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THE POTENT ROLE OF SMART CHITOSAN NANOPARTICLES ON ATTENUATION OF TESTICULAR DAMAGE CIMETIDIN –INDUCED IN SWISS ALBINO MICE

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ABSTRACT
The present study was carried out to investigate the ameliorative effective of chitosan NPS on testicular damage induced by cimetidine. Mice were randomly divided into 4 groups, the first group served as control group, the second group received oral administration of chitosan NPS 12ml/kg, the third group received oral administration of 400mg/kg cimetidine, and the fourth group received oral co-administration of chitosan NPS and cimetidine. Results showed highly significant increase of prolactin and decrease of testosterone in the group received cimetidine in addition to serve pathological alterations in the testicular tissue represented by increasing tubular area due to elongation of seminiferous tubules disorganization of spermatogonia and absence of the most stags of spermatocytes, with low pathological score, in addition to intense incidence of caspase-3 referred to apoptosis. Where as, co-administration of chitosan NPS with cimetidine resulted in significant decrease of prolactin and increase of testosterone besides to improved testicular tissue with raising pathological score and less appearance of apoptosis.

KEYWORDS: Cimetidine – chitosan NPS – testicular pathology- Caspase-3.

INTRODUCTION
Chitosan is a biopolymer can be isolated from the exoskeleton of shrimps and crabs, composed of ß-(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine.[1][2] Chitosan is known to have several biological functions such as anti-diabetic, anti-inflammatory, anti-oxidant and cholesterol reducing agent.[3] Whereas, some undesirable properties of chitosan as high molecular weight and water insolubility stand as barrier upon its biological uses, chemical modifications to over come these problems may cause losing of its biochemical effect, so nanotechnology may be one of the novel solutions.[4] Chitosan nanoparticles have reported more effectiveness, biodistribution, reduction of pharmacological toxicity, improvement of immune effect, antimicrobial and anticancer effects than chitosan itself. Moreover, chitosan nanoparticles have more surface curvatures compared to chitosan large particles which in turn increase dissolution pressure and saturation solubility.[5][6]

Cimetidine is a wide spread anti-gastric and duodenal ulcers and its prophylaxis drug, it is also used to decrease the attendant symptoms of gastrointestinal disorders.[7] Cimetidine is considered one of the major histamine 2 receptors blockers that competing with histamine for H_2 receptors present on parietal cells which in turn responsible for acid production.[8] Recent studies strongly referred to cimetidine as testicular toxicant, it significantly reduced testes weight and injured peritubular cells. The major side effect of cimetidine is the inhibition of dihydrotestosterone via blocking of androgen receptors leading to testicular disorders.[9][10]

Therefore, the present study was achieved to investigate the capability of chitosan nanoparticles to reduce the testicular damage that induced by cimetidine treatment.

MATERIALS AND METHODS
Animals
40 male albino mice weighed (25±5 g) were obtained from El Osman Farm, Cairo, Egypt. Animals were housed in plastic cages and supplied with food and free access of pure water, maintained under environmentally controlled condition of temperature (constant temperature 25-27C with a12 h light/dark cycles). All animals were healthy and clinically free of diseases.

Preparition of chitosan Nps

A chitosan solution was prepared by dissolving chitosan (0.5 mg/ml) in 1% (w/v) acetic acid solution until the solution was transparent. Sodium tripolyphosphate (1.5 mg/ml) was dissolved in deionized water. The chitosan solution was flush mixed with an equal volume of(TPP) solution and the formation of chitosan–TPP nanoparticles began spontaneously via the TPP ionic gelation mechanism under stirring conditions at room temperature.[11] Formed chitosan Nps were examined and micrographed by TEM.

Experimental design

40 male albino mice weighed 25±5 g were divided randomly to 4 groups, 10 per each, the first group served as control, the second received oral administration of 12 ml/kg of formed chitosan Nps, the third one received oral administration of 400 mg/kg of cimetidine and the fourth group received co- oral administration of 12 mg/kg of chitosan Nps and 400 mg/kg of cimitedine, the duration of experiment was 30 days. All mice groups were sacrificed one day-post to the end of experiment.

