspase -3 were done. We assessed the testicular expression of SIRT1 by real-time PCR. Testicular expression of COX-2 was assayed by immunohistochemical staining. A histopathological examination of testicular tissue was done. Our results revealed that cadmium-induced testicular histological injury enhanced oxidative stress and apoptosis, paralleled with down-regulation of SIRT1. Quercetin co-treatment displayed antioxidant/anti-apoptotic efficacy increased SIRT1 level, improved reproductive hormones axis and fertility parameters. We concluded that SIRT1 integrates testicular quercetin-mediated intracellular transduction cascades in cadmium-induced testicular damage.

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INTRODUCTION

1. Introduction:

Male infertility can occur due to various causes. It may be due to genetic and environmental factors such as exposure to heavy metals. Cadmium (Cd) is one of the heavy metals that leads to infertility on prolonged exposure. Cd leads to environmental pollution in different processes of industry and smoking. Exposure to cadmium may occur through air, food or water pollution. (1)

Despite low exposure to Cd, it is eliminated at a very slow rate so that it can accumulate in different body organs. It accumulates in large amounts in testicular tissue. (2) It may be the cause of testicular injury and infertility. (3)

Cadmium-induced testicular toxicity is caused by oxidative stress, an increase in the production of proinflammatory cytokines, and an enhancement of sperm cell death. These consequences result in gonadal malfunction and could lead to infertility. (4)

Oxidative stress is one of the primary mechanisms of Cd-induced testicular injury. Reactive oxygen species (ROS) homeostasis is a state of equilibrium between the antioxidant system and ROS production. Any disruption of this balance causes oxidative stress, impairs the function of the sperm, and triggers cell death. (5)

A naturally occurring food flavonoid called quercetin (3, 3′, 4′, 5, 7-pentahydroxyflavone) is obtained from edible plants, and it contains five hydroxyl groups that are responsible for its biological activities. (6) It has antioxidant, anti-inflammatory, anti-allergic, antimicrobial activities, hepatoprotective and neuroprotective effects (7). Due to its potent antioxidant and free-radical scavenging properties, it has a variety of bio-pharmacological effects and may present intriguing new alternatives for creating more effective chemo-preventive and chemo-therapeutic techniques. (8) Quercetin has a potent antioxidant effect and protects cells against excessive apoptosis. (9)

Silent information regulator 1 (SIRT1) is a class III histone deacetylase, one member of the sirtuin protein family. (10) SIRT1 deacetylates both histones and some non-histone proteins. Nicotinamide adenosine dinucleotide (NAD) controls its activity. (11) It has a vital role in different biological and physiological functions. It controls the expression of genes, metabolic function and the ageing process. (12)

Pancreatic and duodenal homeobox factor 1 (PDX-1), nuclear factor kappa B (NF-kB), and forkhead box class O (FOXO) are among the transcription factors that SIRT1 binds to and deacetylates. (13)

Previous research suggested that SIRT1 regulates reactive oxygen species. So the modulation of the SIRT1 levels may antagonize ROS production, so it seems to be a new target for treating many pathological conditions. (13)

Previous studies stated that if SIRT1 is ablated completely, the reproductive function is severely affected in gonads of male and female mice. (14) Some polyphenols, such as quercetin, can bind to SIRT1 and alleviate inflammation and apoptosis. (15)

In the present study, we hypothesized that Cd might harm the male reproductive system by causing oxidative stress., inflammation and apoptosis. So we aimed in our study to explore the molecular mechanisms of quercetin protective effect against injury to the testes caused by

cadmium and the possible contribution of the SIRT1- dependent signal pathway, with a focus on SIRT1-mediated regulation of oxidative stress and inflammation.

2. Materials and Methods

2.1. Drugs and chemicals

Cadmium chloride ($CdCl_2$) and quercetin were purchased from Sigma-Aldrich (St. Louis, MO, USA). All chemicals were of the highest analytical quality.

2.2. Animals

The study was performed over six weeks in the animal house of the Faculty of Medicine, Tanta University. The study involved forty adult male albino rats weighing 200-250g aged 12 weeks which were obtained from the experimental animal services house of the Faculty of Medicine, Tanta University. Rats were kept for acclimatization in clean cages. All animals were pair-housed in cages. Rats were fed the standard balanced rodent formula with water and libitum. The room temperature was about 25ºC, and a 12h light/dark cycle. The animal experiments were approved by the Ethical Animal Research Committee of Tanta University.

