Effect of Fenugreek seeds "Trigonella foenum graecum" on ethanol-induced renal injury in albino rats

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Abstract:

In this study, the effect of fenugreek seeds on renal injury of experimental rats was studied. A total of 20 male Albino rats, weighing 154 - 169 g, were divided into four groups of 5 each ,were randomly classified into to negative control group and renal injury groups that injected or oral with ethanol (10% dissolved in the drinking water) and then reclassified into 3groups (5 rats each) as follows: Control positive, fenugreek powder 10 % and fenugreek powder 15% in basal diet .At the end of experiment (4 weeks) blood samples were collected. All organs' rat was weight. kidneys were kept in formalin solution (10%) for the histopathological investigation. Blood glucose, glubulin, albumin, bilirubin, and total protein were all measured. The concentrations of creatinine, urea, and uric acid were estimated. In kidney tissues, serum antioxidant levels as well as (SOD) activity, NO, and GR levels were assessed.

Results showed significant differences among initial, final body weights, body weight gain, organs' weights and relative organs' weights, of almost the groups. Results also indicated that the decreases in SOD and the increases in (GR),(NO) levels were more detectable with increases (TSP)level. Also show improvement in the histopathological structure of the kidneys

In conclusion, fenugreek has ameliorative effect against ethanol toxicity in rats due to its antioxidant activity so, drinking or incorporating it into food.

Key words: Trigonella- foenum-graecum ; Leguminosse; Antioxidant enzymes; Nitric Oxide; kidney; ethanol toxicity. Rats

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Introduction

Fenugreek (*Trigonella foenum graecum*) is a medicinal herb that is mostly utilised by people from the Middle East and South Asia. They use it as a spice as well as a medication. Because of its nutritional makeup and bioactive components, fenugreek seeds have a number of health-promoting properties. (Laila et al., 2022)

A green leafy vegetable and medicinal herb used all over the world is fenugreek (*Trigonella foenum graecum*). They lack an alternative and don't have the same adverse effects as chemical drugs. Fenugreek has been shown in several studies to be effective in treating kidney issues as well as a number of other illnesses. (Jagtap et al .,2022)

Fenugreek, or *Trigonella foenum-graecum* L., is a member of the Fabaceae family. Alkaloids, flavonoids, saponins, free amino acids, proteins, glycosides, and mucilaginous fibers were discovered during the phytochemical screening of fenugreek seeds (Wani and Kumar, 2018). Fenugreek seeds have been found to provide a number of medicinal benefits, including those for diabetes, anti-oxidants, hypocholesterolemic, antiulcerogenic, antineoplastic, antipyretic, anti-inflammatory, anti-toxic, and anticancer characteristics (Wani and Kumar, 2018).

Finding natural antioxidants from plant sources has garnered a lot of attention in recent years. Flavonoids and other polyphenols, in particular, have been reported to suppress the spread of free radical reactions and shield the human body from disease. These antioxidant phytochemicals are found in plants. Natural antioxidants exhibit a wide range of metabolic actions, such as the reduction of ROS production, the scavenging of free radicals directly or indirectly, and the modification of intracellular redox potential (MoHFW, 2016) .

Due to its medical, nutraceutical, and pharmaceutical properties, (*Trigonella foenum -graecum*) is well known for its use as an edible species, spice herb, as well as a medicinal plant. (Ahsan et al., 2008). It is regarded as a high source of protein (25%) as well as lysine (5.7 g/116 g N), soluble and insoluble dietary fibers (20%), the alkaloid trigoneline (36%), and

flavonoids with anti-cancer characteristics as ornithine, viticsine, and quercetin. Moreover, the seeds include fix oil, an important component of fenugreek (*Trigonella foenum graecum*).Calcium, iron, and beta-carotene are nutrients, as well as several steroid saponins such diosgenin, ticogenine, and neoticogenine(Moran et al., 2009).

In this review, fenugreek's benefits on lowering cholesterol and having anti-diabetic, anti-fertility, anti-cancer, anti-microbial, and anti-parasitic properties have been examined. Important bioactive chemicals have been discovered to be present in fenugreek. (Wani & Kumar ,2018)

Numerous studies have shown that *Trigonella foenum-graecum*, often known as fenugreek, has antibacterial (Simkhada et al., 2008) anticarcinogenic, (Rahman et al ., 2017) and antioxidant properties (Siddique et al., 2018). *Trigonella foenum-graecum*, also known as fenugreek has phenolic and flavonoid components that contribute to its enhanced antioxidant potential (Choudhury et al., 2012). Fenugreek, or *Trigonella foenum-graecum*, has been used to treat high blood damage from oxidation (Syed et al., (2008). Fenugreek (*Trigonella foenum -graecum*) without causing any negative effects, it also tones the kidneys (Bhutta et al., 2011) and (Wade et al., 2006). Fenugreek (*Trigonella foenum-graecum*) inhibits lipid peroxidation and inhibits the oxidative damage of enzymatic antioxidants. Isolated compounds from the amazing biological properties of fenugreek, *Trigonella foenum-graecum*, including protection against cancer, malaria, allergies, viruses and bacteria . (Hill et al., 2010), (Adams et al., 2020).

