The Journal of Basic and Applied Zoology

RESEARCH

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Assessment ameliorative role of fenugreek seeds and germinated fenugreek seeds on pancreatic and testicular gentamicin toxicity of male Swiss albino mice

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Abstract

Background Gentamicin is one of aminoglycoside antibiotic used for treatment of many infections due to its availability and less cost. The aim of this study aimed to assess the modulation effect of fenugreek seed and its germinated seeds on pancreatic and testicular toxicity induced by gentamicin in male Swiss albino mice. Forty male albino mice were divided into four treatment groups as follows: (1) control group, (2) gentamicin treated group, (3) gentamicin-fenugreek treated group and (4) gentamicin-germinated fenugreek treated group. Pancreatic and testicular tissues were collected for histopathological examinations, histochemical, and biochemical analysis as well as genetic study.

Results Administration of gentamicin resulted in histopathological damage in pancreatic and testicular tissues as well as decreased glutathione peroxides, catalase and total antioxidant activity content in both pancreatic and testicular tissues compared to control group. Histopathological changes and antioxidant/oxidative alterations as well as DNA damage observed in gentamicin treated animals found were moderate improvement by fenugreek seeds administration and marked improvement by treatment with germinated fenugreek seeds.

Conclusions Treated with gentamicin induced histopathological lesions, antioxidant/oxidant imbalance and DNA damage in the pancreatic and testicular. Treatment with germinated fenugreek seeds was more effective than fenugreek seeds in amelioration of pancreatic and testicular lesions, preventing high appearance of carbohydrate and accumulation of collagen fibers as well as oxidative damage and genotoxicity induced by gentamicin administration.

Keywords Gentamicin, Germinated fenugreek, Histopathology, Oxidative stress, Genetic

Background

Gentamicin is an aminoglycoside antibiotic that has been accepted its uses in more than 100 countries worldwide. It is considered as a broad spectrum antibiotic against microbial pathogens, mainly Gram-negative bacteria (Bulman et al., 2020). However, uses of gentamicin have resulted in testicular toxicity as well as having nephrotoxic effects which limits its therapeutic uses (Aly & Hassan, 2018; Edeogu et al., 2019; Khalili et al., 2021).

Earlier studies have established that gentamicin can damage sperm motility, reduce reproductive organ weights and cause apoptosis in the testis, resulting in

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testicular failure (Hamoud, 2019a). The mechanism of gentamicin induced testicular toxicity remains unclear. In general, the generation of toxic reactive oxygen species such as superoxide, hydrogen peroxide, singlet oxygen and hydroxyl radical that lead to oxidative stress is considered as the main reason for gentamicin toxicity (Tewari et al., 2020). Now, there are many food and medicinal plants known for their antioxidant properties. Fenugreek (*Trigonella foenum-graecum*), also known as Methi in Hindi, is a food spice used in India and other Asian, African and European countries (Bulman et al., 2020). Fenugreek is one of the oldest medicinal plants with antioxidant properties and used in many Asian and African countries for its health benefits (Rouag et al., 2021).

Baset et al. (2020), reported hypoglycemic, hypolipidemic, antifertility, antinociception and wound healing properties of fenugreek seeds in rat. In a similar study by Kaur and Sadwa (2020) they in a similar study stated that cytogenetic and testicular damage induced by adriamycin as chemotherapy drug could be prevented by administration of fenugreek seeds in albino rats. Germinated fenugreek seeds have numerous valuable properties over un-germinated fenugreek seeds. Germinated seeds are a good source of important amino acids particularly leucine, lysine and tryptophan that used in biosynthesis of proteins, and play special roles in "anchoring" membrane proteins within the cell membrane as well as tryptophan is also a precursor to the neurotransmitter serotonin, the hormone melatonin, and vitamin B3 (Tewari et al., 2020). In vitro, fenugreek seeds are reported to improve protein digestibility, fat absorption capacity, in addition to, decreasing levels of total unsaturated fatty acids, total lipids, triglycerides, and phospholipids (Omri et al., 2019). The aim in this research is to study the effects of fenugreek, and its germinated seeds on gentamicin induced testicular and pancreatic toxicity in male Swiss albino mice.

Methods

Chemicals

Gentamicin was purchased from Sigma-Aldrich Company (U.S.A), and was freshly dissolved in distilled water. While *Trigonella foenum-graecum* seeds were obtained from the local market, and were finely powdered to prepared solution used in experimental. Others chemicals reagent used were obtained from Sigma-Aldrich Company (U.S.A).

Animals

Male Swiss albino mice weighing 25–30 g, age 9–12 weeks ($n=40$) were obtained from animal house of National Organization for Drug Control and Research, Egypt. The mice were acclimatized under house conditions with a12-h dark/light cycle at 25 ± 2 °C temperature with free access to standard rodent chow and water. The mice were grouped and housed according to the guidelines of the institutional animal ethics committee of National Organization for Drug Control and Research. All experimental procedures were conducted in accordance with the ethical standards and approved by the Institutional Animal Care and Use Committee (IACUC) at NODCAR (approval no. NODCAR/III/39/2019).

Preparation of germinated fenugreek seed

Germinated fenugreek seeds were prepared according to a modified method described by Priyanjali et al. (2014). The seeds obtained from a local market were soaked in water for 24 h to germinate, then kept at 4 °C for 2 days, dried in the shade and ground into a fine powder with a blender.

Preliminary acute toxicity test for germinated fenugreek seed

Single doses of powdered germinated fenugreek seed (5, 50, 300, 500 or 2×10^3 mg/kg bw) were dissolved in distilled water (DIH_2O) and administered orally to mice. The animals were observed for 14 days after administration for clinical changes i.e. weight loss or mortality (Creton et al., 2010). No mortality or body weight changes were observed for all described doses. The safe dose of germinated fenugreek seeds was $\leq 2\times10^3$ mg/kg b.w. Therefore the chosen dose was 500 mg/kg bw.

Experimental design

Forty mice were equally divided into four treatment groups ($n=10$ per group) as follows: (1) control group: Mice ($n=10$) were injected peritoneally (i.p.) with 0.1 ml/10 g b.w. DIH_2O, followed by oral DIH_2O at same dose of i.p. daily for 5 day, (2) Gentamicin treated group: Mice ($n=10$) received 100 mg/ml/kg, i.p for 5 day according to Singh et al. (2012); (3) Gentamicin-Fenugreek treated group: Mice ($n=10$) received gentamicin (100 mg/kg bw, i.p.) daily simultaneous with fenugreek seed powder (500 mg/kg bw) for 5 day according to Seema (2014), and (4) Gentamicin-Germinated Fenugreek treated group: Mice ($n=10$) received gentamicin (100 mg/kg bw,) i.p. daily for 5 day simultaneous with germinated fenugreek seed powder (500 mg/kg bw). Germinated fenugreek seed dosage was used according to results of the preliminary acute toxicity test. Gentamicin (1405-4-0, Sigma-Aldrich Company, USA), and was freshly prepared daily just before use in Groups 2, 3 and 4.

At end of experiment, all mice were euthanized 24 h after the last doses with i.p. injection of sodium

pentobarbital (50 mg/kg bw. i.p.) and decapitation then pancreas and testis were collected for the following examinations.

Histopathological and histochemical examinations

Pieces of pancreas sand testis were preserved fixed in 10% formaldehyde for 48 h and, processed by hand as follows:

Formalin fixed samples washed in water twice, dehydrated in ascending gradient of ethyl alcohol, (E7023, Sigma-Aldrich Company) (50%, 70%,90% and 100%, 3X per change for 10 min), cleared in xylene for 3 times (Sigma-Aldrich Company, catalog number: 247642) for 5 min, infiltrated and embedded in paraffin 3 times for 10–15 min (Sigma-Aldrich Company, catalog number:327212). Paraffin section 5 µm thick were cut and mounted on glass slides.

For all stains, sections were deparaffinzed in xylene, and rehydrated through a descending ethyl alcohol gradient to water. After all stains, sections were dehydrated in ascending ethyl alcohol gradient, cleared in xylene 2×2 min per change and coverslip mounted with DPX mounting medium (215-535-7, Sigma-Aldrich).

All stained sections were examined with a light microscope (BX41, Olympus Corporation, Japan) and photographed with Nikon camera (Nikon Corp, Japan) to evaluate the histopathological and histochemical changes.

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Hematoxylin and eosin (H & E) for histopathological study

Sections were Hematoxylin Solution Modified According to Gill III (105174, Sigma-Aldrich), rinsed 1 min in running tap water; stained 45 s in Eosin (109844, Sigma-Aldrich) according to Malatesta (2016). Sections were dehydrated, cleared and coverslip mounted as described above.