2.3. Ethics statement

All the experiments were carried out in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication no. 85–23, revised 1996). The animal study was reviewed and approved by the Ethical Animal Research Committee of Tanta University approval number 35818/9/22. Animal handling was followed according to Helsinki declaration of animal ethics. Animals in this study will not be used again in other experiments. All the remnants of sacrificed animals were discarded by safe disposal measures in the general incinerator of Faculty of Medicine, Tanta University according to the research and safe disposal rules.

2.4. Experimental design

After 2 weeks of acclimatization, rats were divided into four groups (10 rats each); Group I (control group): received daily injections of the saline vehicle alone. Group II (Quercetin group): received quercetin (15 mg/kg body weight) once daily for 28 days, intraperitoneally. Group III (Cadmium group): injected subcutaneously with $CdCl_2$ dissolved in saline at a dose of 2 mL/kg/ day for 28 days. Group IV (Cadmium + Quercetin group): received quercetin and cadmium chloride as mentioned above starting quercetin 2 days before the cadmium injection (16).

2.5. Blood and tissue sampling

2.5.1 Blood sampling:

At the endpoint of the experiment, all animals were fasted overnight and anaesthetized with thiopental sodium (30 mg/kg body weight, intraperitoneally). (17) Blood was collected from the retro-orbital plexus. After that animals were scarified under anaesthesia by cervical decapitation.

Blood collected allowed to clot by standing for 30 minutes at room temperature, followed by 20 minutes of centrifugation (1000 xg 4^oC). Sera were collected and kept at -80^oC for further biochemical analysis.

2.5.2. Tissue sampling:

Both left and right testes were rapidly removed, weighted, rinsed at their place with saline (0.9% w/v) and then allowed to dry by blotting with filter paper. Left testes were

preserved in 10% phosphate paraformaldehyde (10%) buffered solution to be processed for histopathological and immunohistochemical examination. While the right ones were divided into two halves and then immediately frozen at –80ºC for tissue homogenate and molecular analysis.

2.5.3 Preparation of testicular tissue homogenates

Pieces of testes were allowed to thaw, weighed and homogenized in 5 volumes of cold 50mM phosphate buffer saline PBS (pH 7.4) by a Potter–Elvenhjem tissue homogenizer. Then were centrifuged at 5000×g for 20 min at 4 °C. The resultant supernatant was then divided into aliquots and stored at −80 °C until used for the different estimations. Total protein content was determined in homogenates according to the method of **Lowry et al.** (18)

2.6. Biochemical analysis

2.6.1 Assessment of redox status parameters:

Spectrophotometric assay of testicular tissue malondialdehyde (MDA), reduced glutathione (GSH) and superoxide dismutase (SOD) levels by commercial kits supplied by (Bio-diagnostic, Giza, Egypt).

2.6.2 Assessment of testicular tissue inflammatory and apoptotic biomarkers:

Enzyme-linked immunosorbent assay (ELISA) was used to detect tumour necrosis factor alpha (**TNF-α)** level by ELISA kit supplied by (Sino Biological, catalog number (KRC3011), **Bcl-2** levels by ELISA kit supplied by (Mybiosource, Inc. Southern California, San Diego (USA) (Cat. No: MBS2881713) and **caspase 3** level by ELISA kit supplied by (Elabscience, USA, Cat. No: E-EL-R0160).

2.6.3 Hormonal assay:

Sera were used for colorimetric assay of serum testosterone, LH and FSH levels using ELISA kit (My Bio Source, Inc., CA, USA). Catalog no MBS282195, MBS1609275 and MBS1609301, respectively.

2.7. Sperm parameters evaluation

The epididymal cauda was minced with a scalpel blade in a Petri dish that was pre-heated to 37 °C in order to determine these values. 2 cc of physiological saline solution was used to dilute the epididymal cauda fluid. (19) One drop of evenly mixed sample was applied to a specific slide, and the motility/concentration module of the computer-assisted semen analysis (CASA) system was performed using Mira Lab— Egypt (Mira 9000 sperm Analyzer CASA software). The three criteria utilized to evaluate sperm quality were concentration, motility, and viability. (20)

10 µl of sperm suspension were smeared on a slide, dried by air, and stained with 10 µl of eosin-nigrosin dye (1% eosin Y and 5% nigrosin) to evaluate the viability and morphologic abnormalities. The dead spermatozoa were stained red under the light microscope using × 1000, whereas the live ones were white when viewed under it (21). The sperms were classified into normal and abnormal, and the total sperm abnormality was expressed as percentage incidence. An Olympus trinocular microscope with a plan objective lens, phase contrast optics, and a heated stage (37 °C) was employed for the analysis. Million sperm per milliliter was used to express sperm count. The percentage of sperm motility used to express the number of motile spermatozoa. (22)

