



Thyroidectomy Induced Toxicity of Testis and Possible Amelioration by Chia (*Salvia hispanica*) Seeds Extract

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ABSTRACT

Background: Thyroidectomy or thyroid surgery induces hypothyroidism and causes thyroid and testicular hormone deficiency. The current Study Aimed to examine the therapeutic effect of chia seeds extract against thyroidectomy-induced testicular toxicity, oxidative stress, DNA damage, proliferation in rats.

Methods: 40 male rats were equally divided into 4 groups [Gp1, control; Gp2, CSE; Gp3, thyroidectomies rats; Gp4, treated thyroidectomies rats with chia seeds extract (Thy+CSE)].

Result: Following a thyroidectomy, significant reductions ($P<0.05$) were observed in The Levels of triiodothyronine (T3), thyroxine (T4), sexual hormones (Testosterone (tt), Follicle-Stimulating Hormone and Luteinizing Hormone And prolactin), count of sperm, index of morphology, vitality, progressive motility, total motility (PR+NP), Glutathione, Superoxide Dismutase, Glutathione S-Transferase and PCNA expressions compared to the control and CSE after three weeks. Additionally, thyroidectomy leads to significant ($P<0.05$) increases in the serum of thyrotropin (TSH), alanine transaminase, alkaline phosphatase, non-progressive sperms, immotile sperms, malondialdehyde, DNA damage and injury of testis and significant ($P<0.05$) decreases in blood. Thy+CSE treatment helped to modulate most of these changes and recover testicular injury when compared To The Control and CSE. Rats treated with CSE after thyroidectomy Showed gradual improvements in testicular functions, histology and spermatogenesis due to its beneficial affection in free radicals' scavenger.

Key words: Chia seeds extract, DNA damage, Oxidative stress, PCNA immunoreactivity, Thyroidectomy operation, Toxicity of testes.

INTRODUCTION

Thyroid hormones are essential for controlling a range of metabolic disorders, such as growth and heart rates, blood pressure and the functioning of other endocrine systems (Ibrahim *et al.*, 2012; Elgamasy *et al.*, 2022). In cases of goitre, hyperthyroidism, or thyroid cancer, a thyroidectomy procedure is performed to remove part or all of the thyroid gland by a surgeon (Tousson *et al.*, 2014). The growth and differentiation of non-germ tissues, the release of steroids and the motility of sperm are just a few of the processes that thyroid hormone controls in the testis (Hasan *et al.*, 2024; Singh *et al.*, 2023).

Total T3 controls the growth of Leydig cells and the expression of oestrogen receptors, which control testosterone, as well as acting directly on Leydig cells by controlling the production of steroidogenic factor 1 and indirectly on Leydig cells through paracrine processes involving Sertoli cells (La Vignera *et al.*, 2017; Nittoli *et al.*, 2021). Recently, all research indicates that; the removes all or part of the thyroid tissue-induced hypofunction in patients and animals (Tousson *et al.*, 2012c, 2014., 2022; Lonèar *et al.*, 2022; Hasan *et al.*, 2024; Budhalakoti *et al.*, 2017; Ibrahim *et al.*, 2011). Spermatogenesis is negatively impacted by hypothyroidism, suggesting that the hormone of the thyroid may be important for maintaining normal functioning of the testis, spermatogenesis and monitoring healthy testicular development (Ahsan *et al.*, 2021; Alankooshi *et al.*, 2023; Hasan *et al.*, 2024). A recent study found that hypothyroid testicles showed maturation stoppage of spermatogenesis, a decrease in Sertoli and Leydig cells,

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interstitial oedema and thickening of the basal membrane (Tousson *et al.*, 2012a; Massoud *et al.*, 2012).

