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Fenugreek powder exerts protective effects on alcoholised rats' kidney, highlighted using ultrastructural studies

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Abstract

Trigonella foenum-graecum (TFG) seeds exert a protective antioxidant effect and membrane protector through their rich content in polyphenolic flavonoids. The previous research focused on the hypoglycemic action of the seeds, with scarce studies on the preventive effects in the pathology of the kidney. Our work was conducted on an experimental *in vivo* model; the animals were given two different concentrations of TFG seeds, consequently to alcohol intoxication. Transmission electron microscopy (TEM) analysis showed vacuolation in cytoplasm, edemas at the apical pole of the nephrocytes, diffusion of the cytoplasmic and mitochondrial matrix and the increase in number of the lysozymes and especially peroxisomes, as well as the congestion of blood capillaries. In the case of the groups T5R and T10R, which received *Trigonella* powder together with ethanol, the structural and ultrastructural changes produced by the ethylic intoxication were more reduced, being somewhat improved in the T5R group. Therefore, the majority of the cells nuclei have retained their spherical shape, being at the same time predominantly euchromatic, with little heterochromatin and evenly dispersed. Our results suggest the use of *Trigonella* seeds as a food supplement to prevent cellular deterioration and improve renal morphology and function.

Keywords: ethanol toxicity, rat kidney, renal protective effects, *Trigonella* seeds, ultrastructural studies.

Introduction

Trigonella foenum-graecum, popularly named Fenugreek, is an annual grassy plant from the family of the leguminous plants, whose seeds are used for a long time in the Oriental cuisine as a flavor and condiment. Through their rich content in polyphenolic flavonoids, *Trigonella foenum-graecum* seeds show a membrane protection and antioxidant effect, certified by the studies of some groups of researchers from India, the geographic area where this plant is intensely used. Most researchers have mainly investigated only the anti-diabetic and hypoglycemic action of the seeds [1–3]. There are no structural and ultrastructural studies on their use as a preventive factor in renal pathology in alcoholics.

Lately, plant extracts have been increasingly used as an alternative to drug therapy. The development of phytotherapy is also due to the fact that treatment with certain herbs and plant extracts with phytotherapeutic effect has the advantage of obtaining them and using them at a lower price and with a low toxic potential [4, 5].

Hepatobiliary and renal disorders are currently increasing, being favored by the increase of the environmental pollution, abuse of alcohol and synthetic drugs and viral infections. In pharmacies, one can already find several plant extracts used for the treatment of some liver and kidney diseases, in order to neutralize the negative effects of xenobiotics. In general, a few authors studied the mechanisms of action of these plant products at cellular and subcellular level [6, 7].

The use of plants in treating some diseases has become a tradition especially in Asian medicine, "the natural pharmacy" being an important source for therapy (known as phytotherapy). Currently, the properties of medicinal plants are re-evaluated due to the progress in chemical, clinical and pharmacological research of plants and pharmaceuticals derived from vegetal plants. Among the recognized advantages of phytotherapy, we notice the lack or low toxicity, and showing minor side effects and low price compared to synthetic drugs [8, 9].

It is known that 2% up to 10% of the consumed alcohol is eliminated almost unchanged through kidneys and lungs, while the rest of it suffers a process of oxidation at the level of liver. The ethanol molecules are small and hydrophilic, which explains its rapid absorption from the digestive tract and the alveoli, after a small quantity has been already absorbed in the oral cavity [10–12]. Ethanol also passes relatively easy through the skin and the placental barrier. Earlier studies have shown that the chronic administration of ethanol in experiment/lab animals leads to neurological dysfunctions and dysfunctions of the alimentary tract, of the liver, kidneys, heart and pancreas and causes alterations of fetus [13, 14]. Alcohol intoxication is also considered the main risk factor for inducing chronic pancreatitis in 80% of the cases [15, 16].

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We emphasize the fact that until now, no investigation has been conducted on the effects of Fenugreek flour seeds at cellular and subcellular level upon nephrocytes of alcoholised rats, this way our study is the first of this kind.