2.8. Assay of testicular tissue relative SIRT1 mRNA expression by real-time PCR

Total RNA was extracted from frozen testes using the Qiagen RNeasy Total RNA isolation kit (Qiagen, Hiden, Germany) in accordance with the manufacturer's protocol then the first strand was synthesized using the Super-Script III First-Strand Synthesis System for real-time PCR kit (Life Technologies, Carlsbad, California, USA) in accordance with the manufacturer's instructions. Power SYBR Green PCR Master Mix was used to conduct PCR reactions in accordance with the manufacturer's instructions (Life Technologies, Carlsbad, California, USA). In comparison to the internal control, the housekeeping gene beta-actin, SIRT1 mRNA transcripts were measured. Sequence-specific primers were designed as follows: rat SIRT1 (Gene Bank accession No. XM_039098755.1): up-stream: 5'-CCAGAACAGTTTCATAGAGCC-3',down-stream: 5'TCTTACTTTCAGAGAAGACCCAATA-3'. Rat beta-actin (Gene Bank accession No. EF156276.1): up-stream: 5'-GAGGGAAATCGTGCGTGAC-3 and down-stream: 5'-GGACTCATCGTACTCCTGCTTG-3'. Automatic calculation of relative gene expression was performed using the comparative threshold (Ct) method for the values of the target and the reference genes using the $2^{-\Delta\Delta CT}$ formula.

2.9 Histology and immunohistochemistry

2.9.1 Histological examination of the testis of rats in different groups

The right testes were kept in 10% buffered formalin solution for 24 h, dehydrated in ethanol and embedded in paraffin blocks. Then, 5-µm-thick sections were cut, placed on glass slides, stained with hematoxylin and eosin and examined using Leica DM750 Camera Microscope, Leica Microsystems GmbH, Wetzlar, Germany.

2.8.2 Immunohistochemical examination of COX-2

For immunohistochemistry, 5-µM thickness was cut from the paraffin blocks. The sections were deparaffinized with xylene and rehydrated with ethanol. Non-enzymatic antigen retrieval was performed on each slide and washed with PBS. They were then incubated with primary antibodies for COX-2 (Lab Vision Co.). Positive signals were detected using Lab Vision Universal Detection Kit according to manufacture protocol. (23)

3. Statistical methods

Mean ± standard deviation was used to express data. To evaluate the difference between all studied groups, One-way ANOVA was used. To assess the results between the two groups, post hoc test (Tukey) was used. Results were considered statistically significant with P value <0.05. SPSS was used to perform statistical tests.

4. Results:

4.1. Effect of quercetin on oxidative stress:

Cadmium enhances oxidative stress, which is proved by the significant increase in testicular MDA with a significant decrease in both GSH and SOD levels in the cadmium-treated group compared to the control group. Co-treatment of quercetin with cadmium significantly decreased MDA level and significantly increased GSH and SOD levels as compared to cadmium group (table 1).

4.2. Effect of quercetin on functional sperm parameters:

The cadmium-treated group displayed a significant reduction in total sperm count, sperm motility and viability with an increased percentage of sperm abnormalities compared to control groups. Co-administration of quercetin to cadmium-treated rats significantly increased sperm count, improved sperm viability and motility and decreased sperm abnormality (table 2).

4.3. Effect of quercetin on hormonal assay:

Induction of testicular toxicity by cadmium reduced serum testosterone level, accompanied by decreased gonadotropin levels compared to their levels in the control group. Conversely, Co-treatment of quercetin improved testosterone and gonadotropin levels (table 3).

Table (1): The antioxidant and anti-inflammatory effect of quercetin

	Group I	**Group II**	**Group III**	**Group IV**	**F. test**	**p. value**
MDA (nmol/g tissue)	22.40 ± 0.87	22.51 ± 0.71	42.83±0.98$^{*\#}$	27.75±1.19$^{*\#\$}$	1020.881	0.001*
GSH (mmol/g tissue)	3.77 ± 0.31	3.79 ± 0.29	2.24 ± 0.13$^{*\#}$	3.27 ± 0.18$^{*\#\$}$	93.015	0.001*
SOD(Activity units /g protein	25.02 ± 1.01	25.29 ± 0.69	20.38±0.78$^{*\#}$	25.30 ± 0.45$^{\$}$	110.897	0.001*
TNF- α	41.59± 2.09	41.38± 1.07	84.71± 3.97$^{*\#}$	46.68± 2.65$^{*\#\$}$	617.111	0.001*

Values are represented as mean ± SD (n=10)
$^{*\#\$}$ denote a statistically significant difference at (P < 0.05) using one-way ANOVA with Tukey's post hoc test.
* Denotes statistical significance when compared to group I.
# Denotes statistical significance when compared to group II.
$ Denotes statistical significance when compared to group III.