As the methods by which herbs positively on health and quality of life become better understood, medical herbs are gaining increasing acceptance from both the public and medical fields (mutar *et al.*, 2020; Rohaim *et al.*, 2021). Correlative and elective pharmaceutical use has significantly increased in the last years. Alzheimer's, atherosclerosis, diabetes and arthritis are just a few of the inflammatory disorders that are treated with Chia seeds

extract due to its potent anti-inflammatory and antioxidant features (Salama *et al.*, 2014; Hasan *et al.*, 2023).

Due to their potential uses in the life sciences to increase lifespan and quality of life, chia seeds extract with antioxidative capabilities have lately come to light as strong therapeutic agents (Elgamasy *et al.*, 2022; Hasan *et al.*, 2022). Chia seeds extract can prevent oxidative stress and serves as both hydrophilic particles and a non-homogenous nucleation core. It also looks to be an efficient free radical quencher with antioxidant properties (Zabihi *et al.*, 2015; Alotaibi *et al.*, 2021; Hasan *et al.*, 2021; Saggu *et al.*, 2014). Therefore, this research aimed to examine the affection of Chia seeds extract (CSE) on testicular tissue toxicity, sperm feature abnormalities.

MATERIALS AND METHODS

Thyroidectomy

The rats underwent thyroidectomy under anaesthesia induced by injection of thiopental Na intraperitoneal (50 mg/kg; EIPICO Co., Egypt) and after the surgery, they were treated with Bivatracin (an aerosol antibiotic spray; ECAP Co., Egypt) twice daily for six days, following the steps described by (Tousson *et al.*, 2014; Hasan *et al.*, 2024; Hameed *et al.*, 2023; Singh *et al.*, 2023; Vishen *et al.*, 2021).

Animals

Forty male Wistar rats, a weight of approximately 150 g, were used for the experiments and obtained from the zoology department farm. The male rats were housed in the laboratory room for seven days before the start of the research and provided with a standardized diet ad libitum.

Experimental groups

Male rats were similarly divided into the following four groups.

Gp1: control group in which rats didn't obtain any therapy.
Gp2: (Chia seeds extract); the mice received CSE orally (200 mg/kg bw/day) intramuscularly orally or intramuscularly or both routes please explain over a period of fourteen days (Rohaim *et al.*, 2021).

Gp3: Thyroidectomized group (Thy) in which rats were surgically subjected to thyroidectomy and dissecting after 21 days according to Tousson *et al.* (2014).

Gp4: Treated thyroidectomized rats with CSE (Thy+CSE) in which rats were subjected to thyroidectomy and after 21 days treated with chia seeds extract for another 14 days.

Blood and serum samples

rats After starving each group for ten to twelve hours, the rats were put to sleep with sodium pentobarbital so that they could be thoroughly dissected. The serum from the inferior vena cava was extracted and separated using centrifugation for 15 minutes at 3000 rpm. The obtained serum was maintained at -18°C to facilitate analysis and estimation of blood parameters.

Hormone analysis

Total triiodothyronine (T3) levels were assessed using a commercial test introduced by Diagnostic Systems Laboratories (Taxes, USA), following the method described by Tiirats (1997). Total thyroxine (T4) measurements were determined using a commercial test introduced by the same company, following the approach. Serum levels of thyrotropin (TSH), following the steps described by Engall *et al.* (1980). Total levels of testosterone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in sera were estimated after the approach of (Ezz *et al.*, 2023).

Morphometric analysis of sperms

The caudal part of the epididymis was carefully separated from the testis of each rat and counts of sperm, motility and examination of morphology were estimated following the approach described by Tousson *et al.* (2018).

Oxidative and antioxidants parameters estimations

Oxidative and antioxidant factors were estimated in the homogenate of testis according to the methods described by Mesbah *et al.* (2004) and Aldubayan *et al.* (2019).

Histopathological and Immunohistochemical examination

Fix testicular tissue in 10% neutral buffer formalin, processed for sectioning of paraffin and some sections were used for staining with H and E after Tousson (2016) and the rest were utilized for PCNA expressions after (El-Masry *et al.*, 2020).

