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Uses of Fenugreek (*Trigonella foenum*) leaves alcoholic extract in treatment of nephrotoxicity induced by acetaminophen in male rats

Basheer Ali Al-Janabi

Department of Physiology, College of Veterinary Medicine, University of Kerbala, Karbala, Iraq

* Corresponding author email: dr.basheer.ali96@gmail.com

Ali J. AL-Nuaimi

Department of Physiology, College of Veterinary Medicine, University of Kerbala, Karbala, Iraq

Mousa

Department of Physiology, College of Veterinary Medicine, University of Kerbala, Karbala, Iraq

Rana Fadhil

Department of Physiology, College of Veterinary Medicine, University of Kerbala, Karbala, Iraq

Mahdi S. Hassan

Department of Physiology, College of Veterinary Medicine, University of Kerbala, Karbala, Iraq

Eman Jawad Jaber

Department of Physiology, College of Veterinary Medicine, University of Kerbala, Karbala, Iraq

Sajaa R. AL-Saedi

Department of Physiology, College of Veterinary Medicine, University of Kerbala, Karbala, Iraq

Juman Khaleel Al-Sabbagh

Department of Physiology, College of Veterinary Medicine, University of Kerbala, Karbala, Iraq

Nawras M. Al-Khafaji

Department of Physiology, College of Veterinary Medicine, University of Kerbala, Karbala, Iraq

Rana A. Jawad

Department of Physiology, College of Veterinary Medicine, University of Kerbala, Karbala, Iraq

Ibrahim Alhaitami

Department of Physiology, College of Veterinary Medicine, University of Kerbala, Karbala, Iraq

Mayada S. Hassan

Department of Physiology, College of Veterinary Medicine, University of Kerbala, Karbala, Iraq

Abstract--The current study was proceed to analysis the therapeutic effects of Fenugreek leaves extract (FSE) on renal defect experimentally induced by Acetaminophen exposure. Forty male rats divided into 4 groups as follow: C-, C+,T1 and T2. The result showed detectable depletion in serum creatinine in groups treated with FSE extract. Furthermore there were maximum depletion of serum urea levels statistically increased in group treated with plant as treatment withcontrol. In contrast the high levels of BUN and UN revealed in control negative group which received 2mg/kg.bw of acetaminophen without treatments. For the iron levels there were also increase in animals exposed to FSE in both doses 300 and 150mg/kg.bw comparing with control positive and negative, furthermore there were increased in group of control positive which was healthy animals comparing with those exposed to acetaminophen toxicity, also histopathological slides showed significant damage and loss of architecture on renal tissue and remarkable glomerular necrosis in C+ animals group. In contrast the supportive effects of fenugreek extracts 300mg/kg.bw clearly appeared in kidney section showed normal renal architecture. In conclusion, Fenugreek leaves ethanolic extract revealed good therapeutic effect against nephrotoxicity of Acetaminophen.

Keywords--acetaminophen, fenugreek, rat, nephrotoxicity, Iron.

Introduction

Acetaminophen (N-acetyl-p-aminophenol [APAP]), is an acylated aromatic amide, a metabolite of phenacetin, that was firstly introduced into medicine as an antipyretic/analgesic by Von Mering in 1893 and has been in use as an analgesic for home medication for over 40 years (Hegazy *et al.*, 2021). Although hepatotoxicity is more addressed than nephrotoxicity in paracetamol overdoses, paracetamol -induced renal damages, such as renal tubular damage and acute renal failure are usually life-threatening and there is no specific treatment for them but there are protectants could prevent their happening (Peng *et al.*, 2010). Because APAP (acetyl-para-aminophenol) is a phenacetin metabolite, nephritic syndrome and renal papillary necrosis are possible (chronic analgesic