Periodic acid Schiff's (PAS) stain to detect carbohydrate content

Periodic acid Schiff's stain was done according to according to Layton and Bancroft (2018). Sections were oxidized by 0.5% periodic acid solution (395132, 5 min), washed in distilled water, treated with Schiff's reagent (109033, USA, Sigma-Aldrich) 15 min, and washed in tap H_2O (5 min). Stained sections were counterstained by Mayer's hematoxylin (109249, Sigma-Aldrich, USA) 1 min, washed in tap H_2O 5 min, then dehydrated, cleared and coverslip mounted as described for all stained sections.

Masson's trichrome staining for fibrous connective tissue

Deparaffinized sections were stained with Masson's trichrome as seen in Table 1.

Biochemical analysis of oxidative stress markers, testosterone and anti-Mullerian hormone

Portions of pancreas and testis tissues were washed in ice-cold (4 °C) isotonic saline pH 7 and blotted dry between two filter papers. The tissues were homogenized in ice-cold phosphate buffer pH 7.4 (10010023, Thermo Fisher Scientific Company) to make 10% (w/v) homogenate using the Glass-Col Homogenizer (Fisher Scientific Company, USA). Supernatants from homogenates were used for biochemical determination with the following colorimetry assay kits: Lipid peroxide malondialdehyde (MDA) (MD 25 29, Biodiagnostic Company, Egypt), Catalase (CAT) (CA 25 17, Biodiagnostic), Total Antioxidant Capacity (TAC) (TA 25 13, Biodiagnostic), Glutathione reduced (GSH) (GR 25 11, Biodiagnostic) and Superoxide Dismutase (SOD) (SD 25 21, Biodiagnostic) using Unicam Spectrometer (3050740, England). Elisa tests for

Table 1 Masson's trichrome protocol

Staining reagent and rinses	Time	Catalog number
Weigert's Iron Hematoxylin working solution	10 min	HT107*
Running tap water, warm	10 min	
Biebrich scarlet-acid fuchsin solution	10–15 min	HT151*
DIH_2O	Rinse	
Phosphomolybdic—phosphotungstic Acid solution	15 min	HT153*
DIH_2O Rinse	Brief rinse	
aniline blue solution	10 min	B8563*
1% acetic acid solution	5 min	45,754*
DIH_2O	Rinse	
Sections dehydrated, cleared and coverslipped		

*All reagents are from Sigma-Aldrich

Testosterone (DK0015, Diametra, Italy) and Anti-Müllerian hormone (AMH) (A79765 Beckman Coulter Gen II ELISA, Beckman Coulter, USA) were estimated in the testis homogenate.

DNA fragmentation assay

DNA from mice testis was extracted using the methods and data published by Wlodek et al. (1991) and Aljanabi and Martinez (1997), whereas DNA ladder was used to determine the size of the apoptotic DNA fragments.

Statistical analysis

Values were expressed as means ± standard error (SE) for $n = 10$ samples per each group. p value ≥ 0.05 was considered as statistically significant, while p values ≥ 0.001 were considered highly significant. All statistics were carried out using the analysis of variance with one-way ANOVA IBM SPSS Statistics, USA) and Prism 5.01 (GraphPad, USA) to analyze and graph differences between group means and standards errors.

Results

Hematoxylin and eosin examination of pancreas

Microscopic examinations of pancreas sections from control mice revealed well-developed endocrine as islets of Langerhans and exocrine acinar cells and pancreatic duct. Normal acinar cells are pyramidal with basal nuclei and apical acidophilic cytoplasm (Fig. 1a). In contrast, pancreas sections from the gentamicin treated group had a reduced number and size of most islets of Langerhans. Marked atrophied islets of.

Langerhans showed pyknotic nuclei of its remaining shrunken and degenerated cells. In addition to, shrinkage and degenerated acinar cells, destructive pancreatic duct and widening space between lobules were also seen (Fig. 1b). Others islets of Langerhans revealed moderate reduction, a scattered empty space, degenerative cells,

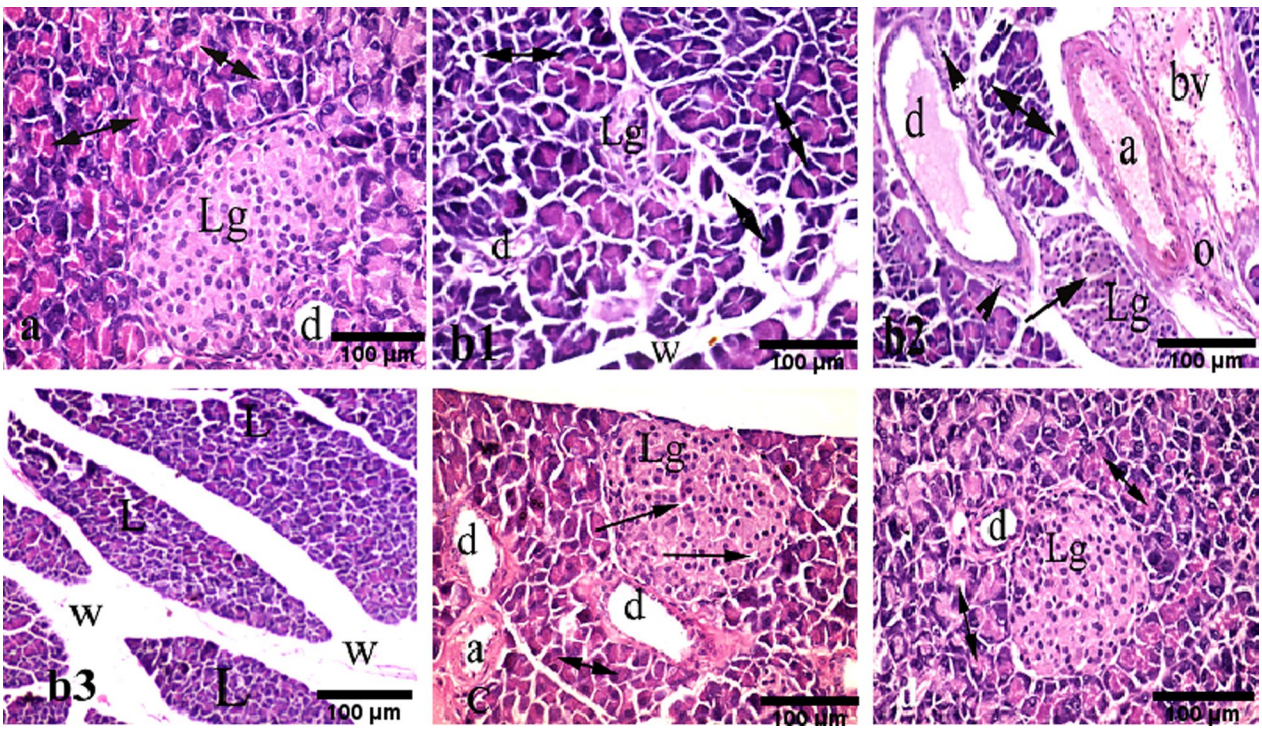


Fig. 1 A photomicrograph of pancreatic tissues staining H &E: **a** From control mouse showing normal appearance of inlet Langerhans (Lg), acini cells (double head arrow) and pancreatic duct (d). **b1** From gentamicin treated mice showing severe atrophied islets Langerhans with pyknosis of remaining cell's nucleus (Lg) as well as widening space between lobules (w). Shrinkage and degenerated acini cells (double head arrow) and distracted pancreatic duct (d) were also seen. **b2** From gentamicin treated mice showing moderate reduced Langerhans (Lg) with pyknotic nucleus of its cells and diffused empty space of degenerative cells (arrow) as well as degenerative acinar cells (double head arrow). mild perivascular inflammatory cells (arrow head), marked dilated pancreatic duct filled with eosinophilia materials (d),thickened haylinized wall of dilated artery (a), dilated congested vein (V) and edema with inflammatory cells infiltrations (o) were seen. **b3** From gentamicin treated mice showing widening space (w) between small lobules (L). **c** From gentamicin–Trigon treated group revealing normal appearance of acini (double head arrow) and well developed islets of Langerhans (Lg) with focally empty space of degenerative cells (arrow). Moderate dilated pancreatic duct (d) and mild hylinized artery (a) were seen. **d** From gentamicin-G.Trigon treated group showing no structural abnormalities in islets of Langerhans (Lg), pancreatic acini (double head arrow) and pancreatic duct (d)

and pyknotic nuclei of its remaining cells in another area. Marked degenerative changes in most acinar cells were also seen. Severe dilated pancreatic duct filled with eosinophilic materials, a thick hyaline wall of dilated artery, a dilated congested vein and edematous area infiltrated with inflammatory cells infiltrations were also seen (Fig. 1c). Prominent areas with widening space were also seen between lobules (Fig. 1d).