Statistical analysis

The results were provided as mean values $\pm$ standard error (SE) and statistical analysis was performed using an unpaired t-test to assess significant differences across experimental groups. For biochemical data, a significance level of 0.05 was used as the cutoff point, signifying statistically significant outcomes. The SPSS statistical version 21 software program (SPSS® Inc., USA) was used for all statistical analyses.

RESULTS AND DISCUSSION

Variation of thyroid hormones

The results presented in Table 1 demonstrate a significant ($P < 0.05$) elevated in serum TSH levels and a significant ($P < 0.05$) decline in T3 and T4 levels in rats subjected to thyroidectomy (Thy) compared to the control and CSE groups. However, management of thyroidectomized rats with Chia seeds extract (Thy+CSE) significantly ($P < 0.05$) elevated T3 and T4 levels and significantly ($P < 0.05$) decline TSH levels compared to the thyroidectomized male rats (Table 1).

Sperms change

Table 2 showed a significant decline ($P < 0.05$) in sperm counts, motility, progressive motility (PR), vitality, morphology index (%) and a significant elevated sperm abnormality,

immotile (IM) sperms in thyroidectomies rats (Thy) as compared to control group and CSE groups. In contrast; a significant elevate ($P < 0.05$) in sperm counts, motility, progressive motility (PR), vitality, morphology index (%) and a significant decrease in sperm abnormality, immotile (IM) sperms in treated thyroidectomies rats with coriander (Thy+CSE) as compared to thyroidectomies rats (Table 2).

Chia seeds extract ameliorated experimentally hyperthyroidism-induced testicular DNA damage

The results presented in Table 3 indicate that thyroidectomized rats (Thy) revealed a significant decline

in sexual hormone levels (testosterone, FSH, prolactin and LH) and a significant elevated in testicular enzyme levels (AST and ALP) compared to the control rats and treatment group with chia seeds extract (CSE). On the other side, operated thyroid rats treated with CSE (Thy+CSE) showed a significant elevation in sexual hormone levels and a significant decline in testicular enzyme levels compared to thyroidectomized rats (Thy).

Furthermore, Table 5 demonstrates that thyroidectomy led to a substantial increase in lipid peroxidation (MDA content) and a significant decline in antioxidant defense markers (GSH content, GST and SOD activity) in testicular

Table 1: Variation in different groups T3, T4 and TSH levels.

	TSH (μ U/ml)	T3 (ng/dl)	T4 (ng/mL)
Control	1.21 [#] ±0.08	126.8 [#] ±6.18	2.23 [#] ±0.16
CSE	1.18 [#] ±0.06	133.0 [#] ±8.15	2.27 [#] ±0.18
Thy	4.98*±0.37	62.7*±3.52	2.07*±0.24
Thy+ CSE	1.56 [#] ±0.19	112.6 [#] ±8.76	2.19 [#] ±0.33

Data are presented as the mean value plus or minus the standard error (SE) of 10 observations. The values of p are both less than 0.05. There is a notable difference between the control and thyroidectomy groups, respectively.

Table 2: Changes in rats' sperm count, morphology index and vitality in different groups.

	Count (million/ml)	Morphology index (%)	Vitality (%)
Control	120.2 [#] ±6.35	46.5 [#] ±1.89	75.2 [#] ±2.68
CSE	126.0 [#] ±1.54	51.5 [#] ±2.05	75.0 [#] ±2.53
Thy	84.70*±5.29	30.8*±1.96	59.3*±1.83
Thy+CSE	109.8 [#] ±6.05	43.20 [#] ±2.22	75.1 [#] ±3.45

Data is presented as the average $\pm$ standard error (SE) of 10 observations. The notation *p < 0.05 and #p < 0.05 signify statistically significant changes compared to the control and thyroidectomy groups.

Table 3: Alterations in serum testosterone (ng/ml), follicle-stimulating hormone (FSH; mIU/ml), luteinizing hormone (LH; mIU/ml) and prolactin (ng/ml) levels across various research groups.