The purpose of the study was to highlight the structural and ultrastructural changes produced at the level of kidneys, especially on the proximal convoluted tubules from the renal cortex where, due to rich vascularization there is the susceptibility of an almost direct action of the alcohol experimentally administered. We have also aimed at the possible renal-protective action of the Fenugreek flour seeds, administered at the same time with the ethylic alcohol.

Our ultrastructural studies were mainly performed on the nephrocytes of the proximal convoluted tubules from the renal cortex, because at their level the re-absorption of water from the filtrate of the primary urine occurs at a rate of 85%, being known the fact that these cells have an intense metabolic activity.

Materials and Methods

Animals

Our experimental research was conducted on white male Wistar rats, weighing 180–200 g. We designed an *in vivo* experimental model, in which the animals were simultaneously given ethanol in water and *Trigonella* seeds powder in food. The animals for the experiment were grouped in four groups, each group consisting of 20 rats, as follows:

▪ the control group (M) that received a standard diet and tap water *ad libitum*;

▪ the group of animals treated only with ethanol (ER), which were given 10% (*v/v*) in their drinking water, and which also consumed the standard diet;

▪ the group of animals that was treated with ethanol as the above group, and the food contained a concentration of 5% *Trigonella* flour seeds (T5R);

▪ the group of animals that was treated with ethanol (as above) and a 10% dosage of *Trigonella* flour seeds in food (T10R).

The experiment lasted for 31 days, after which the animals were sacrificed and we have taken kidney samples that were processed adequately in order to conduct ultra-structural investigations. We mention that all animals were starved 24 hours before being sacrificed. The animals were treated with kindness, being exposed to minimum stress and were sacrificed under anesthesia. The study has been approved by the University's Commission on Research Ethics.

Composition of standard diet

The standard diet contained: casein 200 g/kg, cellulose 50 g/kg, corn oil 155 g/kg, corn starch 393 g/kg, D,L-methionine 3 g/kg, mineral mix 35 g/kg, sucrose 154 g/kg, vitamin mix 10 g/kg, with a total energy of 1631.2 kJ/100 g. The mineral mixture contained the following (mg/kg of diet): $CaHPO_4$, 17 200; KCl, 4000; NaCl, 4000; MgO, 420; $MgSO_4$, 2000; Fe_2O_3, 120; $FeSO_4 \cdot 7H_2O$, 200; trace elements, 400 ($MnSO_4 \cdot H_2O$, 98; $CuSO_4 \cdot 5H_2O$, 20; $ZnSO_4 \cdot 7H_2O$, 80; $CoSO_4 \cdot 7H_2O$, 0.16; KI, 0.32; sufficient starch to bring to 40 g (per kilogram of diet).

The vitamin mixture contained the following (mg/kg of diet): retinol, 12; cholecalciferol, 0.125; thiamine, 40; riboflavin, 30; pantothenic acid, 140; pyridoxine, 20; inositol, 300; cyanocobalamine, 0.1; menadione, 80; nicotinic acid, 200; choline, 2720; folic acid, 10; *p*-amino-benzoic acid, 100; biotin, 0.6; sufficient starch to bring to 20 g (per kg of diet).

Electron microscopy investigation

For ultrastructural investigations, the renal tissue fragments collected were prefixed in a solution of 2.7% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2 for two hours at 4^0C. The samples were then washed in four successive baths, from hour to hour, with phosphate buffer 0.15 M at 4^0C. The methods used are in accordance with the conventional methodologies used by the vast majority of researchers in investigations using transmission electron microscopy (TEM) [17, 18].

For microscopic investigations, the ultrathin sections of 30–60 nm were obtained using an ultramicrotome Leica UC6, with a diamond knife Diatomite Ultra 35^0 type, sections which were collected on electrolytic grilles with 100–200 Mesh, being subsequently double contrasted with an alcoholic solution of 50% supersaturated uranyl-acetate and lead citrate in distilled water, pH 12. Finally, the sections were examined under an electron microscope FEI Tecnai type G2 Spirit TWIN / BioTWIN from the International Centre for Electronic Microscopy of "Vasile Goldiş" Western University of Arad, Romania. The images were recorded using a digital camera type Mega View III.