Table (2): Effect of quercetin on functional sperm parameters

	Group I	**Group II**	**Group III**	**Group IV**	**F. test**	**p. value**
Sperm count(million per ml) (10^6 per ml)	67.10 ± 3.98	64.40 ± 4.90	34.30 ± 2.98$^{*\#}$	52.50 ± 3.03$^{*\#\$}$	153.774	0.001*
Progressive sperm motility (% of the total sperm count)	74.50 ±3.03	74.80±2.90	41.20±2.97$^{*\#}$	54.10±2.28$^{*\#\$}$	342.481	0.001*
Sperm viability(%)	70.87±1.45	72.20±1.33	46.30±1.91$^{*\#}$	63.29±1.45$^{*\#\$}$	589.392	0.001*
Sperm abnormalities(%)	6.53±0.42	6.43±0.50	25.90±1.45$^{*\#}$	9.97±0.65$^{*\#\$}$	1159.931	0.001*

Values are represented as mean ± SD (n=10)
*#$ denote a statistically significant difference at (P < 0.05) using one-way ANOVA with Tukey's post hoc test.
* Denotes statistical significance when compared to group I.
# Denotes statistical significance when compared to group II.
$ Denotes statistical significance when compared to group III.

Table (3): Effect of quercetin on reproductive hormones axis

	Group I	Group II	Group III	Group IV	F. test	p. value
Serum TT(ng/ml)	6.27 ± 0.53	6.22 ± 0.37	1.92 ± 0.23$^{*\#}$	4.32 ± 0.44$^{*\#\$}$	258.159	0.001*
Testicular TT(ng/mg protein)	4.04 ± 0.29	4.02 ± 0.28	1.60 ± 0.30$^{*\#}$	2.46 ± 0.12$^{*\#\$}$	216.364	0.001*
Serum LH(IU L^{-1})	3.96 ± 0.41	4.09 ± 0.41	1.51 ± 0.32$^{*\#}$	2.48 ± 0.10$^{*\#\$}$	136.049	0.001*
Serum FSH mIU/ml	2.43 ± 0.25	2.58 ± 0.20	1.45 ± 0.28$^{*\#}$	2.46 ± 0.12$^{\$}$	57.299	0.001*

Values are represented as mean ± SD (n=10)
*#$ denote a statistically significant difference at ($P < 0.05$) using one-way ANOVA with Tukey's post hoc test.
* Denotes statistical significance when compared to group I.
# Denotes statistical significance when compared to group II.
$ Denotes statistical significance when compared to group III.

4.4. Effect of quercetin on inflammatory and apoptotic biomarkers:

Inflammatory biomarker; TNF-α, showed a significant increase in cadmium treated group when compared to control group. On the other hand, quercetin co-treatment significantly decreased TNF-α level as compared to cadmium group. (table 1).

Regarding apoptotic biomarker caspase 3 level (Fig. 1), administration of cadmium induced a significant increase in testicular tissue caspase 3 level compared to the control group. On the other hand, quercetin induced a significant decrease in tissue caspase 3 level as compared to the cadmium group.

Bcl-2 level was significantly decreased in the cadmium group compared to the control groups. In contrast, Co-treatment of quercetin induced a significant elevation in Bcl-2 level compared to the cadmium group (Fig. 2).

4.5. Effect of quercetin on testicular SIRT1 expression:

A significant decrease in SIRT-1 mRNA expression was observed in the cadmium group compared to the control group. Conversely, the cadmium + quercetin group showed a significant ($P < 0.05$) increase in the SIRT-1 mRNA expression when compared with the cadmium-treated group (Fig. 3).

4.6. Effect of quercetin on histological and immune-histochemical findings.

Examination of H&E stained sections of the testes of control group (group I) displayed the normal structure of seminiferous tubules exhibiting normal spermatogenic series (Fig.4A) and quercetin group (group II) displayed unremarkable histological changes of the testicular specimens (Fig. 4B). While the cadmium group (group III) revealed testicular disruption with basement membrane irregularity, slight desquamation of germ cells and intervening edema of testicular specimens (Fig. 4C). As regards the cadmium + quercetin group (group IV), it showed almost near normal testicular architecture and structure of the testicular specimens (Fig. 4D).