	Testosterone	FSH	LH	Prolactin
Control	3.22 [#] ±0.11	2.40 [#] ±0.15	1.13 [#] ±0.048	0.53±0.021
CSE	3.46 [#] ±0.09	2.73 [#] ±0.10	1.08 [#] ±0.03	0.52±0.035
Thy	2.06*±0.07	1.19*±0.09	0.95*±0.051	0.47±0.012
Thy+ CSE	3.14 [#] ±0.13	2.35 [#] ±0.16	1.02 [#] ±0.011	0.52±0.012

Data is presented as the average $\pm$ standard error (SE) of 10 observations. The notation *p < 0.05 and #p < 0.05 signify statistically significant changes compared to the control and thyroidectomy groups.

Table 4: The levels of malondialdehyde (MDA; nmol/g protein), glutathione (GSH; μ mol/g tissue), superoxide dismutase (SOD; U/mg protein) and glutathione S-transferase (GST; μ mol/h/mg protein) were measured in the testicular tissue of male rats that were treated.

	MDA	GSH	SOD	GST
Control	10.05 [#] ±0.89	3.79 [#] ±0.21	61.8 [#] ±2.45	0.71 [#] ±0.011
CSE	9.39 [#] ±0.90	4.08 [#] ±0.36	63.2 [#] ±3.60	0.78 [#] ±0.009
Thy	23.26*±1.31	1.44*±0.22	37.1*±2.01	0.40*±0.001
Thy+ CSE	12.25 [#] ±1.75	3.66 [#] ±0.36	57.0 [#] ±2.73	0.62 [#] ±0.005

Data is presented as the average value with the standard error (SE) of 10 observations. The notation *p < 0.05 and #p < 0.05 signify statistically significant distinctions compared to the control and thyroidectomy groups.

cells compared to the control and the treatment group treated with CSE. In contrast, treatment of thyroidectomized rats with CSE (Thy+ CSE) resulted in a remarkable decrease in MDA content and a significant increase in GSH content, CAT and activities of SOD compared to the thyroidectomized group (Thy) (Table 4).

A comet assay was done to evaluate the protective affection of Chia seeds extract (CSE) against hypothyroidism-induced testicular DNA damage in rats following thyroidectomy. The aims of the comet assay, presented in Fig 1 and Table 5, demonstrated that thyroidectomized rats exhibited a significant elevate in testicular damage of its DNA as provided by increasing tail length, tail DNA% and tail moment compared to the control group and treatment group with CSE ($p < 0.05$). However, the administration of CSE to treat hypothyroidism resulted in a significant decline in damage to testicular DNA. It is worth noting that no significant variety in DNA damage, specifically in terms of tail length, was marked in rats treated with CSE alone. These results suggest that Chia seeds extract have a protective feature against thyroidectomy-induced hypothyroidism and subsequent damage of testicular DNA in rats, as evidenced by the reduction in DNA damage parameters following treatment with CSE.

CSE ameliorated testicular injury of thyroidectomy

Disturbance in spermatogenic cells arrangements with depletion in sperms counts and leydig cells in testes of thyroidectomies rats as compared to testes in control and CSE that exhibit the natural structure of seminiferous tubules with the natural cycle of spermatogenesis (Fig 2A-2C). Treatments of thyroidectomy rats exhibit recovery in testicular injury and elevate the sperm numbers and Leydig cells (Fig 2D).

PCNA expression in different groups

Strong reactions for PCNA expression were detected in the spermatogenic cell on seminiferous tubules in control and CSE while mild PCNA expressions were detected in the thyroidectomy rats (Fig 3A-3C). In contrast; moderate PCNA expressions were detected in treated thyroidectomies rats with CSE (Thy+CSE) (Fig 3D).