Treatment animals with gentamicin and fenugreek seeds showed some improvement with majority islets of Langerhans appearing normal but empty space still seen, accompanied with mainly acinar appeared normal. However, moderate dilated pancreatic duct and mild hyaline artery with few degenerated acini cells were still noticed (Fig. 1e). However mice treated with gentamicin and germinated fenugreek seeds showed no morphological abnormalities in islets of Langerhans, acinar and duct as compared to gentamicin treated group (Fig. 1f).

Hematoxylin and eosin examination of testis

Microscopic examination of testis from control mice showed normal morphology structure of seminiferous tubules, complete spermatogenic layers, well-developed sperms and normal interstitial Leydig cells (Fig. 2a). On another hand, the gentamicin treated mouse testis sections revealed degenerative changes of many seminiferous tubules with disturbed of spermatogenic layers, absence of sperm and exfoliated spermatogenic cells in the lumen as well as distracted of its basal lamina (Fig. 2b). In other areas, some seminiferous tubules had hyaline degeneration with eosinophilia deposition, cystic degeneration and scattered pyknotic cells in the basal layer together with wide interstitial spaces (Fig. 2c). There were prominent areas with diffused seminiferous tubules showing severe degenerative changes and reduced spermatogenic layers with no sperm (Fig. 2d).

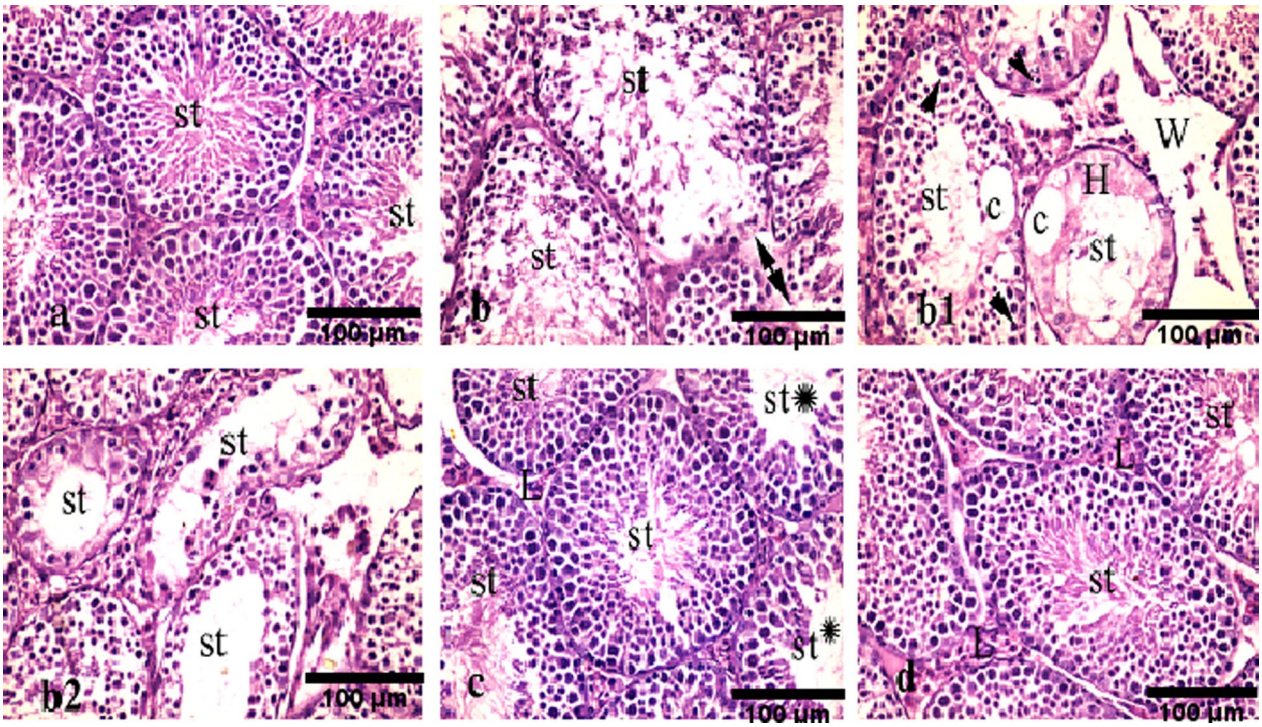


Fig. 2 Photomicrograph of testis section staining with H&E, X200. **a** From normal male Swiss albino mouse showing normal histological structure of seminiferous tubules including complete spermatogenic layers (ST) and Leydig cells (L). **b** Sections of testis from male Swiss albino mice treated with gentamicin showed loss of normal architecture of seminiferous tubules (st), disturbed of spermatogenic layers, absence of sperm and marked degenerative changes with exfoliated spermatogenic cells in the lumen (ST). Distracted basal liminal of seminiferous tubules (double arrow head). **b1** from male Swiss albino mice treated with gentamicin only showing hyaline degeneration with eosinophilia deposition (H), cystic degeneration (c) and scattered pyknotic nuclei in the basal cell layers in seminiferous tubules (st). A widened interstitial space (w) and moderate reduction in spermatogenic series and reduction of sperms in othors seminiferous tubules (st) were seen. **b2** Sections of the testis from male Swiss albino mice treated with gentamicin showing diffused seminiferous tubules with moderate to marked degenerative changes and reduction in spermatogenic layers with absence of sp seminiferous tubules erm (st). **c** From male mice treated with gentamicin- *Trigonella foenum-graecum* showing normal appearance of some seminiferous tubules (st), while mild reduction in spermatogenic layers and absence of sperm in lumens of othors scattered seminiferous tubules still showed (st star). **d** From male mice treated with mice treated with gentamicin-germinated *Trigonella foenum-graecum*showed normal histological structures of layers of seminiferous tubules (st) and normal appearance of Leydig cells (L)

Testis sections from mice treated with gentamicin and fenugreek seeds showed moderate improvements, with normal appearance of some seminiferous tubules but still showed scattered seminiferous tubules with mild reduction in spermatogenic layers and no sperm (Fig. 2e) as compared to gentamicin treated group. However, animals treated with gentamicin and germinated fenugreek seeds revealed obvious improvement with normal testicular morphology, complete normal spermatogenic layers, sperm and normal appearance of interstitial Leydig cells (Fig. 2f).

Periodic acid-Schiff (PAS) for detection carbohydrate content in pancreas

Control mice carbohydrate distribution in pancreatic tissues of control animals is well detected by PAS-Schiffs staining (Fig. 3a) as compared to gentamicin treated mice which exhibited less carbohydrate content in their pancreas (Fig. 3b). In the gentamicin fenugreek treated group, carbohydrate content was seen in some acinar cells but others acinar cells did not show it (Fig. 3c). testis sections from animaaals treated with germinated fenugreek seeds demonstrated a normal distribution of carbohydrate content (Fig. 3d) compared to pancreas from gentamicin treated mice (Fig. 3b).

Periodic acid-Schiff (PAS) for detection carbohydrate content in in testis

Microscopic examination of testis sections from gentamicin treated group stained with PAS for carbohydrate content revealed weakly magenta colored in seminiferous tubules basal lamina, degenerated spermatogenesis layers and completely absence of sperm (Fig. 4b) compared with control group (Fig. 4a). sections of testis from gentamicin fenugreek treated group revealed moderate carbohydrate staining in seminiferous tubules basal lamina, germinal spermatogenic cells and few sperm (Fig. 4c) as compared with gentamicin treated group (Fig. 4b). Testes sections from gentamicin germinated fenugreek group, showed carbohydrate content appeared normal and restored in the spermatogenic layer basal lamina and sperm (Fig. 4d) as compared to the normal control and gentamicin treated groups.