Glutathione reductase (GR) catalyzes the reduction of oxidized glutathione (GSSG) to reduced glutathione(GSH). Glutathione reductase is essential for the glutathione redox cycle that maintains adequate levels of reduced cellular GSH. GSH serves as an antioxidant, reacting with free radicals and organic peroxides, in amino acid transport, and as a substrate for the glutathione peroxidases and glutathione Stransferases in the detoxification of organic peroxide and metabolism of xenobiotics, respectively .Assay of Glutathione Reductase has been used in the detection

of hepatic and malignant disease, nutrition (assessment of riboflavin status) and detection of genetically determined deficiency states. Care must be taken to ensure that apparent enzyme deficiency states are not due to riboflavin depletion. (Goldberg &Spooner 1983)

Nitric Oxide (NO) is synthesized in biological system by the enzyme Nitric Oxide Synthase (NOS). NOS is a remarkably complex enzyme which acts on molecular oxygen, arginine, and NADPH to produce NO, citrulline, and NADP + (Montgomery & Dymock 1961)

ALB and GLB are two readily available, trustworthy biomarkers that are assessed in liver function tests that are regularly carried out before to surgery. They may be completed quickly and cheaply. ALB, a protein with a negative acute phase, is regarded as a biomarker of inflammation and dietary intake (Don & Kaysen (2004), Eckart et al., (2020) and Deng et al., (2019)

One of the major serum protein constituents, albumin (ALB), has been linked to surgical site infections in the field of orthopaedics (Yuwen et al., 2017 and Yang et al., 2020). Globulin (GLB), a substance found in ceruloplasmin and complement, typically rises during the inflammatory process (Li, et al.,2018). The albumin-to-globulin ratio (AGR) is a result. Another promising biomarker for inflammation is one that takes both ALB and GLB levels into account (Ukibe et al., 2020). Additionally, numerous investigations revealed that AGR and GLB were useful biomarkers in the diagnosis of infection of the periprosthetic joint (PJI) (Ye et al., 2020 and Wang et al. 2021). Additionally, plasma fibrinogen and serum D-dimer may be novel markers. In previous investigations, they have demonstrated good performance as biomarkers to identify infected nonunion (Wang et al. 2020 and Wang et al. 2019).

The kidneys are crucial for filtering bodily waste and toxins from the blood and excreting them into the urine. They also keep the body's electrolyte, acidity, and moisture levels balanced and regulate blood pressure (Joo et al, 2020). The use of serum concentrations of creatinine, urea, and uric acid to evaluate renal function is justified because these

substances represent glomerular filtration rate. Renal disease causes the kidney to work less efficiently (glomerular filtration rate decreases), which compromises the kidney's ability to remove creatinine, urea, and uric acid from the blood, causing these substances to accumulate in the blood (Joo et al, 2020 and Salazar, 2014).

The present study was aimed to investigate the effect of different two levels of *Trigonella foenum graecum* Seeds, on the histopathological examination of kidney as well as some parameters in rats including, kidney functions, antioxidants enzymes, serum concentrations of total protein(TP), bilirubin , albumin , glubulin , blood glucose and rat weight.

Material and Methods

Material:

1- Fenugreek Seed "*Trigonella foenum-graecum* "(TSP) were used in the present study. *Trigonella foenum graecum* were obtained from the local market in Cairo

2- A total number of 20 Albino male adult rats, weighing 154 - 169 g, were obtained from the animal laboratory of the Faculty of Veterinary Medicine, Cairo, Egypt.

3-Chemicals: Kits for biochemical analysis of Nitric Oxide, SOD, G. reductase, Kidney functions (Creatinine, Uric acid and Urea), Serum concentrations of total protein (TP), bilirubin , albumin, Glubulin and blood glucose were purchased from Biodiagnostic for Diagnostic and Research Reagents , Dokki, Egypt.

Methods:

Experimental design and diets:

Preparation of Fenugreek Seed "*Trigonella foenum-graecum:*

Seeds were washed with clean water and dried. To make powder, dried seeds were crushed in electrical grinder. The powder was then stored in a clean, dried and covered plastic container at room temperature.

Basal diet:

Diets in all experiment groups contain 14% protein, 4% salt mixture, 1% vitamin mixture and 10% fat (Reeves et al., 1993).

Experimental design:

Rats were divided into four groups of 5 rats each. The rats of each experimental group were individually housed in polypropylene cages in an environmentally clean room, light controlled (12:12 light/dark). Temperature was maintained at 25°C and 60% humidity with unlimited access to food and water. All efforts were made to minimize pain and distress during animal husbandry and experiments assessments. Protocol is approved by the institutional Animal Care and Use Committee, Cairo University,(CU-IACUC) approved number (CU II F 32 17).

Diets were designed and prepared with careful consideration. Rats were fed on basal diet for one week as an adaption period. To induced renal injury 10% ethanol was dissolved in the drinking water. Group 1 as negative control (NC) and group 2 as positive control (PC) were fed on basal diet. While groups 3 and 4 received basal diet with 10% and 15 % *Trigonella foenum-graecum* seed powder. Group I had normal drinking water, while groups 2, 3 and 4 were given ethanol 10% dissolved in the drinking water.