Testes index

Testes weight divided by body weight of the same mice multiplied by 100 then subjected to statistical analysis by spss17.

Estimation of hormones

Testes were collected and washed with cold mannitol buffer (pH 7.4), then fragmented into small pieces, homogenized manually in the mannitol buffer. Homogenized samples for first were centrifuged at 2000 rpm for 10 minutes at 4˚C, then filtered and the supernatant was centrifuged again at 8000 rpm for 15 minutes. The final supernatant was subjected to ELISA to estimate testosterone (mice testosterone ELISA KIT Category No. E90243) and prolactin (mice prolactin hormone ELISA KIT Category No. CK-E90904, -30597) (East Bio Pharm Company).

Histopathological Analysis

Testes samples were fixed in 10% neutral formalin buffer solution, after embedding in paraffin, sections were cut at 5µm thickness stained with Hx&E, examined under light microscopy (motic-2000) and photos were taken. Seminiferous tubules areas were measured by digital microscopic system.

Table 1: Showing the testicular index, tubular area, testosterone and prolactin among control and experimental groups.

Groups	Testicular index	Tubular area Um^2	Testosterone Pg/u	Prolactin Pg/u
Control	0.53±0.1	0.2±0.1	153±0.1	0.04±0.1
Chitosan NPs	0.53±0.1	0.2±0.1	153±0.1	0.04±0.1
Cimetidine	$0.27±0.2^{*a}$	0.6±0.1	$138±0.3^{*a}$	$0.09±0.1^{*a}$
Cimetidine + Chitosan NPs	$0.40±0.1^{*b}$	0.4±0.1	$150±0.3^{*b}$	$0.04±0.1^{*b}$

Histopathological Analysis and Pathological Scoring System

Testes of control group and that received chitosan NPs showed normal testes structure with tubular area (0.2 um^2) characterized by semi oval and rounded seminiferous tubules embedded in connective tissue (figs. 2A,2B). Whereas, administration of cimetidine (400 mg/kg) resulted in severe pathological signs represented by distorted and elongated seminiferous tubules with insignificant increase of tubular area (0.6 um2) besides to marked degeneration of interstitial tissue and destruction of leydig cells (fig. 2C). Moreover, co-administration of chitosan NPs with cimetidine (400 mg/kg) revealed marked reduction of pathological changes resulted from cimetidine administration only manifested by convergent seminiferous tubules (fig. 2D) with insignificant decrease of area (0.4 um2) compared to that group received cimetidine only, and less degeneration of interstitial tissue. Used of high magnification of control and group received chitosan NPs revealed normal seminiferous tubules filled with all stages of spermatogenic cells as spermatogonia, primary spermatocytes followed by secondary spermatocytes, then spermatids which differentiate to spermatozoa (figs. 3A,3B) that scored (10) according to Johnson's pathological scoring system. Seminiferous tubules surrounded by interstitial tissue contains clusters of leydig cells besides to blood supply. While section of group received cimetidine displayed irregular seminiferous tubules with depression of spermatogenesis then absence of most spermatocytes stages and no spermatids or spermatozoa were seen, so the central lumen looked empty that scored (4) according to Johnson's pathological scoring system (fig. 3C). Moreover, co-administration of chitosan NPs resulted in healthy seminiferous tubules filled with most stages of spermatocytes, pathological score showed marked improvement that scored (8), a few spots of vacuolar degeneration, some spermatids fused with each other and formed multinucleated giant cells (fig. 3D).

Immunohistochemistry

Testes sections of control and group received chitosan NPs showed a very weak immune response against caspase 3 (figs. 4A,4B) optical density revealed (0.02), where as, tests sections of group received a very strong immune reactivity against caspase 3, optical density scored (0.1) (fig. 4C) that referred to great apoptosis in

the testes induced by cimetidine while, co-administration of chitosan NPs with cimetidine resulted in weak immune response against caspase 3 (fig. 4D) with optical density (0.03) that represents decreasing of apoptosis.