Detection of collagen deposition in pancreas with Masson's trichrome

Pancreas sections stained with Masson's trichrome from gentamicin treated group revealed high collagen fibers content appeared surrounding wall of ducts and blood vessels in most lobules (Fig. 5b) as compared to control group (Fig. 5a). Examination of pancreas section from gentamicin-fenugreek group, fewer collagen fibers

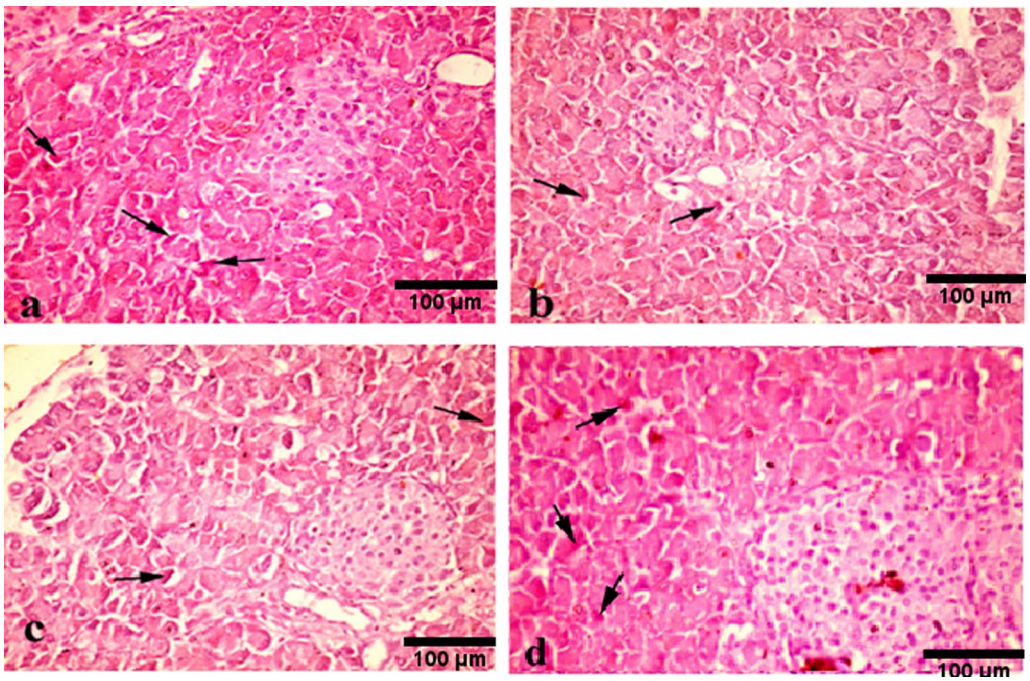


Fig. 3 A Photomicrograph of pancreatic tissues staining with Periodic-Acid Schiff (PAS) for detection carbohydrate (glycogen) content, × 400. **a** From control group showing normal distribution of carbohydrate content (arrow); **b** From gentamicin treated group showing few carbohydrate content; **c** From gentamicin- *Trigon* treated group showing moderate carbohydrate content; and **d** From gentamicin-G. *Trigon* treated group showing restore normal carbohydrate content. Magentic color detected glycogen content (arrow)

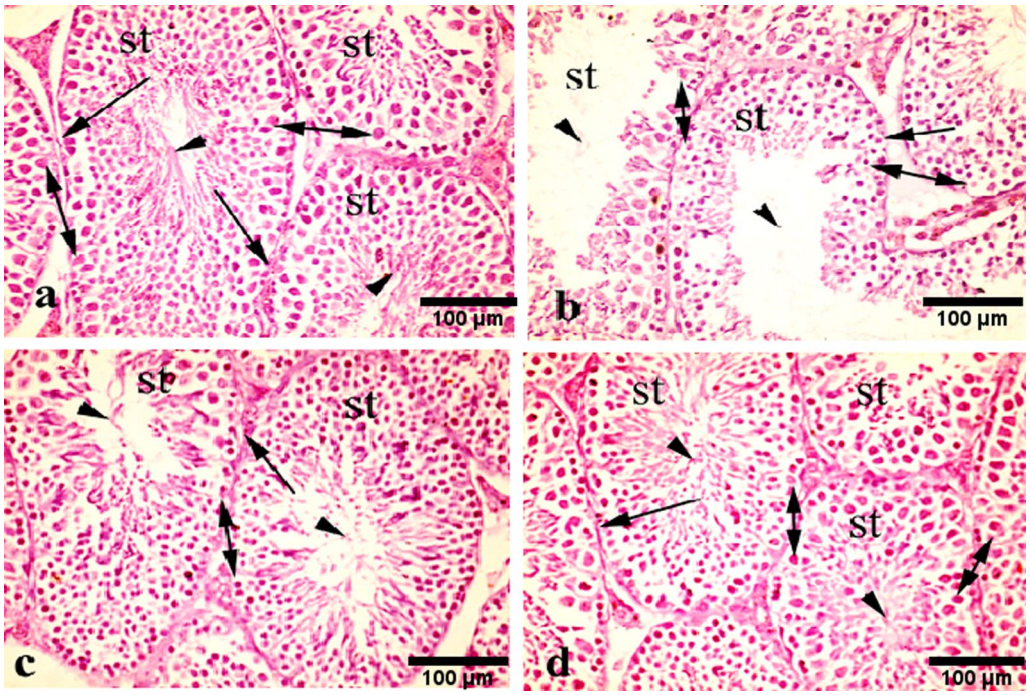


Fig. 4 A Photomicrograph of testis tissues staining with Periodic-Acid Schiff (PAS) for detection carbohydrate (glycogen) content. **a** From control group showing intensely staining periodic acid Schiff in the basal lamina (arrows), germinal spermatogenic layers (double head arrow) and sperm (head arrow) of seminiferous tubules (st). **b** From gentamicin treated group showing weakly staining in basal lamina (arrows), degenerated germinal spermatogenic cells (double head arrow) and negative staining in absence sperm (head arrow) of seminiferous tubules (st). **c** Gentamicin-Trigon treated group showing moderate staining in basal lamina (arrows), and germinal spermatogenic cells (double head arrow) with mild in few sperm (head arrow) of seminiferous tubules (st). **d** Gentamicin-G. Trigon treated group showing restore strongly staining in basal lamina (arrows), germinal spermatogenic cells (double head arrow) and sperm (head arrow) of seminiferous tubules (st)

surrounded duct walls and blood vessels (Fig. 5c) as compared to gentamicin group (Fig. 5b). However pancreas sections from mice treated with gentamicin and germinated fenugreek seeds showed very few to almost no collagen fibers (Fig. 5d) as compared to gentamycin treated group.

Collagen deposition detection in testis tissues with Masson's trichrome

Testis sections from control group staining showed normal distribution of collagen fibers in basal lamina of seminiferous tubules and fewer fibers in the interstitial tissues (Fig. 6a). Testis section of male mice treated with gentamicin revealed an excessive amount of collagen in the walls of seminiferous tubules and congested blood vessel walls in interstitial tissues as compared to the control group (Fig. 6b). Section of testis from animals treated with gentamicin-fenugreek had a low collagen in seminiferous basal lamina wall and some collagen fibers still seen in blood vessels wall as compared with gentamicin treated group (Fig. 6c). The treatment with germinated fenugreek seeds after gentamicin exhibited normal appearance of collagen fiber in seminiferous basal lamina wall, as compared with gentamicin treated group (Fig. 6d).

Biochemical analysis of oxidative stress in pancreas tissue

Gentamicin treated group showed a significant increase in malondialdehyde and a significant decrease in Glutathione reduced, Catalase and Total Antioxidant Capacity content in pancreas tissue compared to control group. However, the gentamicin fenugreek group and gentamicin-germinated fenugreek seed group demonstrated decrease in malondialdehyde and increase in Glutathione reduced, Catalase and Total Antioxidant Capacity content as compared to gentamicin treated group as shown in Fig. 7a–d and Table 2. However, values of malondialdehyde, Glutathione reduced Catalase and Total Antioxidant Capacity content at gentamicin-germinated fenugreek group returned to normal control value.

Biochemical analysis of oxidative stress in testis tissue

In gentamicin treated group has significant decrease in testosterone and AMH of testis as compared to control group. In contrast, there is amelioration in decreasing of testis hormones observed gentamicin treated group