Rats' weight was monitored twice a week throughout the experimental period (4 weeks). At the end of experiment, all the rats were sacrificed by cervical decapitation, and blood samples were collected. Heart, kidney, spleen, lungs and brain of each animal were weighted and Kidney kept in formalin solution (10%) for the histopathological investigation. Kidneys were removed and weighed and its tissue samples were divided; some were also fixed in formalin (10%), others were snap frozen and stored at -80°C until analysis.

During the experimental period (28 days), body weight was recorded every week. Biological evaluations of the different diets were carried out by determination of body weight gain (BWG), Feed intake (FI) and feed efficiency ratio (FER) calculated according to Chapmanet al., (1959), using the following formulas:

Body weight gain (BWG) (g) = Final weight - Initial weight

Feed efficiency ratio (FER) = Body weight gain ÷ Feed intake.

Serum parameter:

Kidney functions parameters:

Kidney functions (Creatinine, Uric acid and Urea) Serum concentrations of total protein (TP), bilirubin, albumin and Glubulin were estimated assessed according to the manufacturer's protocol from Diagnostic kits.

Serum creatinine, serum urea and serum uric acid were determined according to Murray, (1984); Kaplan, (1984) and Fossati et al., (1980), respectively. Determination of serum total bilirubin was assessed by Doumas et al., (1973). Determination of total protein was carried out according to the colorimetric method of Henry (1974). Determination of serum albumin was assessed by Doumas et al., (1971).

Blood glucose:

Blood glucose was determined according to (Braslasu et al.,2007).

Serum antioxidant enzymes:

Serum antioxidant as Superoxide dismutase (SOD) activity, Nitric Oxide and G. reductase were estimated as described in the manufacturer's protocol from Diagnostic kits.

Glutathione reductase (GR) was assessed by (Goldberg &Spooner 1983), serum antioxidant as Superoxide dismutase (SOD) activity, and Nitric Oxide (NO) were determined according to Cao et al., (1993) and (Montgomery & Dymock 1961);, respectively.

Histopathological examination:

Tissue samples of Kidney was removed carefully, and fixed in neutral buffered formalin 10%, dehydrated in ascending grade of alcohol, cleared and embedded in paraffin, sectioned at 5 μm thickness and stained by H & E and examined microscopically (Bancroft et al.,1996).

Statistical analysis:

Statistical analysis was carried out using one way analysis of variance (ANOVA) test followed by Duncan test through the program of statistical packages for the social science (SPSS) version 16. Results were expressed as mean± SD. The differences among means at $p \leq 0.05$ are considered significant (Snedecor and Cochran, 1989).

Results and discussion

Nutritional Indicators:

Effect of TSP on initial weight (g), final weight (g) and BWG (g) of experimental groups rats:

Table 1 illustrated initial body weight, final body weight and body weight gain of rats for all the experimental groups.

Table1. Effect of (TSP) on Initial body weight, final body weight and Body weight gain of rats at the end of experimental study.

Groups	Initial body weight(g)	Fourth week (Final) (g)	Body weight Gain BWG (g)	Food intake (FI)	Feed efficiency ratio (FER)
Negative Control (NC)	154.30±2.02c	225.05±3.88^{c}	70.75±2. 61^{b}	18.98±2.01^{a}	3.72^{c}
Positive Control (PC)	164.25±2.25^{b}	236.23±3.07^{b}	71.98±2.71^{b}	15.34±1.78^{c}	4.69^{b}
Group (3) 10% TSP	161.50±1.153^{b}	239.55±2.95^{ab}	78.05±1.40^{a}	16.6±0.93^{b}	4.7^{b}
Group (4) 15% TSP	169.50±1.50^{a}	258.85±3.45^{a}	89.35±3.82^{a}	17.4±1.08^{ab}	5.13^{a}

Mean values in each column having different superscript (a, b, c & d) are significantly different at P <0.05

Data in Table (1) illustrated the initial weight, final weight and BWG of experimental rats. As for initial weight, it could be noticed that there was a significant difference between control (-ve) & treatment groups.

Concerning final weight, the results indicated that the mean value of final weight of negative control group was lower than positive control group, which were 225.05 (g) and 236.23 (g), respectively. The mean values

of groups 3 and 4, showed a significant difference when compared with positive control group, which were 239.55 and 258.85 (g), respectively.

As for BWG (g), data showed that the mean value of weight gain in negative control group was 70.75g while it was 71.98 g in control positive group. It's clear that weight gain for negative control group was lower than positive control group. As compared to positive control group, BWG of groups 3, 4 was 78.05 and 89.35, the best result was recorded for group 4.

In this regard, Fenugreek, which is high in dietary fibers and proteins, was found to promote the production of anorexigenic, insulinotropic, and glucagon-like peptides, which contributes to body weight loss, according to (Jagtap et al .,2022).

In this research article, it is claimed that ignatia guarantees greater body weight gain with a good feed conversion ratio. Because it is more affordable than ignatia and promotes healthy body weight gain, fenugreek can also be utilised in the production of quail. (Maruf-Ul-Mostakim et al.,2018).

Effect of (TSP) on the absolute organs' weights (g) of rats were illustrated in Table (2).