nephropathy) (Hegazy *et al.*, 2021). *Trigonella foenum-graecum* L. (fenugreek or TFG) is an annual plant that belongs to the Leguminosae family (Eidi *et al.*, 2007). Its leaves and seeds are commonly used as a condiment and seasoning, and as a wheat and maize flour supplement for bread-making. Additionally, TFG is a staple food in Asian and North African regions (Xue *et al.*, 2007), and is also used in traditional medicine owing to its antidiabetic, hypoglycaemic, antioxidant, hypolipidemic and immunomodulatory effects (Bin-Hafeez *et al.*, 2003; Renuka *et al.*, 2009). The most important phytochemicals isolated from TFG are saponins, trigonelline alkaloids, trigocoumarin, phosphates, potassium, proteins (4-hydroxyisoleucine), choline, vitamin C, betacarotene, nicotinic acid, and folic acid (Bin-Hafeez *et al.*, 2003). Number of studies regarding that fenugreek (*Trigonella foenum-graecum*) used as antibacterial (Hamza, *et al.*, 2012), anticarcinogenic (Hassan *et al.*, 2010), antidiabetic (Mokhtari *et al.*, 2008), anti-inflammatory (Morani *et al.*, 2012), and antioxidant (Isahq *et al.*, 2015). It contains phenolic and flavonoid compounds which help to enhance its antioxidant capacity (Rocha *et al.*, 2009).

Materials and Methods

Fenugreek (*Trigonella foenum-graecum*) leaves

The plant leaves purchased from the local market and taken to the laboratory and to clean up with sterile distilled water and then dried on the heat of the laboratory afterwards the leaves were ground by a battery powered mill until it was disintegrated into granules and then retained in opaque cans and wrapped in aluminum foil to prevent the oxidative degradation and then put in the refrigerator stored.

Hydro-Alcoholic extraction of Fenugreek

Hydro alcoholic extraction of Fenugreek leaves powder was carried out according to (Harborne, 1984; Al-Ameedi and Nahi, 2019).

Animals

In this study, forty white male rat were used; aged 2-3 months and average weight 200-230 grams, and were housed and maintained in the animal house/College of Veterinary Medicine/ Al-Qasim green University with optimal conditions. These rats were fed special formula (food pellets) and supplied by clean drinking water. All experimental animals were housed in a clean plastic cages which were contained sawdust as bedding that was changed twice a week to provide a clean environment.

Experimental design

Forty male rats, induced renal toxicity by using acetaminophen 2g/kg.bw orally daily for 2 weeks (Hegazy *et al.*, 2021). Then randomly divided into three groups (3/group):

- Group (C+) 10 nephrotoxic rat, administrating with D.W orally for 2 weeks as control positive .
- Group (T1) 10 nephrotoxic rat, treated with 150mg/kg.bw orally of Fenugreek leaves extract for 2 weeks as therapeutic dose (Gözdeet *al.*, 2019).
- 3-Group (T2) 10 nephrotoxic rat, treated with 300mg/kg.bw orally of Fenugreek leaves extract for 2 weeks as double dose (Gözdeet *al.*, 2019).
- 4-Group (C-) 10 healthy rats without any treatment as control negative group.

Serum creatinine, blood urea nitrogen and Iron concentrations

Kits that made by (Human company/ Spain) were used to determine the concentration of serum creatinine and blood urea nitrogen according to the (Kallneret *al.*, 2008).

Histopathological study

The organs obtained from each mouse were subjected to the following steps (Luna and Lee, 1968).

Statistical analysis

Data were analyzed by using a one-way analysis of variance (ANOVA) then Tukey's multiple comparison tests in GraphPad Prism 8 (free GraphPad Software was performed to determine whether the means for treatment groups differed from those for the controls. The statistical significance for all comparisons was set at a $P < 0.05$.

Results and Discussion

Plant extraction

Weight of the dried extract resulted after evaporation of ethanol was 0.8 g representing 8% of the dried weight of leaves. The extract appeared with greenish brown color, the relative proportion between the amount of plant used for extraction and crude product was variable depending on several factors, such as the method of extraction and solvent used in extraction process as well on plant species (Henning et al., 2003). The specific odor of plant extracts is caused by the high percentage of phenol compounds and terpenes (Cherevaty et al., 1980; Al-Ameedi and Nahi, 2019).