Examination of COX-2 immuno-stained sections of the testes revealed negative expression of COX-2 within testicular tissue in both the control group (group I) and the quercetin group (group II) (Figs. 5A&B). While the cadmium group (group III) showed strong expression of COX-2 within the cytoplasm of testicular tissue (fig. 5 c). in the cadmium + quercetin group (group IV), weak expression of COX-2 was detected (Fig. 5D).

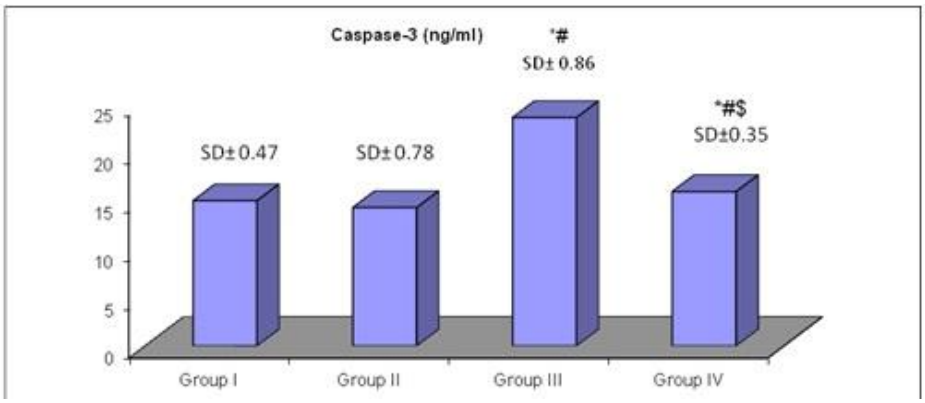

Figure (1): The ameliorative effect of quercetin on caspase-3 among the studied groups

Data are expressed as mean ± SD (standard deviation). P was considered significant at < 0.05* Denotes statistical significance when compared to group I [#]significant change vs Group II (Quercetin-treated group); [$]significant change vs Group III (Cadmium-treated group).

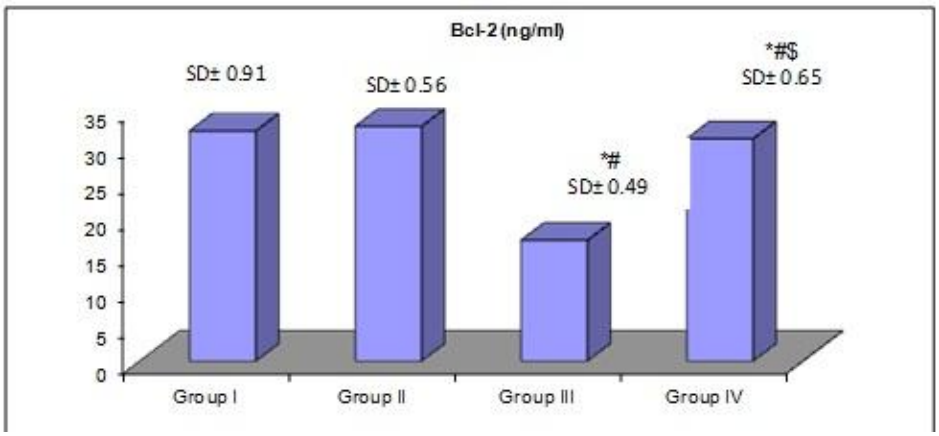

Figure (2): The ameliorative effect of quercetin on Bcl-2 among the studied groups

Data are expressed as mean ± SD (standard deviation). P was considered significant at < 0.05.* Denotes statistical significance when compared to group I [#]significant change vs Group II (Quercetin-treated group); [$]significant change vs Group III (Cadmium-treated group).

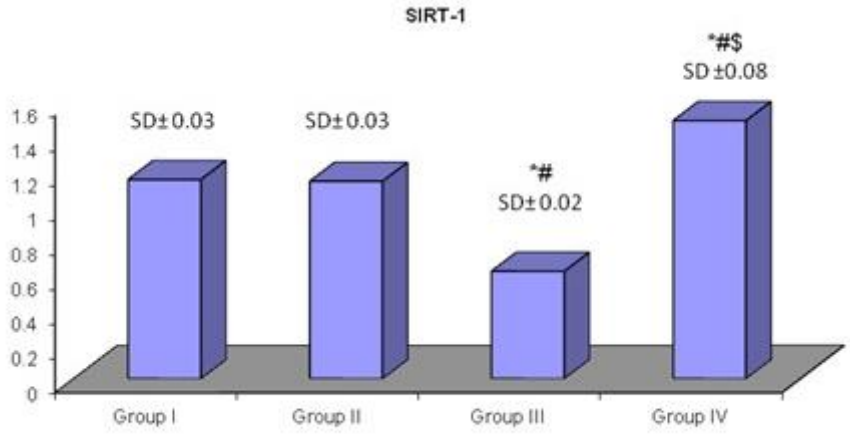

Figure (3): The ameliorative effect of quercetin on SIRT1 among the studied groups

Data are expressed as mean ± SD (standard deviation). P was considered significant at < 0.05. * Denotes statistical significance when compared to group I [#]significant change vs Group II (Quercetin-treated group); [$]significant change vs Group III (Cadmium-treated group).