Table 2. Effect of (TSP) on the absolute organs' weights (g) of rats

Groups	Liver	Spleen	Lungs	Kidneys	Heart	Brain
Negative Control	8.82 ± 1.02^{ab}	1.36 ± 0.53^{a}	1.56 ± 0.07^{a}	2.44 ± 0.09^{a}	0.76 ± 0.09^{b}	1.58 ± 0.12^{a}
Positive Control	7.80 ± 0.92^{b}	0.96 ± 0.12^{a}	1.35 ± 0.21^{a}	2.04 ± 0.32^{a}	0.92 ± 0.06^{ab}	1.65 ± 0.06^{a}
Group (3) 10% TSP	9.97 ± 1.36^{ab}	1.06 ± 0.20^{a}	1.48 ± 0.10^{a}	2.45 ± 0.35^{a}	0.94 ± 0.09^{ab}	1.70 ± 0.06^{a}
Group (4) 15% TSP	10.52 ± 1.23^{a}	1.08 ± 0.13^{a}	1.59 ± 0.24^{a}	2.51 ± 0.18^{a}	1.08 ± 0.13^{a}	1.75 ± 0.12^{a}

Mean values in each column having different superscript (a, b, c & d) are significantly different at $P < 0.05$

Table (2): illustrated the effect of feeding tested plant (TSP) in concentration 10% & 15% for 4 Wk. on organ weight (liver, spleen, lungs, kidney, Heart and Brain) in rat of the different experimental groups. In our study, weight most of organ in all groups showed a non significant

difference except Liver &Heart show a significant difference between positive Control and Negative Control, also with 10% & 15% (TSP) .

The outcomes showed that the addition of fenugreek seed oil improved body weight on average (Hussein & Zaki 2022)

Effect of (TSP) on relative organ weight (%) of rats presented in table 3.

Table 3. Effect of (TSP) on relative organ weight (%) of rats.

Groups	Liver	Spleen	Lungs	Kidneys	Heart	Brain
Negative Control	0.040±0.005^{a}	0.006±0.002^{a}	0.007±0.00٠^{a}	0.011±0.00٠^{a}	0.003±0.000^{a}	0.007±0.00٠^{a}
Positive Control	0.032±0.004^{a}	0.003±0.000^{a}	0.005±0.001^{a}	0.008±0.00٠^{b}	0.003±0.000^{a}	0.006±0.00٠^{ab}
Group (3) 10% TSP	0.040±0.006^{a}	0.004±0.001^{a}	0.005±0.00٠^{a}	0.009±0.001^{ab}	0.003±0.00٠^{a}	0.006±0.00٠^{ab}
Group (4) 15% TSP	0.039±0.002^{a}	0.004±0.00٠^{a}	0.005±0.00٠^{a}	0.009±0.00١^{ab}	0.004±0.00٠^{a}	0.006±0.00٠^{b}

Mean values in each column having different superscript (a, b, c & d) are significantly different at P <0.05

Relative organ is shown in table (3) no significant differences among relative organs' weights of the experimental groups were noticed except kidneys & brain show a significant difference between positive control and negative control, also with 10% & 15% (TSP).

In this respect (Hussein & Zaki 2022) showed that fenugreek seed oil significantly increased average body weight

Antioxidant enzymes:

Activities of Nitric Oxide, anti-oxidant superoxide dismutase (SOD), and Glutathione reductase (GR) parameters were illustrated in Table (4).

Table 4: Effect of (TSP) on Nitric Oxide, SOD and Glutathione reductase

Groups	Nitric Oxide	SOD	G. reductase
	(u/l)		
Negative Control	33.33±2.5٠^{c}	220.66±2.08^{a}	75.00±3.0٢^{a}
Positive Control	56.00±3.0١^{a}	169.00±3.0٣^{d}	35.66±2.51^{c}
Group (3) 10% TSP	41.33±2.5٢^{b}	176.33±2.5٣^{c}	46.00±3.0٤^{b}
Group (4) 15% TSP	20.33±2.5٤^{d}	210.33±2.51^{b}	50.33±2.5٣^{b}

Mean values in each column having different superscript (a, b, c & d) are significantly different at P <0.05

Nitric Oxide (NO) is synthesized in biological system by the enzyme Nitric Oxide Synthase (NOS)

Results showed that untreated rats (positive control) showed significant increase in serum (NO) level, compared with those of the normal and (TSP) treated rats.

Glutathione reductase (GR) catalyzes the reduction of oxidized glutathione (GSSG) to reduced glutathione(GSH). Glutathione reductase is essential for the glutathione redox cycle that maintains adequate levels of reduced cellular GSH

Values of serum (GR) level in untreated rats were lower than those of the normal and treated rats. Supplemented diets with the two different levels of *Trigonella foenum graecum* seeds demonstrated higher serum (GR) level than that of positive control rats, while slightly lower than those in rats of negative control group fed on normal diet.

As the first line of defense in cells against the damaging effects of oxygen radicals (ROS), superoxide dismutase (SOD) catalyses the dismutation of superoxide into hydrogen peroxide and oxygen. (Hussein et al., 2014)

Data presented in table (4) indicated that SOD levels diminished in control (+) group. However, the reverse recorded for treated rat, in particular for 10 and 15% (TSP) group. The best result was found in treated groups with high doses of (TSP) .