Results

The ultrastructural investigations were mainly carried out on the proximal convoluted tubules, given both the importance of the nephrocytes in absorbing water from the filtrate of the primary urine, as well as of the proximity to numerous blood capillaries from the renal glomeruli, to which the presence of blood alcohol, in experimental administration conditions, is relatively constant and at the highest concentration.

Control group (M)

In the control group (M), the nephrocytes of the proximal tubes are arranged, within each tube, on a relatively thick basal membrane (Figures 1 and 2). Each nephrocyte has a spherical nucleus, with a clear nucleolus. At the apical pole, the nephrocytes are provided with a thick microvilli border that facilitates filtering and absorption of water and of some electrolytes from the primary urine (Figures 1 and 2). In contrast, from the basal membrane into the interior of the cell, numerous basal folds emerge, which are inserted with numerous elongated mitochondria, suggesting the intense process of transit of the reabsorbed water from the level of the microvilli of the apical pole to blood capillaries that are under the basal membrane of the tubules.

Numerous elongated mitochondria are present in the cytoplasm as well as relatively few dense formations, represented primarily by peroxisomes and partially by lysosomes (Figures 1 and 2).

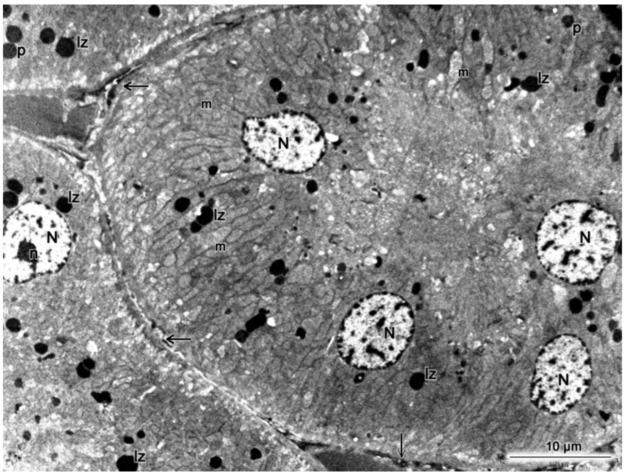

Figure 1 – ***M group. Nephrocytes of the proximal tubules are arranged, inside each tube, on a relatively thick basal membrane (black arrow); N – nucleus, n – nucleolus, m – mitochondrion, lz – lysosome, p – peroxisome; TEM, ×2500.***

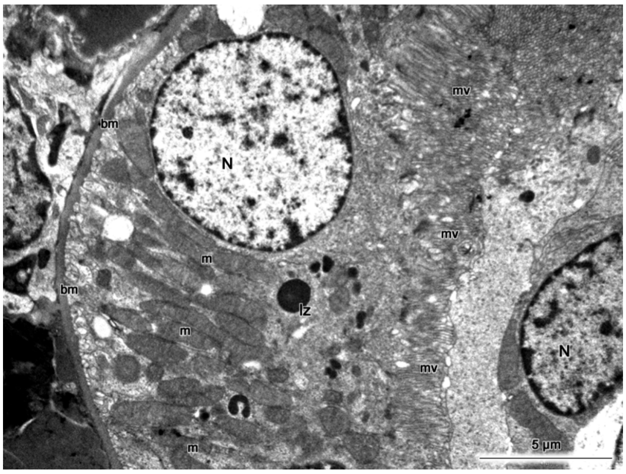

Figure 2 – ***M group. Each nephrocyte possesses a spherical nucleus, with a visible nucleolus. At the apical end, nephrocytes present a dense border of microvilli; N – nucleus, n – nucleolus, m – mitochondrion, lz – lysosome, p – peroxisome, bm – basal membrane, mv – microvilli; TEM, ×6000.***

ER group

In the group treated only with alcohol (ER), we mainly noticed the alteration of the nephrocytes of the proximal convoluted tubules, compared to other areas of the kidney. This alteration is due to the fact that at their level the filtered plasma reaches the maximum pressure and the blood alcohol has a relatively constant presence here, being in the area where the most significant changes occur, such as: rarefaction of cytoplasm, of the mitochondria, alteration of the basal membrane and of the apical pole of the nephrocytes, increase of the number of peroxisomes, causing the alteration of the normal functioning of the kidney. Please note the fact that keeping the integrity of the basal membrane is the premise, which favors the recovery of the structure of the uriniferous tubules.