Creatinine and blood urea nitrogen

The therapeutic effects of Fenugreek extract on kidney functions represented by serum creatinine and blood urea nitrogen, illustrated in the tables 1. A significant decrease ($P \leq 0.05$) in serum creatinine in groups treated with plant extract when administered in two doses, in comparison with control negative group, for creatinine the data showed significant ($P \leq 0.05$) decrease in T2 group treated with 300mg/kg.bw of plant extract comparing with those in T1 which received

150mg/kg.bw. The maximum depletion of serum creatinine and urea levels found significantly ($P \leq 0.05$) in control positive group.

Iron concentration

The results in table (1) showed that there were significant ($P \leq 0.05$) increase in animals exposed to Fungreekethanolic extract in both doses 300 and 150mg/kg.bw comparing with control positive and negative, furthermore there were significant increases ($P \leq 0.05$) in group of control positive which was healthy animals comparing with those exposed to acetaminophen toxicity.

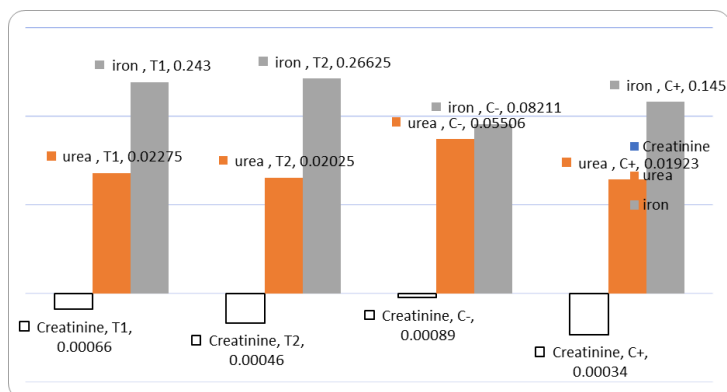
Histopathological study

Sections of kidney slides in the current study showed of animals treated with acetaminophen (C+ group), revealed sever infarcted renal tubules(coagulative necrosis) pale tubules with pink cellular borders, significant damage and loss of architecture on renal tissue and remarkable glomerular necrosis with inflammatory cells infiltration. In contrast the supportive effects of fenugreek extracts 300mg/kg.bw clearly appeared in kidney section showed normal renal architecture, mild degenerative changes in renal tubules epithelia and slight glomerular inflammatory cells infiltration with slight interstitial tissue inflammation.

Table 1
Showing the therapeutic effect of Fungreek extract on kidney functions

Groups parameters	Serum creatinine mean $\pm$ S.E	Blood nitrogen urea mean $\pm$ S.E	Serum Iron mean $\pm$ S.E
T1	0.66 $\pm$ 0.02 B	22.75 $\pm$ 0.85 B	243.00 $\pm$ 16.30 A
T2	0.46 $\pm$ 0.03 C	20.25 $\pm$ 1.89 B	266.25 $\pm$ 17.53 A
C-	0.89 $\pm$ 0.02 A	55.06 $\pm$ 0.64 A	82.11 $\pm$ 1.22 C
C+	0.34 $\pm$ 0.01 D	19.23 $\pm$ 0.87 B	145 $\pm$ 3.98 B
LSD	0.08	4.00	43.55

Capital letters denote the significances between groups



Histogram 1. Biochemical concentration of different groups exposed to acetaminophen