The ability of the galactomannan (F-GAL) and aqueous extract (FS-AE) of fenugreek seeds to prevent liver and kidney damage extracts in streptozotocin (STZ)-induced T1DM in rats was examined by Alsuliam et al., (2022). However, both treatments significantly boosted Nrf2 transcription, translation, and nuclear localization, as well as hepatic and renal superoxide dismutase (SOD) in the livers and kidneys of both the control and diabetic-treated rats. According to the toxicology studies, FS-

AE and F-GAL both have antioxidant and anti-inflammatory benefits that are unrelated to their potential to lower blood sugar.

Our results are in harmony with Darwish et al.,(2020) Who showed that the kidney levels of glutathione reduced (GSH), glutathione reductase (GR), superoxide dismutase (SOD), and catalase (CAT) activities were significantly lower in the gentamicin group than in the control group (P<0.001) as well. However, the significance value for (SOD) was (P0.005) when compared to the control group. Simultaneous treatment of germinated fenugreek generated significant ameliorative restoration in levels of (GSH), (GR), and (CAT) to their normal values (P<0.001). However, when fenugreek (*Trigonella foenum -graecum*) and gentamicin were administered simultaneously, the levels of (GSH), (GR), (SOD), and (CAT) were significantly lower than they were in the control group (P<0.001, P0.029, P0.023, and P<0.001, respectively).

Kidney Functions

Kidney functions (Creatinine, Uric acid and Urea) in rats of the different experimental groups after 4 Wk. presented in table(5)

Table 5: Effect of (TSP) on Serum concentrations of Kidney functions (Creatinine, Uric acid and Urea) in normal and alcoholic induced rats.

Groups	Creatinine	Uric acid	Urea
	mg /dl		
Negative Control	0.83 ± 0.02^{a}	1.47 ± 0.16^{b}	18.00 ± 2.00^{a}
Positive Control	0.77 ± 0.02^{b}	1.50 ± 0.15^{a}	21.50 ± 1.50^{a}
Group (3) 10% TSP	0.66 ± 0.02^{c}	1.38 ± 0.04^{c}	21.00 ± 1.00^{a}
Group (4) 15% TSP	0.66 ± 0.01^{c}	1.20 ± 0.04^{c}	22.50 ± 4.50^{a}

Mean values in each column having different superscript (a, b, c & d) are significantly different at P <0.05

Table 5 presented that serum levels of Urea of the treated rats did not significantly differed compared to the normal control rats (negative control group), except creatinine and uric acid that was significantly different.

Significantly higher value of creatinine and uric acid was noticed for positive & negative control than those of the other experimental groups.

In this respect , Darwish et al., (2020) found that the male mice treated with fenugreek (*Trigonella foenum -graecum*) alone or germinated fenugreek alone did not show any significant changes in all kidney function parameters that were measured in the serum, as compared with the control group ($P>0.05$). However, gentamicin treated mice showed a significant increase ($P<0.001$) in Urea, creatinine and uric acid levels compared with the control group.

Also , Alsuliam et al .,(2022) Who investigated the potential of the galactomannan (F-GAL) and aqueous extract (FS-AE) of the Fenugreek seed aqueous to prevent liver and kidney damage extracts in streptozotocin (STZ)-induced T1DM in rats. Results showed that there no significant differences in the serum levels of urea, creatinine, and albumin were depicted when a comparison was made between the control, FS-AE, (aqueous extract (FS-AE) of the Fenugreek seed) and F-GAL-treated rats

The kidney of arsenic-treated rats showed a considerable biochemical and cellular recovery following the administration of fenugreek seed extract. The levels of urea, uric acid, creatinine, and albumin have significantly normalised, according to the biochemical criteria. (Kumar et al., 2021)

This is consistent with studies by Inegbedion et al., (2022) Who found that albino rats' serum levels of creatinine, urea, and uric acid were affected by the aqueous extract of fenugreek seeds. When compared to the control, no dose of fenugreek seed aqueous extract substantially ($P>0.05$) altered the levels of serum urea, creatinine, or uric acid. Implying that fenugreek seed aqueous extract may prevent renal disorders at all doses.

By measuring blood urea, creatinine, and uric acid levels, this study explored the potential preventive effects of fenugreek and curcumin consumption on nicotine-induced kidney damage in rats (Azab et al., 2022)

Effect of (TSP) on Serum concentrations of total protein, bilirubin albumin and glubulin in normal and alcoholic induced rats presented in table(6).

Table 6. Effect of (TSP) on serum concentrations of total protein, bilirubin albumin and glubulin in normal and alcoholic induced rats.