Table 2: Showing Johnson's scoring system among control and experimental groups.

Groups	Johnson's scoring system
Control	10
Chitosan NPs	10
Cimetidine	4
Cimetidine + chitosan NPs	8

Table 3: Showing the optical density among control and experimental groups.

Groups	Optical Density
Control	0.02±0.01
Chitosan NPs	0.02±0.01
Cimetidine	0.1±0.03^{*a}
Cimetidine + chitosan NPs	0.03±0.02^{*b}

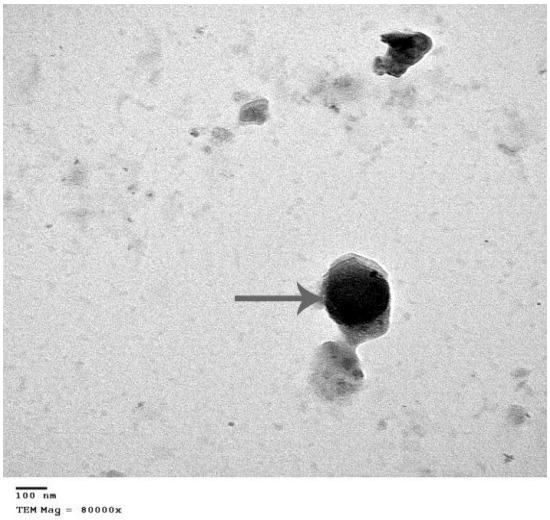

Fig. 1: Photomicrograph of TEM showing morphology and size of formed chitosan NPs. (TEM-80000).