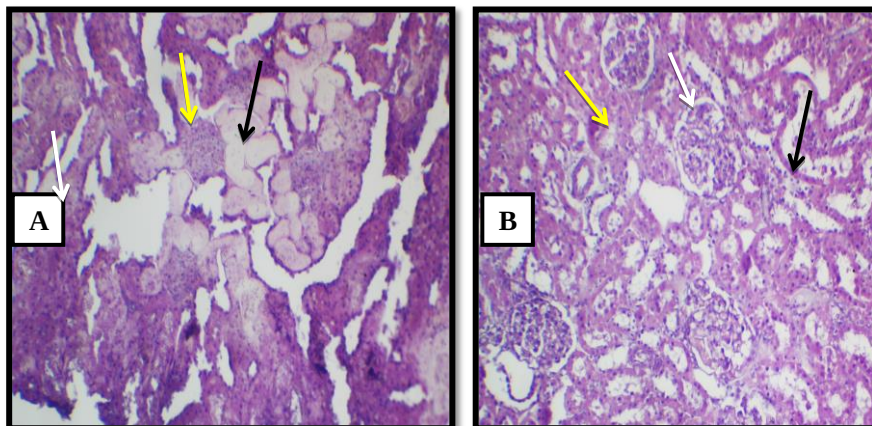


Figure 1. A; kidney section from a group of animal treated with Acetaminophen , revealed sever infarcted renal tubules(coagulative necrosis) pale tubules with pink cellular borders (black arrow), significant damage and loss of architecture on renal tissue (white arrow)and remarkable glomerular necrosis with inflammatory cells infiltration(yellow arrow).B: kidney section from a group of animal treated with Fenugreek 300mg/kg.bw , showed normal renal architecture , mild degenerative changes in renal tubules epithelia (black arrow), slight glomerular inflammatory cells infiltration (white arrow)with slight interstitial tissue inflammation (yellow arrow) (H and E,10 X)

Discussion

Reduction of the ability of the kidney to eliminate the toxic metabolic substances is indicated by elevation of serum levels of creatinine and urea nitrogen (Hummadi, 2012). The kidney is very sensitive to the adverse effects of drugs and chemicals. Walker and Duggin (1988) proved that even low concentrations of any chemical or its metabolites could generate a certain degree of nephrotoxicity. we aimed in our experiment to observe the nephrotoxic effect of acetaminophen on kidney which caused reduction in the renal function, oxidative stress and also iron level changes in serum. As well as to detect the therapeutic role of two doses of ethanolic extract of fenugreek leaves extract. In a study of Abdul Hamid et al. (2012), which performed to investigate the paracetamol-caused oxidative kidney damage, 750 mg/kg paracetamol was orally administered to rats for seven consecutive days. According to the results of the study, MDA, BUN and creatinine levels increased and GSH levels decreased in the paracetamol group compared to the control group. Another study showed that a single dose of paracetamol (2 g/kg) caused to a rise on lipid peroxidation and decreased by 66% of GSH stores in the liver cells [22]. To examine the effects of paracetamol on nephrotoxicity, Naguib et al. (2014) administered 500 mg/kg dose of paracetamol to mice intraperitoneally.

Zaher et al. (2007), examined the toxic effects of paracetamol in kidney tissues of rats. In the same study, the researchers revealed that paracetamol caused to damage of centrilobular areas in the liver which is resulted in induction of the NO

synthesis due to increase in NOS expression (Abdul-Zaher et al., 2007). Similarly, another study showed that a single dose of 250 mg/kg paracetamol administration resulted with rise in NO level of liver and kidney tissues due to the increased iNOS expression in rabbits (Cigremis et al., 2009). The results of the current study in accordance with El-Tawil, (2009) determine the possible protective effect of fenugreek, against γ -radiation-induced oxidative stress in kidney tissues of rats. Fenugreek treatment has significantly attenuated radiation-induced oxidative stress in kidney tissues, which was substantiated by the significant amelioration of serum creatinine, urea, glucose, and insulin levels. The author concluded that fenugreek would protect from oxidative damage and metabolic disturbances induced by ionizing irradiation. In another clinical trial (El-Tawil, 2009).

Fenugreek alcoholic extract was compared with the equal volume of distilled water given to two groups of five healthy adult male albino rabbits. Fenugreek caused a significant increase in sodium and potassium excretion with a significant hypocalciuric effect. No significant changes were observed in serum sodium, potassium, chloride, calcium, pH, and osmolality with respect to the control values. In order to the impairment in kidney functions was accompanied by either increase in serum creatinine and urea levels or kidney tissue (Salvatore et al., 2002; Atessahin et al., 2005). In addition, uric acid might be a true mediator of renal disease and progression (Azab et al., 2017).

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