The ultrastructural investigations fully confirm the results of the structural examinations and provide very clear additional details regarding the increase in number of the peroxisomes. The rarefaction of the cytoplasm and the dilatation of the base of the microvilli, the dilatation of the basal folds (Figures 3 and 4), the shape alterations of the nuclei, vacuolation of the cytoplasm, decrease of the number of mitochondria, damages of the apical pole of some nephrocytes, the presence of cellular detritus in the lumens of the tubes resulting from the affected tubules (Figure 4). In relatively small number of nephrocytes, all the mitochondria suffered a process of vesiculation, and the apical border has united microvilli, so that these nephrocytes are practically dysfunctional. Note also the fact that vascular congestions with red cells clusters occurred both at the level of the capillaries placed at the base of the tubules (Figure 4), as well as in the capillaries of the renal glomeruli. However, we mention that all these changes due to alcohol administration in the dose and time of our experiment are reversible overtime if its administration is interrupted.

T5R and T10R groups

In the groups T5R and T10R, we observed a much more improved situation, due to the concurrent administration of the Fenugreek seed flour, which proved to provide protection against the toxic action of the alcohol, more effective in the case of the dose of 5% than of the dose of 10%. However, we note the fact that in some nephrocytes some changes of the cellular components are still present, but at the same time we observe nephrocytes with a very similar look to that of the nephrocytes of the proximal convoluted tubules from the control group.

T5R group

In the Figures 5 and 6, the nephrocytes present an ultrastructural aspect, which is very similar to that of the nephrocytes that come from the control group, except that the nuclei are not completely spherical. The mitochondria are elongated and have normal structure (Figures 5 and 6). There are also nephrocytes where the mitochondria have rarefied matrix and the base of the microvilli show slight dilatations. In such nephrocytes, we also found edemas (evaginations) of their apical pole and few vacuolations in cytoplasm, suggesting the persistence of some affectations due to the influence of alcohol.

T10R group

Like the previous group, we can distinguish both the presence of the nephrocytes with ultrastructural aspect very close to the nephrocytes in the control group, which demonstrates the protective effect towards the action of alcohol, as well as of the nephrocytes that show different levels of damage due to the action of alcohol.

In the nephrocytes with an almost normal aspect, we can observe spherical euchromatic nuclei, few vacuolations in the cytoplasm, a moderate number of peroxisomes (Figure 7), mitochondria, the basal membrane and folds with a normal structure (Figure 8). Other nephrocytes present different levels of damage, which refer to dilatation of the basal folds and of the microvilli, edemas of their basal pole and the presence of cellular detritus in the lumen. At the same time, there are relatively few nephrocytes, which have spherical mitochondria and slightly rarefied matrix. A number of blood vessels, which are located especially in the chunks of the tubes, show edemas and red cells clusters.

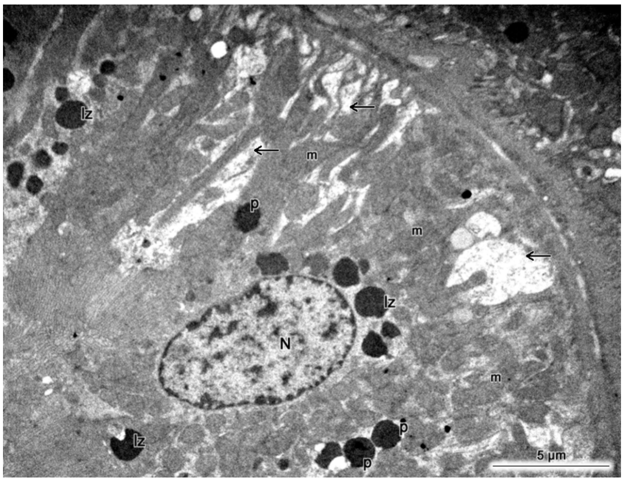

Figure 3 – ***ER group. Dilation of the basal folds (black arrow); N – nucleus, n – nucleolus, m – mitochondrion, lz – lysosome, p – peroxisome; TEM, ×6000.***