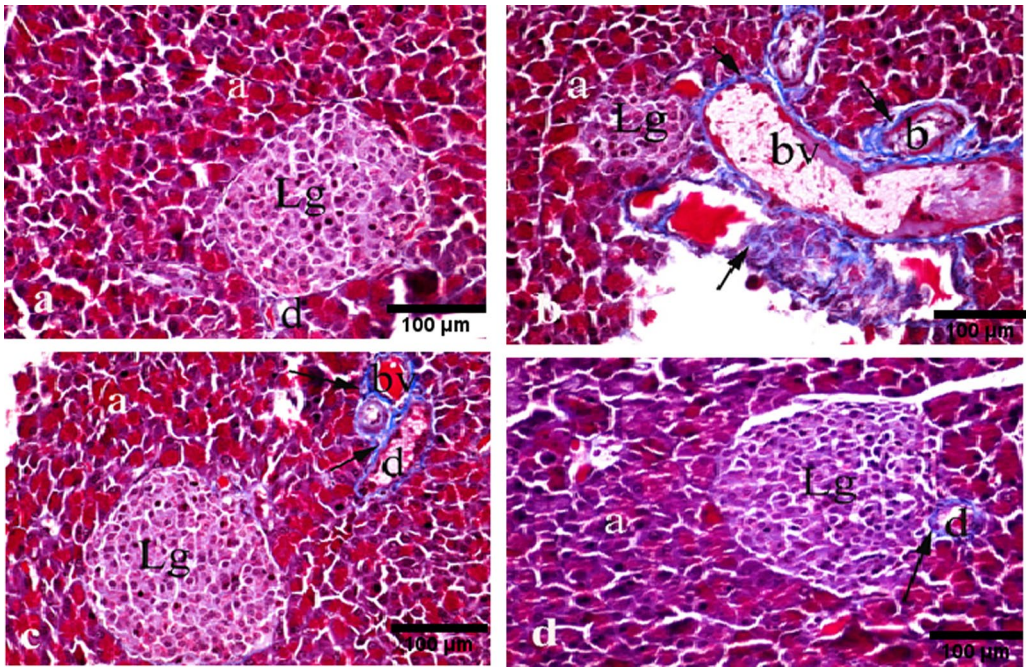


Fig. 5 Photomicrograph of pancreatic tissues staining with Masson's trichrome stain. **a** Control group showing almost absent fibers. **b** Gentamicin treated group showing high collagen fibers content appeared around ducts (d) and blood vessels (bv). **c** Gentamicin-*Trigon* treated group showing moderate collagen fibers around ducts (d) and blood vessels (bv); and **d** Gentamicin-G. *Trigon* treated group showing very minimal around wall of ducts (d). Ducts (d) and blood vessels (bv). Blue color detected collagen fibers (arrow)

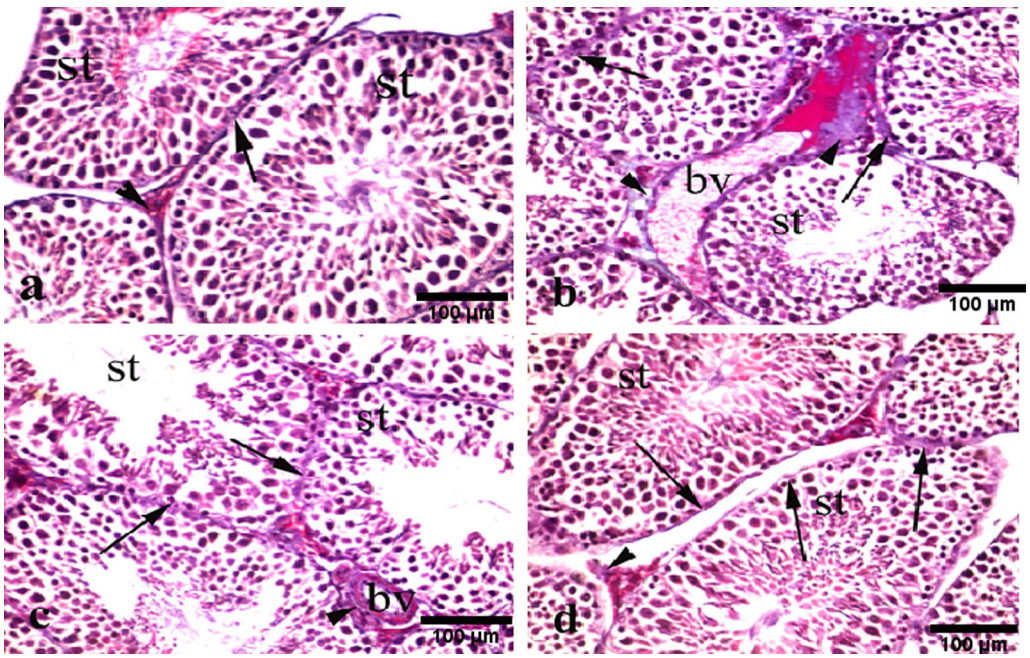
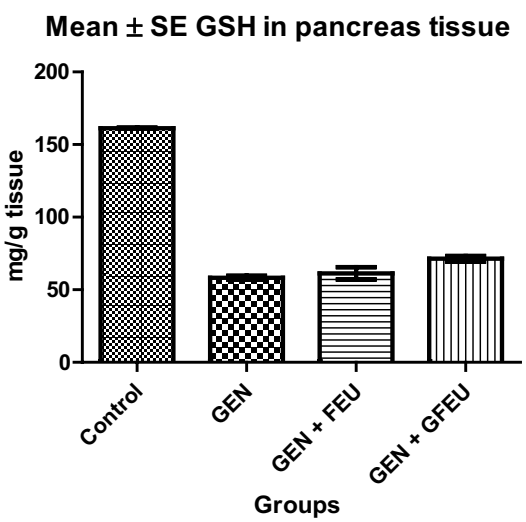
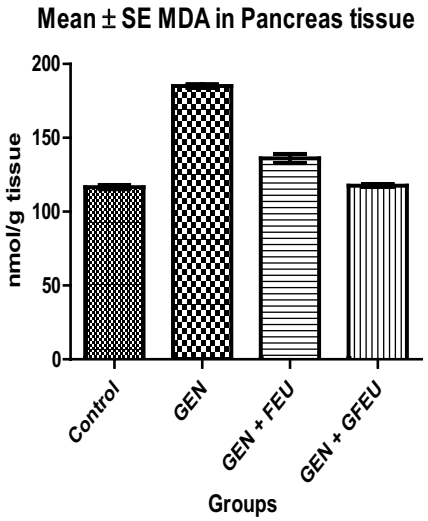


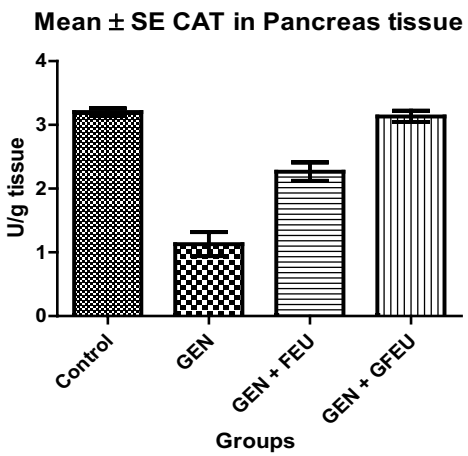
Fig. 6 A Photomicrograph of testicular tissues staining with Masson's trichrome stain. **a** Control group showing collagen fibers in basal lamina separating seminiferous tubules (arrow) and minimal in the interstitial tissues (head arrow). **b** Gentamicin treated group showing abundant collagen fibers in basal lamina (arrow) and congested blood vessels (bv) in the interstitial tissues (head arrow). **c** Gentamicin-*Trigon* treated group showing moderate collagen fibers in basal lamina (arrow) and mild collagen fibers blood vessels (bv) in the interstitial tissues (head arrow).and **d** Gentamicin-G. *Trigon* treated group showing normal collagen fibers in basal lamina (arrow) and minimal in the interstitial tissues (head arrow). Blue color detected collagen fibers



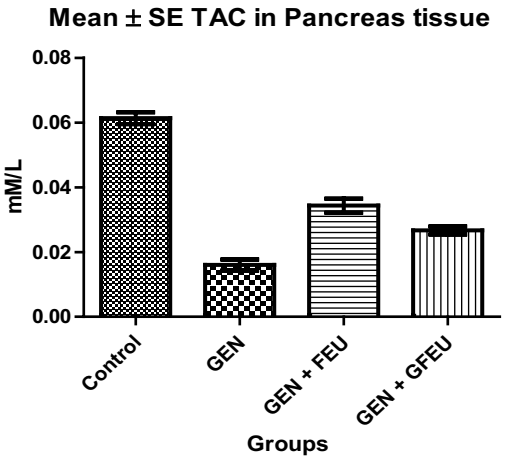
(a): Mean ±SE of GSH content of Pancreatic tissue



(b): Mean ±SE of MDA content of Pancreatic tissue



(c): Mean ±SE of CAT content of Pancreatic tissue



(d): Mean ±SE of TAC content of Pancreatic tissue

Fig. 7 Biochemical analysis in mice administration gentamicin with *Trigonella foenum-graecum* and its germinated form in pancreatic tissue. In each group, ten animals were used. Data are represented as mean. **a** Mean ± SE of GSH content of Pancreatic tissue. **b** Mean ± SE of MDA content of Pancreatic tissue. **c** Mean ± SE of CAT content of Pancreatic tissue. **d** Mean ± SE of TAC content of Pancreatic tissue

when mice administration gentamicin and fenugreek seeds or germinated fenugreek seeds (Fig. 8, Table 3).