Sex hormones are significantly and significantly impacted by thyroid gland health, particularly in men. The thyroid gland in both hypothyroidism and hyperthyroidism is crucial for sperm quantity and quality, which are both aspects of fertility (Beltagy *et al.*, 2016; Alankooshi *et al.*, 2023). The current study's main goal was to examine the potential of Chia seeds extract (CSE) in mitigating testicular

Table 5: Comet test values were determined using image analysis in cells from all experimental groups after the treatment.

	Tailed %	Untailed %	Tails length μm	Tail DNA%	Tail moment
Control	2.0	98.0	1.75 [#] ±0.21	1.72	2.33
CSE	1.4	98.6	1.35 [#] ±0.10	1.48	1.95
Thy	35	65	13.08 [*] ±0.37	8.55	65.68
Thy+CSE	5.6	94.4	2.62 [#] ±0.29	2.04	5.17

Based on five observations, the data are presented as the mean value plus or minus the standard error (SE). The values ^{*} $p < 0.05$ and [#] $p < 0.05$ indicate statistical significance at a confidence level of 95%. There was a notable difference in tail length between the control and thyroidectomy groups.

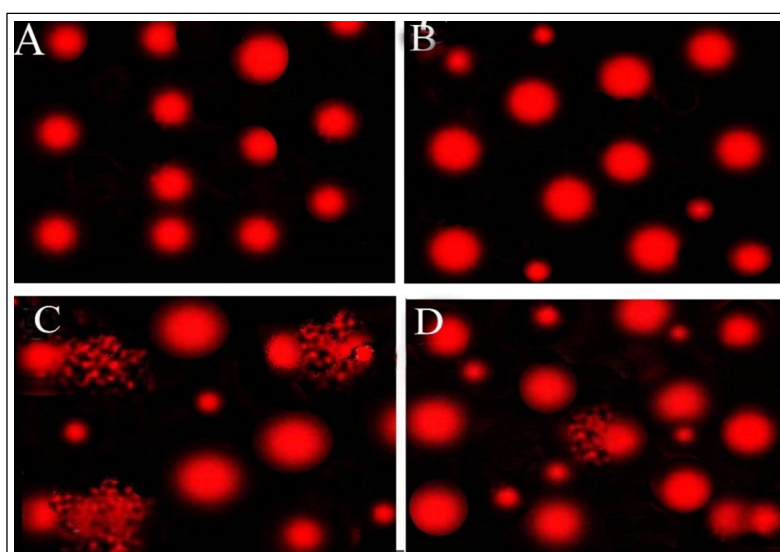


Fig 1: Comet assay for assessment of testicular tissue DNA damage. Where A, Control; B, Chia seeds extract (CSE); C, thyroidectomies (Thy); D, hypothyroidism with CSE.

injury, damage of DNA and oxidative Stress resulting from thyroidectomy operation in male rats. the Results of the research align with the Results of a previous Study By which demonstrated the critical protective effects of Chia seeds extract against the effects of hypothyroidism and thyroid damage of tissue induced by potassium dichromate.

Current results revealed that; thyroidectomy induced hypothyroidism and therefore it elevated TSH and depleted T3 and T4 levels in contrast to rats used as controls. Treatments with CSE (Thy+CSE) enhanced these differences in thyroid hormone concentrations and brought normal hormone conditions to operated thyroidectomy rats.