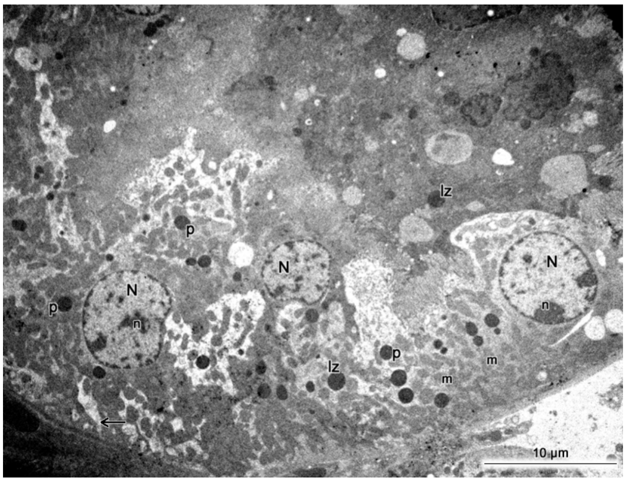

Figure 4 – ***ER group. Dilation of the basal folds, decrease of mitochondria and alterations of the apical end of some nephrocytes; N – nucleus, n – nucleolus, m – mitochondrion, lz – lysosome, p – peroxisome; TEM, ×2500.***

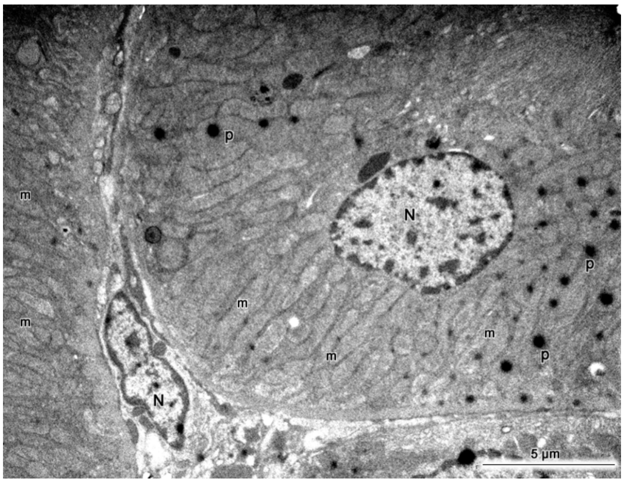

Figure 5 – ***T5R group. The nephrocytes show a similar ultrastructure to the nephrocytes of the control group, with the difference that here the nuclei are not completely spherical; N – nucleus, m – mitochondrion, p – peroxisome; TEM, ×6000.***

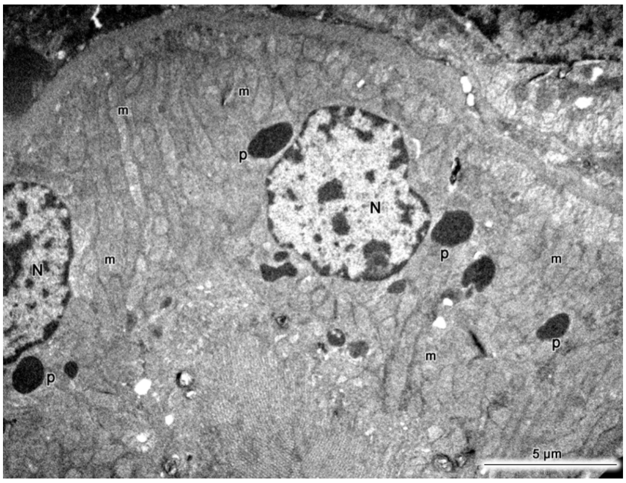

Figure 6 – ***T5R group. The mitochondria are elongated but overall with a normal structure; N – nucleus, m – mitochondrion, p – peroxisome; TEM, ×6000.***