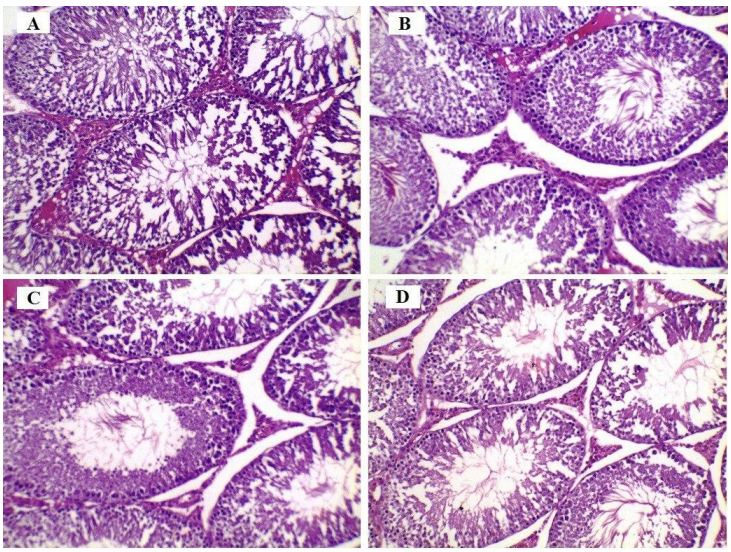

Figure (4): Hematoxylin and eosin staining of testicular tissues in different experimental groups:
Normal structure of seminiferous tubules exhibiting normal spermatogenic series with unremarkable intervening tissue of testicular specimens of the control group (A), unremarkable histological changes of the testicular specimens of quercetin group (B), testicular disruption with basement membrane irregularity, slight desquamation of germ cells and intervening edema of testicular specimens of cadmium group (C) near normal testicular architecture and structure of the testicular specimens of cadmium + quercetin group (D)

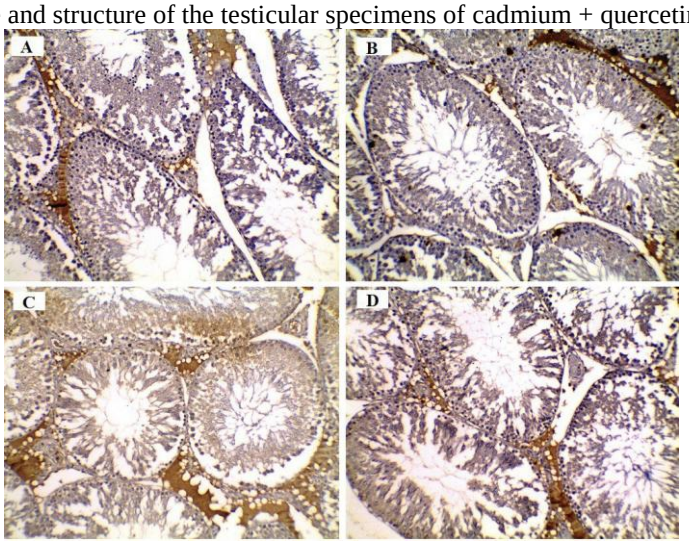

Figure (5): Immunohistochemical expression of COX 2 in different experimental groups:
Negative staining in testicular tissue of control group (A), negative staining of testicular tissue of quercetin group; reminiscent of the control group (B) positive cytoplasmic staining in testicular tissue of cadmium group (C) weak cytoplasmic staining in testicular tissue of cadmium + quercetin group (D)

5. Discussion

Cadmium is an environmental toxicant that is harmful to organs and tissues. Testis are adversely affected when they are exposed for long time to cadmium, it may cause inflammation and impairment of spermatogenesis that may lead to infertility (2).

Quercetin is a plant polyphenol having antioxidant, antiproliferative, and anti-inflammatory properties; previous research stated that it has biological effects and different pharmacological actions (24).