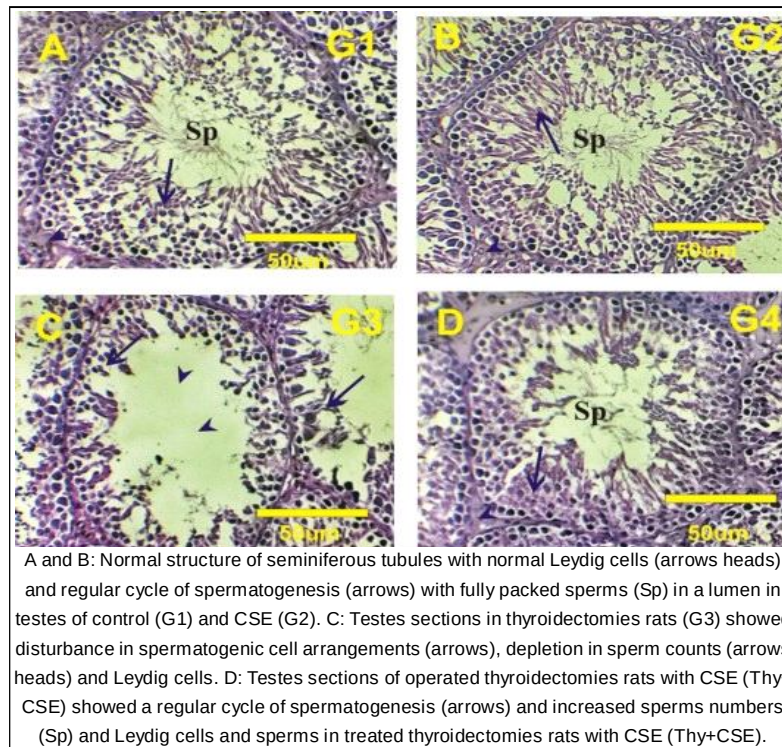


Fig 2: Photomicrographs of testes parts stained with HE in different groups.

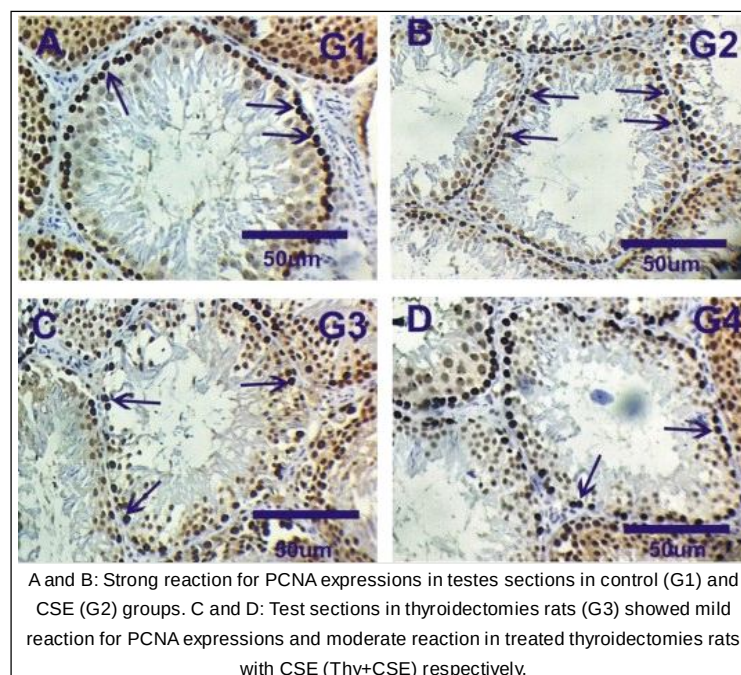


Fig 3: Photomicrographs of testes sections stained with PCNA in different groups.

The current finding is accepted by those of Tousson *et al.* (2012c, 2014) and Alankooshi *et al.* (2023), who found that; thyroidectomy induces hypothyroidism.

The findings of the current research demonstrated that thyroidectomy led to a decline in sexual hormones (FSH, LH, total testosterone and prolactin) as well as sperm count, morphological index, vitality and progressive motility while increasing non-progressive and immotile sperm. These findings are consistent with the study conducted by Jalilvand *et al.* (2019), which found that the effects of hypothyroidism resulted in damage to testicular cells and decreased sperm counts. Similarly, Alankooshi *et al.* (2023) reported that hypothyroidism induced reproductive toxicity.