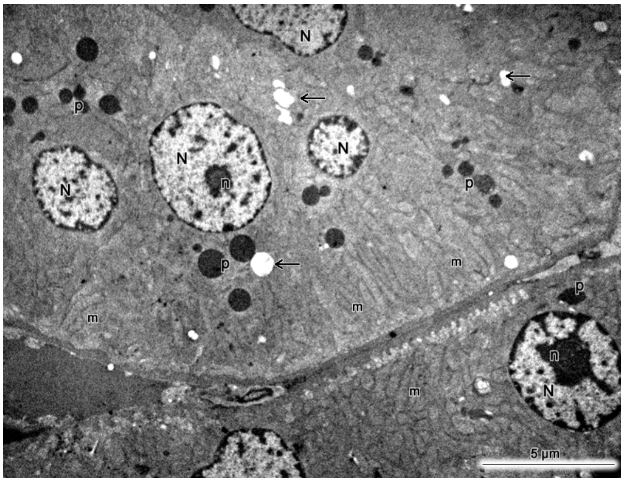

Figure 7 – ***T10R group. Nephrocytes showing an ultrastructure close to the control group, with euchromatic spherical nuclei, few vacuoles in the cytoplasm (black arrow) and a moderate number of peroxisomes; N – nucleus, n – nucleolus, m – mitochondrion, p – peroxisome; TEM, ×6000.***

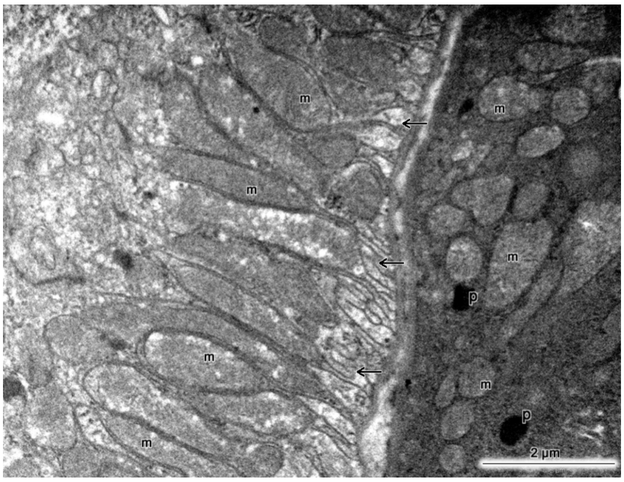

Figure 8 – ***T10R group. Nephrocytes with an aspect close to the control group; mitochondria, basal membrane and basal folds (black arrow) with normal structure; m – mitochondrion, p – peroxisome; TEM, ×15 000.***

As we mentioned earlier, all these damages described in these last two groups are not irreversible damages because, if the administration of alcohol stops, the respective nephrocytes restore in time their ultrastructural aspect and their normal specific functions.

Discussion

Our results have shown an improvement on ultrastructural morphology of the kidneys, due to the concurrent administration of the Fenugreek seed flour, proving to provide protection against the toxic action of the alcohol. The improved reactions were shown also in previous studies, under different conditions. One of these studies, conducted in 2013, under $AlCl_3$ toxicity, has shown alterations in rat kidney similar to those of ethanol toxicity, including inflammation of distal and proximal tubules, epithelial cell degeneration of the convoluted tubules and metaplasia of simple cuboidal epithelium with a simple squamous epithelium and loss of Bowman's spaces in the majority of glomeruli. In addition, similar to our results, has been observed nuclei pyknosis along with the vacuolation of the cytoplasm. Treatment with Fenugreek seed powder showed an improvement of the kidney morphological aspect, similar to our study, by regeneration of the cuboidal form of the epithelium, recovery of Bowman's spaces and reduction of pyknotic cells [19].

In our study, we found that ethanol affects the Bowman's capsule by altering its basement membrane. Similar to our observations, aluminum changed the morphology of the glomerulus filtration membrane, a membrane acting as a selective barrier and diminished the Bowman's space [19].