Groups	**Bilirubin (mg/dl)**	**Total Protein (mg/dl)**	**Albumin** ALB **(mg/dl)**	**Globulin** GLB **(mg/dl)**	**A/G ratio** AGR
Negative Control	0.28 ± 0.025^{b}	7.05 ± 0.25^{ab}	2.50 ± 0.20^{a}	3.55 ± 0.25^{a}	0.70 ± 0.01^{a}
Positive Control	0.32 ± 0.005^{a}	7.75 ± 0.75^{a}	2.40 ± 0.70^{a}	3.60 ± 0.10^{a}	0.67 ± 0.21^{a}
Group (3) 10% TSP	0.25 ± 0.010^{c}	6.50 ± 0.70^{b}	2.27 ± 0.25^{a}	3.60 ± 0.40^{a}	0.64 ± 0.14^{a}
Group (4) 15% TSP	0.29 ± 0.020^{b}	7.55 ± 0.25^{ab}	3.00 ± 0.30^{a}	3.35 ± 0.05^{a}	0.89 ± 0.10^{a}

Mean values in each column having different superscript (a, b, c & d) are significantly different at $P < 0.05$

ALB= albumin; GLB = globulin; AGR = albumin-to-globulin ratio

Table 6 showed that, the male rat treated with (*Trigonella foenum - graecum*) did not show any significant changes in albumin, glubulin and **A/G ratio** that were measured in the kidney, as compared with the control group. A significant increase in

bilirubin, total protein in both Positive Control & Negative Control respectively.

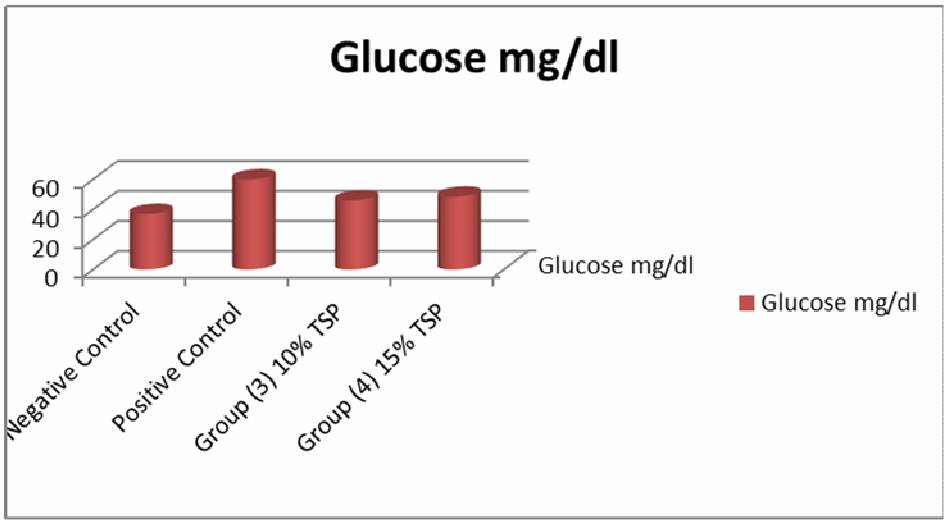
On the other hand, Group (3) 10% TSP showed the lowest significant decrease in the same pervious parameters .

Our findings are consistent with those of (Kumar et al.,2021), who found that arsenic-treated rats had significantly higher serum levels of SGPT, SGOT, ALP, and total bilirubin than the control group ($p< 0.001$). The levels of SGPT, SGOT, total bilirubin, and ALP were significantly ($p< 0.001$) lower in the arsenic-treated group than in the arsenic-pretreated control group rats, who were allowed 90 days to recover on their own. But after giving arsenic-pretreated rats fenugreek seed extract at a dose of 250 mg/kg body weight per day for 90 days, the levels of SGPT, SGOT, and

AST significantly decreased ($p < 0.001$), indicating that the supplement had a protective effect against arsenic-induced hepatotoxicity.

In addition, Kumar et al. (2021) discovered that after administering fenugreek seeds extract to arsenic-pretreated rats for 90 days at a dose of 250 mg/kg body weight per day, there was a significant ($p > 0.001$) decrease in the levels of urea, uric acid, and creatinine, as well as a significant increase ($p > 0.001$) in the levels of albumin, indicating the protective effect of fenugreek

Figure 7, illustrated the effect of feeding diets supplemented with different levels of TSP seeds (10 and 15%) on serum levels of glucose



From the results presented in Figure (7) it could be noticed that glucose was significantly higher in the serum of positive control rat receiving ethanol than those in the negative control group. Whereas, these levels in rat receiving ethanol together with (*Trigonella foenum -graecum*) were significantly lower than those in rat that were subjected to ethanol treatment alone. Administration of *Trigonella foenum -graecum* has a protective effect against ethanol -induced some blood parameters elevation.

By reducing the rate of glucose absorption in the gut, enhancing the activity of the beta islet cells of the pancreas, fostering insulin sensitivity,

and enhancing glucose utilisation inside cells, fenugreek seeds are linked to blood sugar regulation (Dilworth et al., 2021)

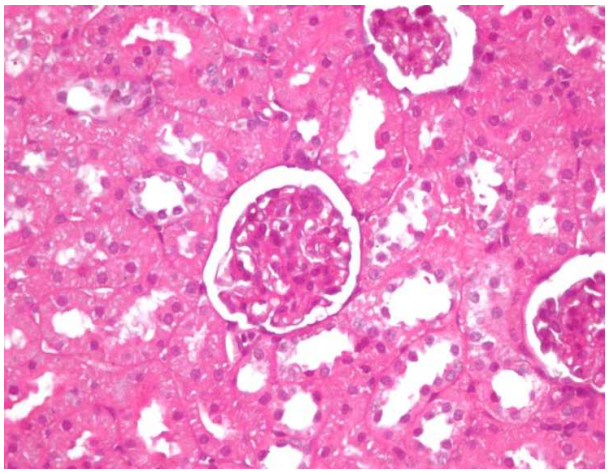
The plant contains bioactive substances such steroids, saponins, flavonoids, alkaloids, and others Laila et al., (2022). Additionally, it has a lot of soluble fiber, which prevents the absorption and retention of carbohydrates, hence decreasing blood sugar levels (Shashikumar et al., 2019).