The use of chia seeds extract was found to have a steroidogenic effect, potentially by indirectly increasing testosterone levels through the inhibition of testosterone metabolism. The mechanism behind chia seeds extract testosterone-increasing properties is believed to involve its role as an aromatase inhibitor, an enzyme responsible for converting testosterone to estrogen (Dhawan *et al.*, 2002). Treatment with chia seeds extract in thyroidectomized rats resulted in the modulation and improvement of sexual hormones and semen parameters. This included an elevation in testosterone levels, increased sperm production, a decrease in sperm abnormalities and a reduction in testicular tissue oxidative stress, possibly due to the antioxidant activity of chia seeds extract.

These findings are similar to studies by Alankooshi *et al.* (2023), which reported similar outcomes. Furthermore, the results align with the research by Patat *et al.* (2007) which highlighted the protective effects of chia seeds extract against spermatogenic damage and decreased sperm count and testosterone levels induced by chromium, as well as its role in enhancing antioxidant defence demonstrated that chia seeds extract had a protective effect on sperm abnormalities by improving the percentage of rapid progressive motility and slow progressive motility types for sperms while significantly decreasing the percentage of non-progressive and immotile sperms in metronidazole-treated mice. These findings are consistent with Fetouh and El-Saied Azab (2014), who found that chia seeds extract improved daily sperm production in guinea pigs with gentamicin-induced reproductive toxicity.

Furthermore, our results align with the result by Noorafshan *et al.* (2010), which showed that chia seeds extract reduced the production of highly reactive oxygen species (ROS), which are known to produce oxidative stress and damage to cells. Increased ROS production disrupts the redox balance and leads to oxidative stress, as highlighted by Altwaijry *et al.* (2020). In our analysis, thyroidectomy significantly changed the condition of oxidant/antioxidant balance, consistent with previous findings by Tousson *et al.* (2014). Specifically, thyroidectomy led to elevated levels of malondialdehyde (MDA) and declined levels of reduced glutathione (GSH), glutathione-S-transferase (GST) and superoxide dismutase (SOD) activity in testicular tissues. However, treatment with Chia seeds

extract (CSE) in thyroidectomized rats improved these changes.

These observations are in line with the results of Noorafshan and Ashkani-Esfahani (2010), who showed that Chia seeds extract acts as an antioxidant by preventing the formation of free radicals and lipid peroxidation in various tissues.

The current findings indicate that thyroidectomy leads to DNA damage in testicular tissues, disruption in the arrangement of spermatogenic cells, decreased sperm counts and reduced expression of proliferating cell nuclear antigen (PCNA) in spermatogonia. These effects are likely responsible for the testicular damage and reduction in sperm production observed after thyroidectomy. However, treatment with Chia seeds extract (CSE) improved sperm quality, which can be attributed to the enhancement of testicular histology and increased testosterone concentration. Chia seeds extract serves as a scavenger for oxygen-derived free radicals, thereby protecting the testis from damage (Altwaijry *et al.*, 2020). Consequently, CSE treatment may reduce genotoxicity and decrease sperm abnormalities.

These findings are similar to the study by Kanter *et al.* (2013), which reported that Chia seeds extract supplementation protects against testicular damage in diabetic rats and reduces the presence of apoptotic cells compared to a group of diabetic rats. Therefore, Chia seeds extract can be recommended as a dietary spice and a supplement for patients undergoing thyroidectomy to improve their reproductive health.

CONCLUSION

Thyroidectomy has been shown to cause alterations in the structure and functions of the testis. However, treatment with Chia seeds extract has been found to improve these changes and reverse the modifications to testicular structure and functions following thyroidectomy. The extract is effective in reducing damage to cells and increasing sperm counts. Additionally, the supplementation with an extract of Chia seeds has been shown to enhance spermatogenesis, making it a promising adjuvant therapy in such conditions.

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Ethics

The study design was approved by the Institutional Ethical Committee for Animal Care and Use (code: IACUC-SCI-TU-0241).

Declaration of conflicting interest

The authors declare that they have no possible conflicts of interest.

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