We have investigated mainly the proximal convoluted tubules because these seemed to be the most affected representing the initial segment of the renal tubule that reestablishes much of the filtrate into the blood, reabsorbing minerals and nutrients. We have found that ethanol, similar to $AlCl_3$ study, altered the proximal tubule cells by decreasing the brush border density and consequently determined a reduction of the surface area responsible for absorption [19].

Remarkably, Fenugreek seed powder was found to have protective effects on the kidneys. In the ethanol-treated group, associated with *Trigonella* 5% and 10% respectively has proven to counterbalance the weight loss of kidneys (data not shown), similar to the study of Belaïd-Nouira *et al.*, conducted in 2013 [19].

Additionally, Fenugreek treatment has considerably improved the histological and ultrastructural parameters. *Trigonella* supplementation produced an important restoration of the kidneys ultrastructure among the T5R and T10R groups compared to those of the ER group. This beneficial effect on the kidneys is just a part of various properties of *Trigonella foenum-graecum* (TFG), particularly its potential as a free radicals scavenger. This antioxidant activity of TFG seeds, mentioned in various studies, is considered to be exerted by polyphenols, especially flavonoids. Taking into consideration that flavonoids, mainly quercetin extracted from other medicinal plants with nephroprotective effects, have been described to inhibit nephrotoxicity induced by xenobiotics in some experimental animal models [20], we can suggest that the nephroprotective effects of TFG could be described by a concurrent antioxidant and free radicals scavenging effect. This hypothesis has been validated by Xue *et al.* [21], who confirmed that TFG seed was able to re-establish the kidney function of diabetic rats, through its antioxidant activity. Their study has shown that the ultramorphological abnormalities in the kidney of diabetic rats, including the patchy thickening of the glomerular membrane, were evidently improved by TFG treatment, concluding that TFG has protective effects against functional and morphologic injuries in the kidneys by increasing activities of antioxidants, suggesting a potential drug for the prevention and therapy [21].

Alternatively, Fenugreek flavonoids could be responsible for its beneficial influence on collagenous structures, like basement membrane and filtration membrane. It has been shown that TFG improved the properties of collagen and restored the collagen content in the ethanol-injured liver, probably by acting as a regulator on reactive oxygen species (ROS) production [8].

In a recent study has been shown that Fenugreek alleviated glomerular morphological alterations in rats, being similar to our results. As conducted in our study, morphological changes in glomeruli were assessed under electron microscopy. Three types of alterations were detected in the glomeruli, including segmental thickening of glomerular basement membranes, extensively joined foot processes podocytes and extremely deposited mesangial matrix, even though these ultrastructural anomalies were evidently prevented by Fenugreek in the treatment group. Further investigations, glomerular volume was measured under electron microscopy and quantified to be as the measure index of glomerular hypertrophy. The study showed that glomerular volume was significantly reduced by Fenugreek treatment. This suggested that Fenugreek could evidently mitigate glomerular hypertrophy. Nevertheless, Fenugreek has a protective effect on the kidney and prevents the nephropathy development [22].

Another research, showing similar results, has evaluated the effects of two synergic plant extracts, which consisted of *Trigonella foenum-graecum* L. (TFG) and *Psoralea corylifolia* L. (PC). The study has revealed that the renal tissues of rats with untreated nephropathy showed remarkable glomerular hypertrophy and fibrosis, effacement of the podocyte foot processes and hyperplasia of mesangial area. However, treatment with TFG and PC restored these morphology changes. In summary, similar to our study, Zhou *et al.* demonstrated that TFG improves renal function and ameliorate renal histopathological alterations. The suggested mechanism may be related to reducing oxidative stress [23].