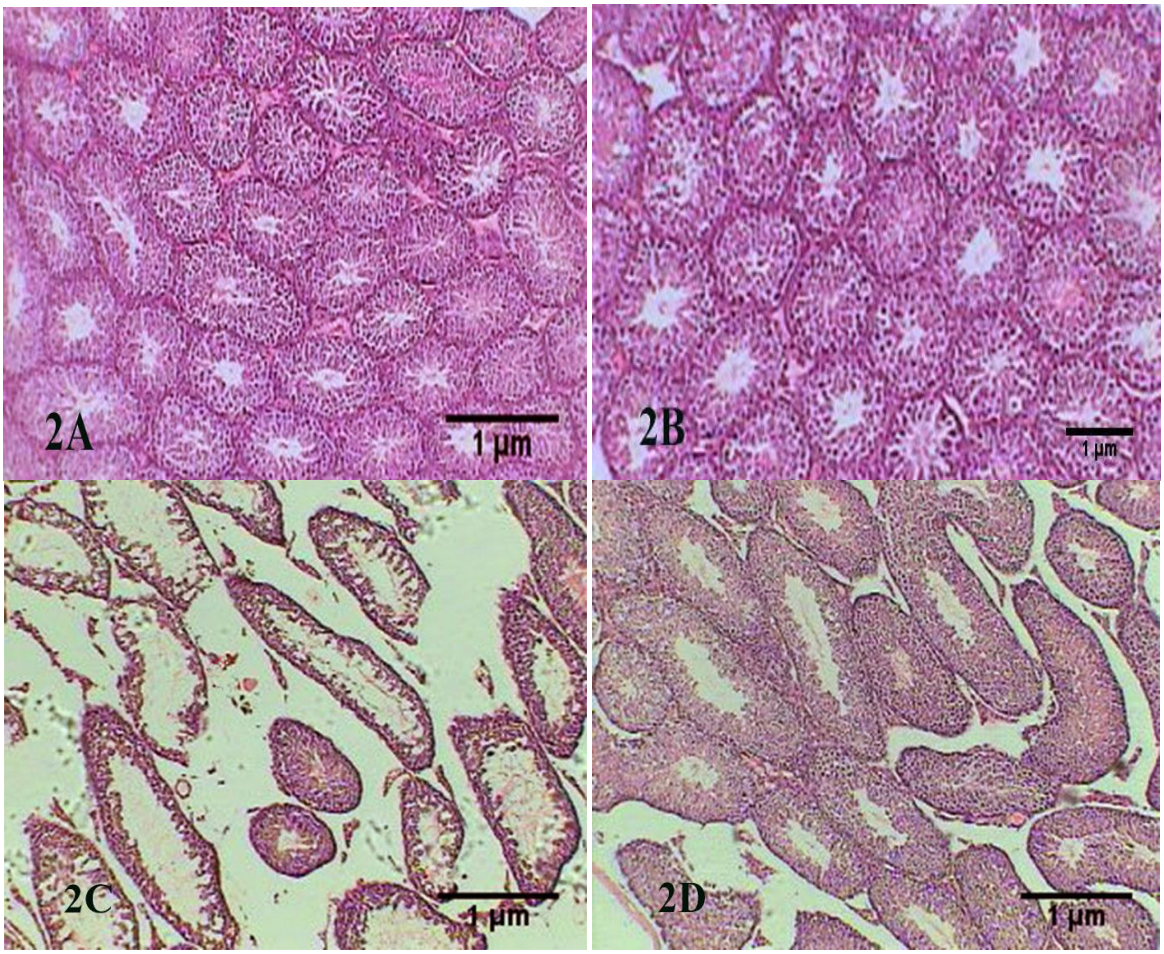

Fig. 2: Photomicrograph showing control testes section (A), chitosan NPs revealing normal section (B), Cimetidine displaying elongated and destructed seminiferous tubules besides to severe degeneration of interstitial tissue (C), chitosan NPs with cimetidine showing improved section with convergent tubules (D). (H&E-100X).