DNA fragmentation assay

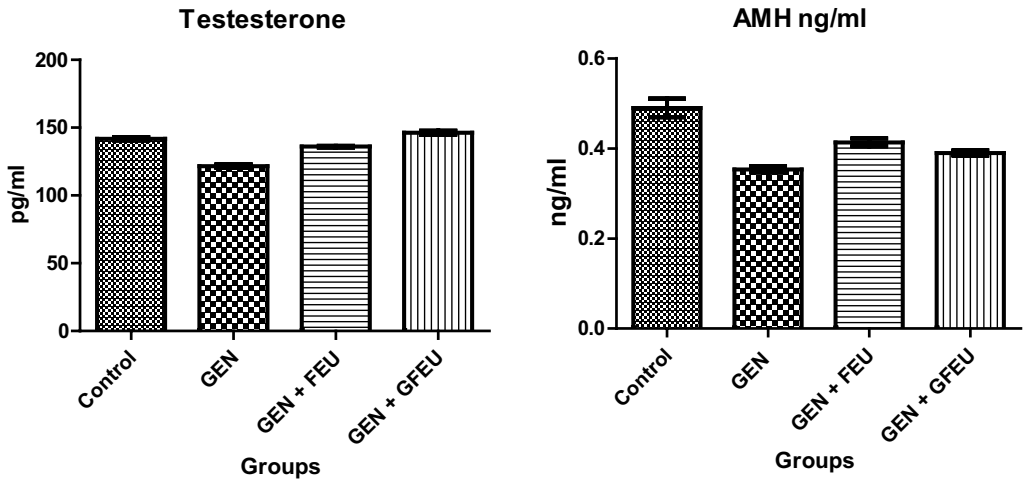
Gentamicin caused apoptotic DNA fragmentation in the testicular tissues of mice (Fig. 9). No ladder was observed on the agarose gel in the DNA of the control testis (Fig. 9, lane 1). Genomic DNA ladder formation was observed in the DNA of mice treated with GEN (100 mg/kg b.wt.) for 5 days (Fig. 9 lane 2). Also, the degradation of DNA into oligonucleotide fragments was also observed in the GEN group treated with fenugreek extract (500 mg/kg) (Fig. 9 lane 3). Administration of germinated fenugreek (500 mg/kg) to mice treated with gentamicin for 5 days restored the DNA laddering profile induced by the toxic effect of gentamicin treatment alone (Fig. 9, lane 4), to normal. These results suggest that germinated fenugreek was more effective than fenugreek in protecting against the toxic effect of gentamicin on testis of male mice.

Table 2 Effect of gentamicin with fenugreek or fenugreek germinated seeds on oxidative stress estimated values for glutathione reduced, malondialdehyde, catalase and total antioxidant capacity content in pancreatic homogenate from Swiss albino mice

Oxidative parameters	Control $n=10$	Gentamicin treatment 10	Gentamicin-fenugreek treatment $n=10$	Gentamicin-Germinated Fenugreek treatment $n=10$
Glutathione reduced (mg/g tissue)	165.3 ± 1.1	69 ± 0.6	89 ± 0.4	135 ± 0.8
Malondialdehyde (nmol/g tissue)	157 ± 1.2*	181 ± 0.9*	122 ± 1.5*	150 ± 2.5*
Catalase (U/L)	3 ± 0.03 [a]	1.7 ± 0.08	2.3 ± 0.09	2.9 ± 0.05
total antioxidant capacity (Mm/l)	0.08 ± 0.001	0.04 ± 0.003	0.06 ± 0.001	0.07 ± 0.002

n = number of mice per. Data are represented as mean ± standard errors for each group

*($P<0.01$) is significant. Group superscripted with same latter are not significant while groups superscripted with different letters are significant



(a): Mean ±SE of testosterone in testicular tissue

(b): Mean ±SE of AMH in testicular tissue

Fig. 8 Level of testosterone and AMH in in mice administration gentamicin *Trigonella foenum-graecum* and its germinated form in testicular tissue. In each group, ten animals were used. Data are represented as mean. **a** Mean ± SE of testosterone in testicular tissue. **b** Mean ± SE of AMH in testicular tissue

Table 3 Level of testosterone and anti-Müllerian hormone in in mice administration gentamicin, gentamicin-fenugreek and gentamicin-germinated fenugreek in testis tissue

Biochemical parameters	Control $n=10$	Gentamicin-treatment $n=10$	Gentamicin-fenugreek treatment $n=10$	Gentamicin-germinated fenugreek treatment $n=10$
Testosterone (pg/ml)	150 ± 1.8[a]	120 ± 2.4*	130 ± 1.2	135 ± 2.7
Anti-Müllerian hormone ng/ml	0.52 ± 0.8	0.28 ± 0.3*	0.45 ± 0.9	0.46 ± 1.1

n = number of mice per. Data are represented as mean ± standard errors for each group

*($P<0.01$) is significant. Group superscripted with same latter are not significant while groups superscripted with different letters are significant

Discussion

The present study evaluated the ameliorative effect of fenugreek and its germinated seed form against gentamicin toxicity on pancreas and testis in Swiss albino mice using histopathological and histochemical examination as well as biochemical analyses and DNA study. This works revealed that, gentamicin resulted in several histopathological lesions in pancreas and testis tissues, decrease carbohydrate content and enhances accumulation of collagen fibers. The biochemical analyses

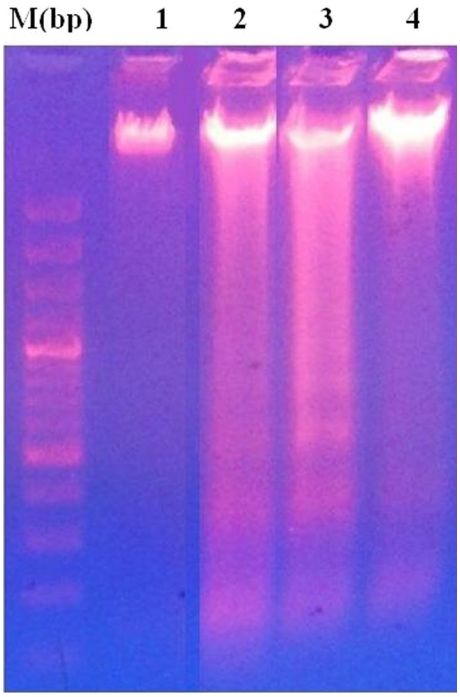


Fig. 9 Determination DNA fragmentation by oral administration of fenugreek and germinated fenugreek on animals treated with gentamicin in testis tissues

reported a decrease in glutathione reduced, catalase and total antioxidant capacity content with an increase in the malondialdehyde level of pancreatic and testicular tissue of gentamicin treated group. In addition to, genetic studies referred apoptotic DNA fragmentation in the testicular tissues. These were in agreement with work done by Elsawah et al. (2020), and Hamoud et al. (2019b), Aly (2019), El-Sayed et al. (2022).

The observed histopathological lesions observed in pancreases and testis in the present study, could be attributed to elevation of reactive oxygen species (Udupa et al. 2019; El-Sayed et al., 2022), oxidative stress, lipid peroxidation and activity of caspases-3 and 9 (Aly & Hassan, 2018; Elsawah et al., 2020) combined with decreasing activity of antioxidant enzymes (catalase, superoxide dismutase, glutathione peroxidase) as recorded in our study and previews studies El-Sayed et al. (2022). Oxidative stress containing enriched generation of reactive oxygen species has been caused the etiology of over one hundred human diseases (Dixit et al., 2005).

The complete absence of sperm in gentamicin treated group in the current study could be attributed to high productions of free radicals that lead to deterioration of sperm through lipid peroxidation and polyunsaturated fatty acids oxidation. Peroxidation of sperm lipids can damage the matrix of spermatozoa membrane lipids. These were confirmed by Aly and Hassan (2018), Agarwal et al. (2007), who stated that polyunsaturated fatty acids in plasma membranes and a few scavenging enzymes in cytoplasm make spermatozoa extremely sensitive to damage of reactive oxygen species.

This study also improved the ameliorative effect of fenugreek seeds in preventing pancreatic and testicular lesions induced by gentamicin as recorded previously with work done by Alsuliam et al. (2022). Moreover, our study also showed that treatment with germinated fenugreek seeds was more effective than treatment with fenugreek in preventing pancreatic and testicular lesions induced by gentamicin. These observed beneficial effects of fenugreek seeds and its germinated seeds form against toxicity of gentamicin could be attributed to more phenolic and flavonoid compounds in germinated fenugreek seeds than non-germinated, which mostly responsible for antioxidant activity, anti-inflammatory properties and scavenging free radicals (Dhawi et al., 2020).

Fenugreek has been reported to be an important medicinal plant with nutritional properties as an appetite stimulant, along with hypocholesterolemic, antidiabetic, antileukemic and antimicrobial effects (Ibrahim et al., 2020; Syed et al., 2020).