It is not quite clear how cadmium causes the harm to the testicles. The biochemical process for cadmium-induced testicular degeneration may involve the production of free radicals together with an accumulation of lipid peroxides. Additional potential molecular mechanisms include increased production of pro-inflammatory

cytokines and promotion of apoptosis in spermatogenic cells. (25)

Here, we gave a proof that quercetin guards against testicular harm brought on by cadmium by reducing oxidative stress. Additionally, this study showed that quercetin reduces testicular damage driven on by cadmium via the SIRT1 signaling pathway.

According to our findings, cadmium exposure significantly increased the content of lipid peroxides in the testes, as evidenced by raised MDA levels, and significantly decreased GSH and SOD activity. This is in line with the findings of **Farombi et al.** (26), who reported that rats exposed to cadmium experienced higher MDA levels and a substantial decrease in testicular and spermatozoal SOD, CAT, and GPX activity.

Elmallah et al. also demonstrated that adult rats exposed to Cd for five days experienced increased oxidative stress, increased formation of lipid peroxides and nitric oxide, and lower levels of catalase, superoxide dismutase (SOD), glutathione peroxidase, and GSH. (27)

In our study, quercetin administration considerably reduced high MDA levels and also significantly increased SOD and GSH, providing protection against Cd poisoning.

According to **Unsal et al.,** quercetin significantly protected rats from Cd-induced neurotoxicity. It reduces lipid peroxides and prevents neuronal cell death, enhancing the activity of antioxidant enzymes in brain tissue. (28)

The antioxidant-sparing action of quercetin can be credited with improving the antioxidant capacity of testicular tissues. In the current work, quercetin's antioxidant properties may be regulated by SIRT1 that it directly upregulates. Increased testicular SOD activity, GSH, and decreased lipid peroxidation are all caused by an increase in SIRT1 activity. These findings are consistent with earlier findings that dietary polyphenols increase SOD activity, and SIRT1 controls this activity via deacetylating forkhead box class O (FOXO) to detoxify ROS. (29)

During the process of spermatogenesis, cells undergo apoptosis, or programmed cell death. Increased apoptosis and impairment of testicular functioning are brought on by radiation exposure, endocrine disruption, and infection. Administration of quercetin provided considerable testicular protection by reducing the apoptotic impact brought on by cadmium (16).

SIRT-1 is a conserved NAD+ dependent deacetylase enzyme. It play a crucial role in both inflammation and apoptosis. (30) SIRT-1 controls a variety of cellular processes that control many physiological processes and illnesses. PGC-1 alpha is deacetylated by SIRT-1, which boosts its activity, encourages mitochondrial biogenesis, and maintains mitochondrial function. (31) Prior studies established SIRT-1 as a significant marker in the control of testicular physiology. Since SIRT1 is regarded as a redox-sensitive molecule, the amount of thiol inside of cells affects both its level and its activity. Additionally, SIRT1 is inactivated by the phosphorylation of SIRT1 by free radicals produced during oxidative stress (32). Cadmium decreased testicular SIRT1 expression and activity in this study. This could be due to the depletion of testicular GSH (thiol) content.

Quercetin, on the other hand, increased SIRT1 expression and activity to counteract these effects. These results support the assertion made by **Zhang et al.** (33), who claimed that the

activation of SIRT1 is what causes quercetin to have an antioxidant impact. In light of this, it might be said that quercetin is a testicular SIRT1 activator.

A harmful signal for cell death is produced when the caspase family of cysteine proteases is activated. Caspase-3 is regarded as the apoptosis' final executor (34). BCL-2, on the other hand, is a cell survival factor that all cells require. As it safeguards mitochondrial integrity and blocks apoptotic pathways, it prevents caspase activation. In the current study, we found that the group that received cadmium treatment had lower testicular Bcl-2 expression and higher caspase-3 expression. The Bcl-2 expression was higher and caspase-3 expression was close to normal in the quercetin plus cadmium group.

As per **Pang et al.**, SIRT1 overexpression prevents apoptosis in preadipocytes and reduces camptothecin-induced apoptosis through the caspase-3 pathway, but silencing of SIRT1 results in apoptosis. We therefore came to the conclusion that SIRT1 had a function in the inhibition of apoptosis. (35)

Furthermore, our findings demonstrated that the mechanism of Cd poisoning on the testes was related to both the inflammatory response and testicular tissue death.

An essential indicator of inflammation is COX-2 (36). NF-B is activated in the COX-2 promoter by TNF, which starts the expression of COX-2 (37). Through the activation of the P38 mitogen-activated protein kinase, a cellular signaling molecule that is susceptible to oxidative stress, Cd causes the production of COX-2 (p38 MAPK). (37).