Natural medicinal herbs have been proven to be effective anti-diabetic medications, according to Jagtap et al., (2022). It has been demonstrated that fenugreek, or common fenugreek, lowers blood sugar and increases insulin sensitivity. Diabetics who take galactomannan, a form of dietary fiber, can drop their blood sugar levels.

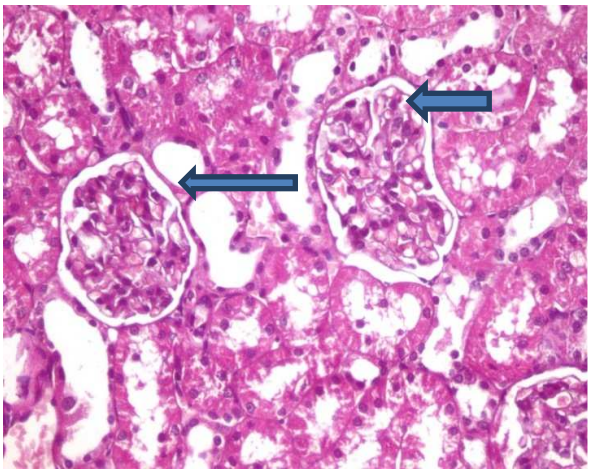
In this regard, Reimer and Russell (2008) said that fenugreek, which is abundant in dietary fibers and proteins, has been demonstrated to promote the release of anorexigenic, insulinotropic, and glucagon-like peptides, which aids in lowering body weight and enhancing glucose levels.

Histopathological results

Kidneys histopathologies are shown in Picture's (1-4).

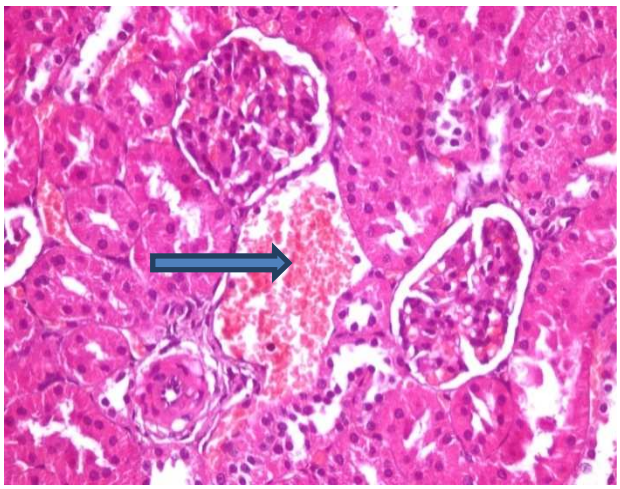


Picture 1: Photomicrograph of rat kidneys from control negative group showing normal renal parenchyma; note the normal glomeruli and renal tubules, (H&E X 400).

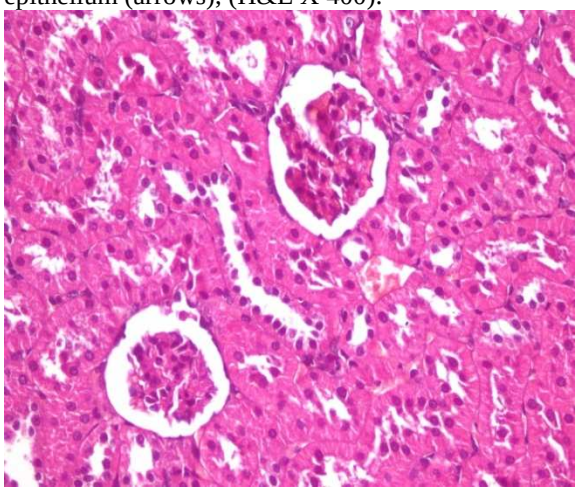


Picture 2: Photomicrograph of rat kidneys from control positive group showing vacuolated glomerular tuft epithelium and renal tubular

epithelium (arrows), (H&E X 400).



Picture 3: Photomicrograph of rat kidneys from low dose diet group showing normal renal parenchyma with congestion in the interstitial blood vessel (arrow), (H&E X 400).



Picture 4: Photomicrograph of rat kidneys from high dose diet group showing normal renal parenchyma with no congestion in the interstitial blood vessel improved than low dose diet group, (H&E X 400).

Histopathological Results

Control negative group showed **that renal tissue** examination revealed normal renal parenchyma with normal glomeruli and normal renal tubules **(Picture 1).**

Group II (control positive group) showed that **renal tissue** examination revealed vacuolated glomerular tuft epithelium and renal tubular epithelium **(Picture 2)**.