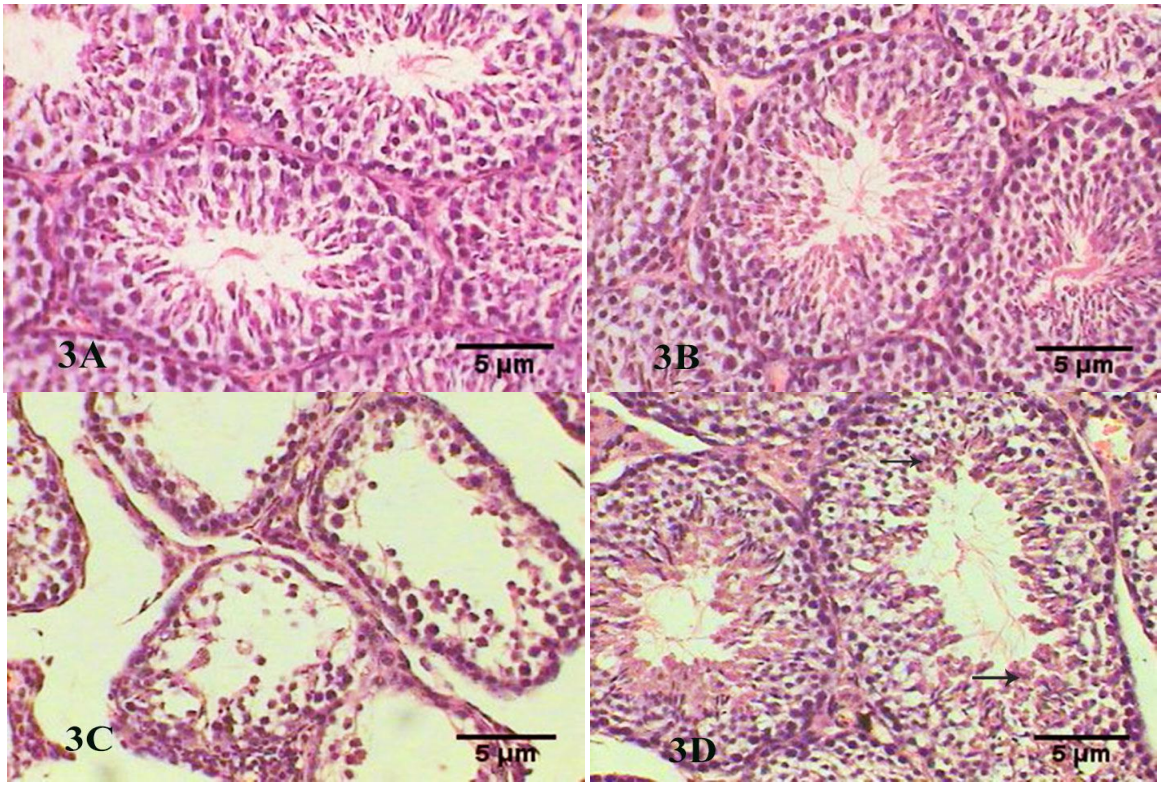

Fig. 3: Photomicrograph showing normal seminiferous tubules of control (A), chitosan NPs displaying normal seminiferous tubules with all stages (B), cimetidine administration showing disorganization of spermatogenic cells, absence of the most spermatogenic stages (C), chitosan NPs co-administration with cimetidine showing more healthy seminiferous tubules, the most stages of spermatogenesis were seen, fusion of many cells to form multinucleated cell (D). (H&E-400X).