The findings of our investigation showed that Cd might increase TNF- α and COX-2 mRNA expression, showing that Cd causes inflammation of the testicular tissue. Quercetin co-treatment helped to reverse this. This is in agreement with **Lim et al.** who stated that Cd induces COX-2 expression and apoptosis in astrocytes. (38)

Quercetin blocks the inflammatory enzymes cyclooxygenase (COX) and lipoxygenase, so it lowers inflammatory mediators such as prostaglandins and leukotrienes. (39)

According to earlier studies, quercetin treatment dramatically lowers the levels of inflammatory mediators including NO synthase, COX-2, and CRP in a cell line derived from human hepatocytes. (40). Quercetin reduces TNF-α production in macrophages. (41); it also inhibits COX and lipoxygenase enzymes (42). According to **Zhang et al.,** SIRT1 may reduce COX-2 expression, which modifies macrophage activity (43).

Quercetin prevents chondrocyte death induced by endoplasmic reticulum stress by activating the SIRT1/AMPK signaling pathway in vitro, as shown by **Feng et al.** (44). Through antioxidative and anti-inflammatory properties that were shown to be linked to the SIRT1/NF-B signaling pathway, quercetin was shown to be beneficial against atherosclerosis in high-fat diet rats' carotid arteries. (33)

A crucial biomarker of sperm dysfunctions is sperm morphology. If more than 10% of the sperm cells in the man's semen are abnormal, the male is infertile. (45)

In the present study, cadmium-induced modification in sperm parameters, including a decline in epididymal sperm count, sperm motility, and increased sperm abnormalities, which results in reproductive failure; these

observations are consistent with earlier reports by **Adamkovicova et al.** (46). However, co-treatment with quercetin was able to undo these alterations.

According to **Farombi et al.** quercetin dramatically halted the decline in sperm motility, sperm count, and antioxidative parameters in cadmium-treated rats, (26) These findings agree with those of **Yelumalai et al.** (47), they demonstrated that in vivo injection of quercetin reduces sperms with morphological defects, enhances sperm motility, and mitigates the decline in sperm count in nicotinamide-induced DM.

Additionally, Cd treatment resulted in a drop in the rats' testosterone, LH, and FSH levels. This outcome is consistent with that of **Pillai et al.** (48), who discovered a substantial drop in testosterone, FSH, and LH levels in rats given CdCl2 treatment. Cd may, depending on the dose, induce anterior pituitary apoptosis both in vivo and in vitro, so decreasing FSH and LH expression. (49) In addition to its effects on Leydig cells, cadmium can alter hormone levels by impacting the hypothalamus pituitary-testicular axis in many ways. For instance, Cd affected the circadian pattern release of noradrenaline, a regulator of hypothalamus hormone secretion, which lead to changes in the daily pattern of plasma testosterone and LH levels. (50) Gunnarsson et al. demonstrated that rat testosterone production is impacted by Cd exposure by suppressing the expression of the LH receptor mRNA. Steroidogenic acute regulatory protein (StAR), cAMP, LH receptor levels were all lowered in the testis by Cd (51)

The most remarkable finding of our study was the progressive improvement in Leydig cell function brought on by quercetin administration, which was reflected in increased serum and testicular testosterone levels.

So, the current study offers proof that quercetin has a protective effect on the testicles. It might lessen the severity of biochemical and histological problems brought on by cadmium. This hopeful effect might be caused by a mechanism that increases testosterone secretion, SIRT1 expression and activity, and total antioxidant power while decreasing caspase and COX 2 activity and inhibiting apoptosis as a result. It is necessary to do additional research to confirm the current findings in clinical settings.

6. Conclusion

Testicular functions were affected by cadmium administration, as shown by a drop in testosterone levels, a disruption in histoarchitecture, and an increase in oxidative stress, inflammatory burden, and apoptosis. On the other hand, quercetin administration improved testicular antioxidant status (GSH) and reduced oxidative stress (MDA), inflammation (TNF & COX-2), and apoptosis (caspase-3), maybe through the elevation of SIRT-1 expression. Consequently, quercetin might be a helpful compound that aids in shielding the testes from deteriorating changes brought on by toxins or infection.

7. Declarations and statements

Ethics approval and consent to participate: We conducted the study protocol according to The Local Committee of Research and Medical Ethics of the Faculty of Medicine, Tanta University.

Consent to publication: Not Applicable.

Availability of data and material: The corresponding author can provide the datasets used and/or analysed during the current work upon request.

Competing interests: The authors declare to have no conflicts of interest.

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Authors' contributions: All authors contributed to the data analysis and interpretation of the data, drafting, and revising the manuscript, and approved the final version of the manuscript.

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