Sakr and Shalaby (2014), reported that an amelioration effect of fenugreek against testicular toxicity of carbofuran may be due to the antioxidant activity of flavonoids and polyphenols constituents. Similarity, Ibrahim et al. (2020), stated that medicinal properties of fenugreek are related to phytochemicals component such as galactomannans, phenolic compounds, alkaloids, proteins, vitamins (A, B1, C and nicotinic acid) and volatile oils.

The observed higher activities of germinated fenugreek seeds on fenugreek seeds may be attributed to germinated seeds have several beneficial properties over ungerminated fenugreek seeds. Germinated fenugreek seeds are a good source of essential amino acids especially leucine, lysine and tryptophan. Germination improves in vitro protein digestibility, as well as fat absorption capacity (Sakr & Shalaby, 2014). This was supported by Dixit et al. (2005), who stated that germinated fenugreek seeds showed a good antioxidant, radical scavenging properties, and inhibition of lipid peroxidation due partly to the presence of flavonoids and polyphenols.

Germinated fenugreek seeds have abundant in bioactive antioxidant substances and were used extensively as an important ingredient in daily food preparations and herbal formulations (Idris et al., 2021; Syed et al., 2020). Germination, fenugreek sprouts have shown to be richer in polyphenols, reducing sugars and minerals (K, Zn and Fe) than fenugreek seed (Randhir et al., 2004;

Shakuntala et al., 2011). Moreover, Dhawi et al. (2020), reported that water extract of germinated fenugreek seeds have higher bioactive compounds, antioxidant, DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity, and antimicrobial activities than ethanol, methanol and aqueous extract of ground fenugreek seeds. The highest phenolic, flavonoid and vitamin C contents were found in aqueous extract of germinated fenugreek seeds. This group also reported that aqueous extract of germinated fenugreek seeds had greater antimicrobial activity against tested bacterial pathogens (*Bacillus subtilis, Staphylococcus aureus, and Escherichia coli*). Further, Darwish et al. (2020), stated that the administration with germinated fenugreek more effective than fenugreek (*Trigonella foenum-graecum*) in the reverse the histopathological changes, and restored antioxidant/oxidant balance induced by gentamicin in kidney tissue.

Recently, many studies reported Antifungal properties (Sudan et al., 2020), as well as effficacy and safety of fenugreek Seeds on treatment of testosterone deficiency syndrome (Mansoori et al., 2020; Park et al., 2019). Similarly Syed et al. (2020), reported nutrition and therapeutic effect of fenugreek while, Bruce-keller et al. (2020), reported that fenugreek ha ability to counter the effects of high fat diet on gut microbiota in mice. Similarity, Kaur and Sadwa (2020), reported the phytomodulatory potential of fenugreek (*Trigonella foenum-graecum*) on bisphenol-A induced testicular damage in mice.

Conclusions

It was concluded that, gentamicin acts as a cytotoxic agent and its administration induces several histopathological lesions, reduced antioxidant enzymes as well as decrease carbohydrate content and enhances accumulation of collagen fibers compared to control treated group. It also elevated oxidative damage in both pancreatic and testicular tissues as evidenced by decrease in glutathione reduced, catalase and total antioxidant capacity content with an increase in the malondialdehyde level of pancreatic and testicular tissue of gentamicin treated group. In addition to, genetic studies referred apoptotic DNA fragmentation in the testicular tissues.

The treatment with germinated fenugreek seeds is more effective than ground fenugreek seeds in improving histopathological lesions, preventing high appearance of carbohydrate and accumulation of collagen fibers as well as antioxidant/oxidant imbalance and DNA damage induced by gentamycin toxicity in pancreatic and testicular tissue. Theses could be attributed to germinated seeds have several beneficial properties over ungerminated fenugreek seeds. Germinated fenugreek seeds are a good source of essential amino acids especially leucine, lysine and tryptophan and contain high amount of flavonoids and phenolic compounds.

Acknowledgements

The author (SRH, HMA, MSM) thanks, greatly honors, and expresses deep gratitude to Egyptian Drug Authority Operations and Control Sector (Previously National Organization for Drug Control and Research (NODCAR), Cairo, Egypt, for providing laboratory of work.

Author contributions

SR has done the practical of the experimental, histopathological examinations. She also wright most of methods, and results of histopathological examinations as well as declarations, discussion and references of manuscript working of the manuscript as well as read and approved the final manuscript. HMA done biochemical analysis and wright most part of introduction, method of biochemical analysis and related results and references while, MSM done practice of DNA fragmentation assay and wright its method and results. All authors have read and approved the manuscript.

Funding

The authors declare that they have no funding support during this study.

Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate

The mice were grouped and housed according to the guidelines of the institutional animal ethics committee of National Organization for Drug Control and Research. All experimental procedures were conducted in accordance with the ethical standards and approved by the Institutional Animal Care and Use Committee (IACUC) at National Organization for Drug Control & Research (NODCAR) (approval no. NODCAR/III/39/2019).

Consent for publication

Not applicable.

Competing interests

The author declares that he/she has no competing interests.

Received: 22 August 2022 Accepted: 1 January 2023
Published online: 17 January 2023

References

Agarwal, A., Makker, K., & Sharma, R. (2007). Clinical relevance of oxidative stress in male factor infertility: An update. *American Journal of Reproductive Immunology, 59*(1), 2–11. https://doi.org/10.1111/j.1600-0897.00559.x

Aljanabi, S. M., & Martinez, I. (1997). Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucleic Acids Research, 25*(22), 4692–4693.

Alsuliam, S. M., Albadr, N. A., Almaiman, S. A., Al-Khalifah, A. S., Alkhaldy, N. S., & Alshammari, G. M. (2022). Fenugreek seed galactomannan aqueous and extract protects against diabetic nephropathy and liver damage by targeting NF-κB and Keap1/Nrf2 axis. *Toxics, 10*, 362. https://doi.org/10.3390/toxics10070362

Aly, H. A. A. (2019). Testicular toxicity of gentamicin in adult rats: Ameliorative effect of lycopene. *Human and Experimental Toxicology, 38*(11), 1302–1313. https://doi.org/10.1177/0960327119864160

Aly, H. A. A., & Hassan, M. H. (2018). Potential testicular toxicity of gentamicin in adult rats. *Biochemical and Biophysical Research Communications, 497*, 362–367. https://doi.org/10.1016/j.bbrc.2018.02.085

Baset, M. E., Ali, T. I., Elshamy, H., El Sadek, A. M., Sami, D. G., Badawy, M. T., Abou-Zekry, S. S., Heiba, H. H., Saadeldin, M. K., & Abdellatif, A. (2020). Anti-diabetic effects of fenugreek (*Trigonella foenum-graecum*): A comparison between oral and intraperitoneal administration: An animal

study. *International Journal of Functional Nutriation, 1*(2), 1–9. https://doi.org/10.3892/ijfn.2020.2

Bruce-Keller, A. J., Richard, A. J., Fernandez-Kim, S.-O., Ribnicky, D. M., Salbaum, J. M., Newman, S., Carmouche, R., & Stephens, J. M. (2020). Fenugreek counters the effects of high fat diet on gut microbiota in mice: Links to metabolic benefit. *Scientific Reports, 10*(1245), 1–10. https://doi.org/10.1038/s41598-020-58005-7

Bulman, Z. P., Cirz, R., Hildebrandt, D., Kane, T., Rosario, Z., Wlasichuk, K., Park, M., & Andrews, L. D. (2020). Unraveling the gentamicin drug product complexity reveals variation in microbiological 2 activities and nephrotoxicity. *Antimicrobial Agents and Chemotherapy*. https://doi.org/10.1128/AAC.00533-20

Creton, S., Dewhurst, I. C., Earl, L. K., Gehen, S. C., Guest, R. L., Hotchkiss, J. A., Indans, I., Woolhiser, M. R., & Billington, R. (2010). Acute toxicity testing of chemicals: Opportunities to avoid redundant testing and use alternative approaches. *Critical Reviews in Toxicology*. https://doi.org/10.3109/10408440903401511

Darwish, M. M., Shaalan, S., Amer, M. A., & Hamad, S. R. (2020). Ameliorative effects of dried and germinated fenugreek seeds on kidney failure induced by gentamicin in male mice. *American Journal of Biomedical Science & Research, 9*(6), 459-466A. https://doi.org/10.34297/AJBSR.2020.09.001452

Dhawi, F., El-Beltagi, H. S., Aly, E., & Hamed, A. M. (2020). Antioxidant, antibacterial activities and mineral content of bu_alo yoghurt fortified with fenugreek and *Moringa oleifera* seed flours. *Foods, 9*(1157), 1–16. https://doi.org/10.3390/foods9091157