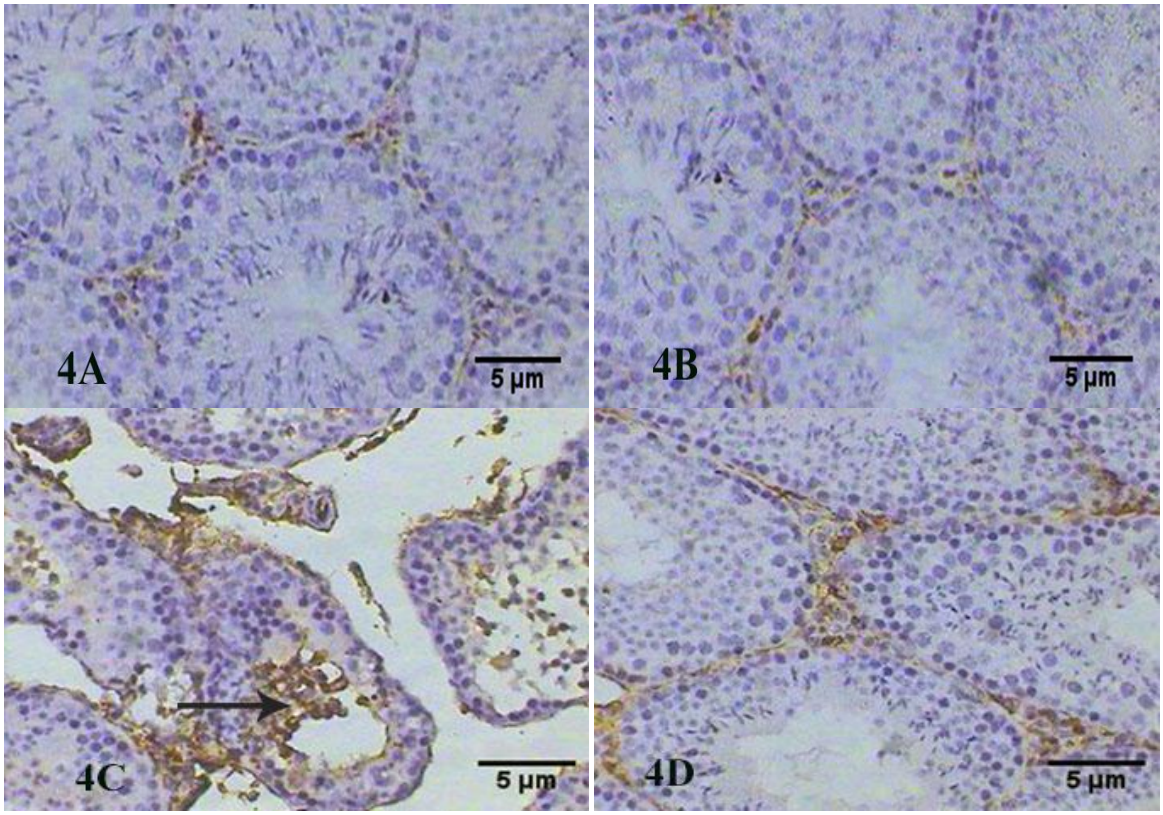

Fig. 4: Photomicrograph of control and chitosan NPs showing weak immune response to caspase-3 (A,B), cimetidine testes section displaying intense immune response (arrow) (C), co-administration of chitosan NPs with cimetidine revealing weak immune response to caspase-3. (ABC-400X).

DISCUSSION

Cimetidine is an ulcer inhibitor by reduction of stomach acid production with marked side effects as diarrhea, muscle pain, sexual dysfunction and testicular damage.[12] More recent studies showed that cimetidine administration resulted in significant reduction of testicular index[13][14], which coincided with the present findings that administration of cimetidine caused significant decrease of testicular index, may be attributed to the general atrophy of testes with absence of the most spermatogenic cells.

The present investigation showed significant elevation of prolactin and decreasing of testosterone concentrations due to cimetidine administration that run in agreement with many previous studies who gave an evidence that cimetidine considered as dopamine antagonist at the receptors of dopamine sites in the anterior part of pituitary gland causing hyper secretion of prolactin hormone (hyperprolactinmea) which in its turn caused inhibition of gonadotrophins that associated with hypogonadism gets to appear in the form of testicular atrophy and general degeneration. In addition, gonadotrophin such as LH stimulates leydig cells of testes to produce testosterone and hence LH gonadotrophin hormone decrease lead to dropping of testosterone production. Moreover, cimetidine competitivety blocks dihydrotestosterone DHT receptors in the pituitary gland, hypothalamus and other tissues that require DHT which is a sterol transformed from testosterone that ended with reduction in the testosterone production[15][16], that agreed with the present investigation results as significant elevation of prolactin and decreasing of testosterone concentrations due to cimetidine administration were observed.

Many studies displayed that cimetidine caused significant changes in seminiferous tubules including disorganization of spermatogonia absence of spermatocytes, spermatid and spermatozoa. Also, blocked tubular lumen by deciduous germ cells, atrophy and leydig cells hyperplasia.[17] The present study illustrated that cimetidine administration caused severe pathological changes in testicular tissue represented by increasing tubular area due to its elongation, irregular seminiferous tubules with absence of most spermatocytes stags, low pathological score and degeneration of interstitial tissue and leydig cells.

About the immunohistochemistry, the present study revealed intense incidence for caspase 3 indicating severe apoptosis in seminiferous tubules that agreed with previous studies indicated that cimetidine induced structural and ultrastructural apoptosis in seminiferous tubules and surrounded vascular cells, leading to testicular atrophy.[8]

More recent studies revealed that chitosan NPs could protect testes from pathological changes, oxidative stress and apoptosis caused by lead acetate by increasing antioxidant and inhibiting caspase 3 expression.[18] The potential biological effect of chitosan may be due to its active ingredients that chitosan treatment combined with Bisphenol A significantly decreased testes damage and caspase 3 activity, chitosan seams to act as antioxidant against toxicity of testes.[19] Our present data show that chitosan NPs with cimetidine which might be the first study in this topic, displayed raising of testicular index and testosterone levels besides to significant decreasing of prolactin concentration to reach the normal levels, moreover reduction of pathological signs that seminiferous tubules looked healthy with raising of pathological score, in addition to less apoptic features in the seminiferous tubules.

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