Group III (10% TSP diet**)** showed general improvement in all the examined tissues than the control positive group, , **Renal tissue** examination revealed improve drenal parenchyma with slight congestion in the interstitial blood vessel **(Picture 3)**.

Group IV (15% TSP treated group**)** showed general improvement in all the examined tissues than the low dose diet group, **Renal tissue** examination revealed normal renal parenchyma with no congestion in the interstitial blood vessel **(Picture 4)**.

In this regard, Kumar et al. (2021) discovered that the administration of fenugreek seeds extract to arsenic-pretreated rats led to a significant (p>

0.001) decrease in the amount of arsenic in the blood, liver, and kidney tissues as compared to the arsenic-pretreated control group.

Fenugreek seeds have been shown to enhance the histological changes against sodium nitrite intoxication in kidney and liver damage, according to a study by USLU et al. (2019).

Conclusion

The present study was aimed to investigate the effect of different two doses of *Trigonella foenum graecum* Seeds, on the kidney as well as some parameters in rats including, kidney functions, antioxidants enzymes, serum concentrations of total protein(TP), bilirubin , albumin , glubulin , blood glucose and rat weight. Therefore, including *Trigonella foenum graecum* in one's daily diet may be a first step in preventing Kidney illnesses and damage. The use of seeds or extract, concentration, or the length of time could be to blame for the variations in results. To ascertain the dose necessary for the greatest protective impact of *Trigonella foenum graecum* in humans and to make its use appropriate as an effective functional food with therapeutic potential, additional studies utilising various doses and longer time periods would be required. Additionally, adding this plant to your diet can improve your Kidney's histology by reducing the Kidney damage that hepatotoxicity-induced rats' Kidneys experience.

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تأثير بذور الحلبة على الإصابة الكلوية التي يسببها الإيثانول في الفئران البيضاء

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الملخص العربي:

ركز البحث على دراسة تأثير إضافة مسحوق بذور الحلبة بنسبة ١٠ و١٥ ٪ الى الوجبة على الإصابة الكلوية في الفئران . كما تم الفحص الهستوباثولوجي لأنسجة الكلى وكذلك تقدير الإنزيمات المؤكسدة والمضادة للأكسدة في الفئران المصابة بالتسمم الكحولي. تم إعطاء تركيزين مختلفين من بذور الحلبة . تم تقسيم عشرين من ذكور الفئران الألبينو، التي يتراوح وزنها بين ١٥٤ - ١٦٩ (جم)، إلى أربع مجموعات (خمسة / مجموعة). حصلت المجموعتان ٣ و ٤ على الوجبة الأساسية، بينما كانت المجموعات ١ ككنترول سالب (NC) والمجموعة ٢ ككنترول موجب (PC). في حين اعطيت المجموعتين الثالثة والرابعة الوجبة الأساسية المضاف اليها مسحوق بذور الحلبة بنسبة 10و 15 ٪ على التوالي. أعطيت المجموعة الأولى مياه شرب العادية ، بينما المجموعات الثانية والثالثة والرابعة أضيف كحول الإيثانول بتركيز 10 ٪ الى ماء الشرب. واستمرت التجربة لمدة ٤ أسابيع . تم أخذ عينات الدم في نهاية التجربة وكذلك تسجيل اوزان الاعضاء لكل حيوان. لغرض الفحص الهستوباثولوجي. تم حفظ الكلى في محلول فورمالين ١٠٪ .تم فصل سيرم الدم لتقدير تركيزات جلوكوز الدم ، الجلوبيولين ، الألبومين ، البيليروبين ، والبروتين الكلي. تم تقدير تركيزات الكرياتينين واليوريا وحمض البوليك في أنسجة الكلى ، تم تقييم مستويات مضادات الأكسدة في الدم وكذلك نشاط الإنزيمات المضادة للأكسدة (SOD) وأكسيد النيتريك (NO) ومستويات الجلوتاثيون GR.

أظهرت النتائج اختلافات معنوية بين أوزان الجسم الأولية والنهائية وزيادة وزن الجسم وأوزان الأعضاء وأوزان الأعضاء النسبية في معظم المجموعات التجريبية تقريبًا. كما أشارت النتائج إلى أن الانخفاض في مستوى SOD في الدم والزيادات في مستويات المصل (GR) و (NO) كانت أكثر وضوحا مع زيادة مستوى الحلبة .حيث وفرت الوجبات المدعمة ببذور الحلبة حماية واضحة على التركيب الهستوباثولوجي للكلي وقدمت الدراسة أدلة على التأثير الوقائي لمسحوق الحلبة على تلف الكلى نتيجة لنشاطها المضاد للأكسدة

لذا توصي الدراسة بتناول الحلبة اما كمشروب أو اضافتها للاغذية لما لها من تأثير إيجابي ضد سمية الإيثانول في الفئران

الكلمات الدالة : بذور الحلبة ؛ الإنزيمات المضادة للأكسدة ؛ وظائف الكلى ؛ أكسيد النيتريك ؛ الفئران ؛ سمية الإيثانول .

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