Dixit, P., Ghaskadbi, S., Mohan, H., & Thomas, P. A. (2005). Devasagayam. Antioxidant properties of germinated fenugreek seeds. *Phytotherapy Research, 19*, 977–983. https://doi.org/10.1002/ptr.1769

Edeogu, C. O., Kalu, M. E., Famurewa, A. C., et al. (2019). Nephroprotective effect of *Moringa oleifera* seed oil on gentamicin-induced nephrotoxicity in rats: Biochemical evaluation of antioxidant, anti-inflammatory, and antiapoptotic pathways. *Journal of the American College of Nutrition, 2019*(12), 1–9. https://doi.org/10.1080/07315724.2019.1649218

Elsawah, H. K., Kandiel, M. M., Amin, A. A., Mokhimar, H. M., & El Mahmoudy, A. M. (2020). Gentamicin and amikacin adversely affect male infertility indicated by pharmacological, andrological and pathological evidence. *International Journal of Basic and Clinical Pharmacology, 9*(2), 218–225. https://doi.org/10.18203/2319-2003.ijbcp20200167

El-Sayed, K., Ali, D. A., Maher, S. A., Ghareeb, D., Selim, S., Albogami, S., Fayad, E., & Kolieb, E. (2022). Prophylactic and ameliorative effects of PPAR-γ agonist pioglitazone in improving oxidative stress, germ cell apoptosis and inflammation in gentamycin-induced testicular damage in adult male albino rats. *Antioxidants, 11*(191), 2–25.

Hamoud, A. E. M. M. (2019a). Possible role of selenium nano-particles on gentamicin-induced toxicity in rat testis: Morphological and morphometric study. *Egypt Journal of Histology, 42*(4), 861–873. https://doi.org/10.21608/ejh.2019.9926.1093

Hamoud, A. E. M. M. (2019b). Possible role of selenium nano-particles on gentamicin-induced toxicity in rat testis: Morphological and morphometric study. *Egypt Journal of Histology, 42*(4), 861–873. https://doi.org/10.21608/ejh.2019.9926.1093

Ibrahim, A. M., Anwar, A. Y., Sani, M. A., Ya'u, S. A., Tasi'u, A. M., Sani, M. Y., Abdulmumin, Y., Murtala, M., Musa, H., Sadiya, A. B., Abdullahi, N., Maimuna, D. M., Salisu, A. A., & Tasi'u, M. (2020). Assessment of antioxidant activity and mineral elements composition of fenugreek seed extract. *Dutse Journal of Pure and Applied Sciences, (DUJOPAS), 6*(2), 75–84.

Idris, S., Mishra, A., & Khushtar, M. D. (2021). Recent therapeutic interventions of fenugreek seed: A mechanistic approach. *Drug Research (stuttg), 71*(04), 180–192. https://doi.org/10.1055/a-1320-0479

Kaur, S., & Sadwa, S. (2020). Studies on the phytomodulatory potential of fenugreek (*Trigonella foenum-graecum*) on bisphenol-A induced testicular damage in mice. *Andrologia, 52192*(e13492), 1–10. https://doi.org/10.1111/and.13492

Khalili, N., Ahmadi, A., Azadi, H. G., Moosavi, Z., AbedSaeedi, M. S., & Baghshani, H. (2021). Protective effect of betaine against gentamicin-induced renal toxicity in mice: A biochemical and histopathological study. *Comparative Clinical Pathology, 30*, 905–912.

Layton, C., & Bancroft, J. D. (2018). Carbohydrates. In S. K. Suvarna, C. Layton, J. D. Bancroft (Eds.) *Bancroft's theory and practice of histological techniques* (7th ed., p. 215). London.

Malatesta, M. (2016). Histological and histochemical methods: Theory and practice. *European Journal of Histochemistry, 60*, 173–170. https://doi.org/10.4081/ejh.2016.2639

Mansoori, A., Hosseini, S., Zilaee, M., Hormoznejad, R., & Fathi, M. (2020). Effect of fenugreek extract supplement on testosterone levels in male: A meta-analysis of clinical trials. *Phytotherapy Research, 34*(8), 1–6. https://doi.org/10.1002/ptr.6627

Omri, B., Manel, B. L., Jihed, Z., Durazz, A., Lucarini, M., Romano, R., Santini, A., & Abdouli, H. (2019). Effect of a combination of fenugreek seeds, linseeds, garlic and copper sulfate on laying hens performances. *Egg Physical and Chemical Qualities. Foods, 8*(311), 2–10. https://doi.org/10.3390/foods8080311

Park, H. J., Lee, K. S., Lee, E. K., & Park, N. C. (2019). Efficacy and safety of a mixed extract of *Trigonella foenum-graecum* seed and lespedeza cuneate in the treatment of testosterone deficiency syndrome: A randomized, double-blind, Placebo-controlled Trial. *Journal of Urology*. https://doi.org/10.1097/01.JU.0000555756.74398.32

Priyanjali, D., Saroj, G., Hari, M., & Thomas, P. A. D. (2014). Antioxidant properties of germinated fenugreek seeds. *Phytotherapy Research, 19*, 977–983. https://doi.org/10.1002/ptr.1769

Randhir, R., Lin, Y.-T., & Shetty, K. (2004). Phenolics, their antioxidant and antimicrobial activity in dark germinated fenugreek sprouts in response to peptide and phytochemical elicitors. *Asia Pacific Journal of Clinical Nutrition, 13*(3), 295–307.

Rouag, F., Djemli, S., Boussena, M., Memouni, R., Refes, I., Ferhati, H., & Tahraoui, A. (2021). The effect of oral gavage (force-feeding) administration of fenugreek seeds (*Trigonella foenum-graecum* L.) on biochemical and neurobehavioural parameters in male Wistar rats. *Journal of Animal Behaviour and Biometeorology, 9*(2107), 1–7. https://doi.org/10.31893/jabb.21007

Sakr, S. A., & Shalaby, S. Y. (2014). Effect of fenugreek seed extract on carbofuran-inhibited spermatogenesis and induced apoptosis in albino rats. *Journal of Infertility and Reproductive Biology, 2*(2), 36–42. ID: 51766170.

Seema, Z. (2014). Protective effect of Fenugreek on thioacetamide induced hepatotoxicity in rats. *Saudi Journal of Biological Sciences, 21*, 139–145. https://doi.org/10.1016/j.sjbs.2013.09.002

Shakuntala, S., Naik, J. P., Jeyarani, T., & Naidu, M. M. (2011). Characterisation of germinated fenugreek (Fenugreek L.) seed fractions. *International Journal of Food Science & Technology, 46*(11), 2337–2343. https://doi.org/10.1111/j.1365-2621.2011.02754.x

Singh, A. P., Muthuraman, A., Jaggi, A. S., et al. (2012). Animal models of acute renal failure. *Pharm. Rep., 64*, 31–44. https://doi.org/10.1016/s1734-1140(12)70728-4

Sudan, P., Goswami, M., & Singh, J. (2020). Antifungal potential of fenugreek seeds (*Trigonella foenum-graecum*) crude extracts against microsporum gypseum. *International Journal of Research in Pharmaceutical Sciences, 11*(1), 646–649. https://doi.org/10.26452/ijrps.v11i1.1870

Syed, Q. A., Rashid, Z., Ahmad, M. H., Shukat, R., Ishaq, A., Muhammad, N., & Ur Rahman, H. U. (2020). Nutritional and therapeutic properties of fenugreek (*Trigonella foenum-graecum*): A review. *International Journal of Food Properties (IF 1.808), 10*, 6. https://doi.org/10.1080/10942912.2020.1825482

Tewari, D., Jóźwik, A., Łysek-Gładysińska, M., Grzybek, W., Adamus-Białek, W., Bicki, J., Strzałkowska, N., Kamińska, A., Horbańczuk, O. K., & Atanasov, A. G. (2020). Fenugreek (*Trigonella foenum-graecum* L.) seeds dietary supplementation regulates liver antioxidant defense systems in aging mice. *Nutrients, 12*(2552), 1–12. https://doi.org/10.3390/nu12092552

Udupaa, V., & Prakash, V. (2019). Gentamicin induced acute renal damage and its evaluation using urinary biomarkers in rats. *Toxicology Reports, 6*, 91–99. https://doi.org/10.1016/j.toxrep.2018.11.015

Wlodek, D., Banath, J., & Olive, P. L. (1991). Comparison between pulsed-field and constant-field gel electrophoresis for measurement of dna double-strand breaks in irradiated chinese hamster ovary cells. *International Journal of Radiation Biology, 60*(5), 779–790.

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