Trigonella foenum-graecum is a well-known hypoglycemic agent used in traditional Indian medicines. Thus, several studies have been conducted to establish to effects of TFG on different types of diabetic nephropathy in rats. In these studies, there were investigated the effects of several drugs that induced diabetes and has been established the histological structure and function of the kidney and the protective effect of TFG seed powder using enzyme analysis and light and transmission electron microscopy. The histopathological studies showed early nephropathic changes in diabetic rats and the ultrastructure of the diabetic kidney revealed the same aspects as it has been shown in the current study. Alloxan administration showed symptoms of severe nephropathy (presence of glomerular matrix formation, tubular necrosis, interstitial inflammation and fibrosis). TFG treatment to the diabetic rats effectively prevented the structural abnormalities thus suggesting a protective effect of TSP (*Trigonella foenum-graecum* seed powder) on kidneys of the diabetic rats. The role of TFG in reversing the diabetic state at the cellular level proves its potential as an antidiabetic agent with beneficial nephroprotective effects [24–26].

Other studies have used streptozotocin (STZ) (50 mg/kg) to induce diabetes mellitus and investigated the effects of trigonelline (an active compound of TFG) on the renal functional, morphological changes and renal apoptosis in neonatal diabetic rats, a model of non-insulin-dependent diabetes mellitus. TFG treatment showed significant beneficial effects on all the biochemical and histological parameters. The degenerative changes in kidney tissue and fibrosis were alleviated in the TFG treated groups [27].

Another drug used for inducing nephropatic injuries was Adriamycin (ADR), an anticancer drug used in treatment of a variety of neoplastic lesions. Its use is limited due to diverse toxicities including cardiotoxicity, hepatotoxicity and nephrotoxicity. The administration of a single dose of ADR (10 mg/kg body weight) induced histopathological, immunohistochemical and biochemical alterations. Kidneys of ADR-treated rats showed many histopathological alterations. The renal tubules were degenerated and the glomeruli were atrophied. The inter-tubular spaces were infiltrated by inflammatory leukocytic cells. These changes were time-dependent, similar to our current study. Treating animals with ADR and seed extract of Fenugreek led to an improvement in the histological structure of the kidney. The results presented by Sakr & El-Gamal, in 2011, are consistent with our data shown by us indicating that Fenugreek seeds have ameliorative effect on kidney damage and this may be mediated by its potent antioxidant effects [28].

Similar studies on Wistar rats have used carbon tetrachloride (CCl_4) as a toxicity agent that was supplemented with apidiet formulations containing honey, pollen, propolis, Apilarnil, with or without royal jelly (RJ). The results have shown, after six weeks, alterations of the renal tissue, such as hyaline cylinders, interstitial infiltrate, medullar and glomerular congestion. After nine weeks, the results were even more compelling showing interstitial and glomerular congestion, granular dystrophy of contort tubes, peritubular congestion with granular dystrophy, peritubular congestion with damage to the brush border, granulo-vacuolar dystrophy of contort tubes, inflammatory infiltrate. However, upon treating the lab animals with apidiet with/without RJ there were minor renal lesions after six or nine weeks. This study has shown the beneficial effects of natural products having nephroprotective effects and could be considered in association with TFG for further studies and as a part of a more complex diet formulation [29].

Regarding its adverse effects, it seems that TFG exerts minor or no side effects as shown in our results. An exhaustive overview has evaluated critically the evidence regarding the adverse effects of 50 different herbal medicines, showing *T. foenum-graecum* as being part of the group exerting minor adverse effects [30].

Conclusions

The experimental administration of ethylic alcohol in rats induces various structural and ultrastructural changes of the kidney, more pronounced at the level of the renal cortex and especially at the level of the proximal nephrocytes of the convoluted tubules. Regarding the effects of *Trigonella foenum-graecum* on ethanol nephrotoxicity, the current study has clearly shown that the treatment with Fenugreek powder did not induce any side effects on animals, as it has been shown through kidney ultrastructural parameters. Our study, according to similar studies, has proven that the ultrastructural kidney alterations were clearly re-established by Fenugreek treatment, concluding that *T. foenum-graecum* has protective effects against functional and morphological alterations of the kidneys, and we suggest the use of TFG as a potential drug for the prevention and therapy. Fenugreek seeds can be used as a regular nutrient to alleviate the side effects of ethanol ingestion, in the kidneys, especially for chronic renal failure patients who are more susceptible to developing ethanol toxicity.

Conflict of interests

The authors declare that they have no conflict of interests.

Author contribution

All authors contributed equally to this work and all may be considered as main authors.

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