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USES OF FENUGREEK (*TRIGONELLA FOENUM*) LEAVES ALCOHOLIC EXTRACT IN TREATMENT OF HEMATOTOXICITY INDUCED BY ACETAMINOPHEN IN MALE RATS

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Abstract

The current study was proceed to analysis the therapeutic effects of Fenugreek leaves extract (FSE) on renal defect experimentally induced by by acetaminophen oral administration for 14 consecutive days. After that T1 and T2 received 150mg/kg.bw and 300mg/kg.bw respectively for 14 consecutive days while control positive group (C+) leave without any treatment. Forty male rats divided into 4 groups as follow: C-, C+,T1 and T2. The results showed Significant increase ($P \leq 0.05$) in blood parameters such as RBC, MCH% , , Hb, Granulocyte and MID%, in animals groups were treated with FSE. On the other hand WBCs and lymphocytes significantly ($P \leq 0.05$) decrease in T1 and T2 groups when compared with C+ group. In conclusion, fenugreek extract has good therapeutic effects on biochemical and hematological parameters depending on dose and concentration of extract as well as arise clearly in nourishment of kidney and liver tissue.

Keywords: Acetaminophen, fenugreek, rat, RBCs, WBCs.

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). Trigonellafoenum-graecum L. (fenugreek or TFG) is an annual plant that belongsto the Leguminosae family (Eidi*et al.,* 2007). Paracetamol poisoning causes no significant differences in the hematological profile of Wistar rats (Payasi *et al*., 2010). However, some studies have reported that rats treated with paracetamol showed significant decrease in red blood cells count and hemoglobin (Adedapo *et al*., 2007; Ikpi and Nku, 2008; Juma *et al*., 2015). Similarly, paracetamol has been reported to cause significant decrease in mean values of haemoglobin, haematocrit and total erythrocyte count (Nwodo *et al*., 2010). Haematopoetic system is susceptible to xenobiotic attack since blood is involved in transportation of substances (Adeniyi *et al*., 2010). Treatment of rats with paracetamol causes non-

significant changes in total white blood cells, neutrophil, eosinophil, monocyte and lymphocyte counts as well as platelet count (Oyedeji *et al*., 2013).

Materials and Methods

Fenugreek (*Trigonellafoenum-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).Thenrandomly divided into three groups (3/group)

1-Group (C+) 10 nephrotoxic rat, administrating with D.W orally for 2 weeks as control positive .

2-Group (T1) 10 nephrotoxic rat, treated with 150mg/kg.bw orally of Fenugreek leaves extract for 2 weeks as therapeutic dose (Gözde*et al.,* 2019).

3-Group (T2) 10 nephrotoxic rat, treated with 300mg/kg.bw orally of Fenugreek leaves extract for 2 weeks as double dose (Gözdetali *et al.,* 2019).

4-Group (C-) 10 healthy rats without any treatment as control negative group.

Hematological Parameters: The hematological parameters were determined using a VET Hematological auto analyzer (count 60) manufactured by Genex in the Laboratory of Research and Studies / College of Veterinary Medicine / Al-Qasim Green University. The tool is capable of measuring and calculating 22 different parameters. This tool only used two reagents (Dilute and Lyse) and one maintenance reagent (Probe cleanser), It also has a mechanical image inside made of thermal paper. The device's estimated hematological parameters were (RBC, WBC, Plat, PCV, Hb, Lymphocyte, Granulocyte).

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:

1-RBC, Hb and HCT: The results of RBC and Hb counts of the nephrotoxic rats treated with two different doses of150mg/kg.bw and 300mg/kg.bw of Fenugreek seeds extract in table 4.1 seen a significant increase ($P \leq 0.05$), in T1 and T2 groups compared with C+ and C- groups respectively. Furthermore, there were significant increase ($P \leq 0.05$) in RBC and Hb levels in C- group comparing with C+ group. While the results of HCT levels revealed that there were significant increases ($P \leq 0.05$) in T2 and T1 comparing with C+ and C- respectively, as well as there were statistically differences between C+ and C- groups.

Table (1): Table 4.1: Showed the therapeutic effect of Fenugreek extract on red blood cell

Parameter Groups	RBC (10^12/L) Mean± Std. Error	Hb (g/dl) Mean± Std. Error	HCT % Mean± Std. Error
T1	6.71±0.14 A	12.78±0.39 A	34.05±1.36 A
T2	6.62±0.12 A	12.89±0.19 A	36.67±0.77 A
C-	5.98±0.09 B	11.52±0.17 B	32.34±0.48 B
C+	4.21±0.07C	8.45±0.08C	29.87±0.56C
LSD	0.38	0.87	3.02

2- WBC, GRN%, LYM% and MID%: Table (4-2) illustrate the WBC count that was significant increase ($P \leq 0.05$) in the group of male rats induced nephrotoxicity (C+) group comparing with those C-, T2 (300mg/kg.bw) and T1 (treated with 150mg/kg.bw) of fenugreek extract respectively. Furthermore, the statistical analysis of granulocytes (GRN%) indicated that there was significant increase ($P \leq 0.05$) in T2 and T1 groups comparing with C+ and C- groups respectively. For the lymphocytes percent (LYM%) the results showed that the statistical higher levels appeared in C+ group when compared with those in C-, T2 and T1 respectively. MID% means all types of WBCs except (LYM and GRN), they were significantly ($P \leq 0.05$) higher in T2, C- and T1 respectively comparing with C+ group.

Table (2):Showed the therapeutic effect of Fenugreek extract on white blood cell.

Parameters Groups	WBC (10^9/L) Mean± Std. Error	GRAN (%) Mean± Std. Error	LYM (%) Mean± Std. Error	MID (%) Mean± Std. Error
T1	5.07±1.50 B	3.90±1.80 A	59.13±3.02 B	18.98±1.32 A
T2	5.11±0.41 B	4.67±3.09 A	65.27±5.74 B	20.07±3.11 A
C+	7.25±0.06 A	3.30±0.03 B	90.62±1.34 A	7.21±0.11 B
C-	5.61±0.87B	2.90±4.22 C	69.45±87B	19.65±2.89A
LSD	2.87	2.61	12.23	6.24

Many studies have reported that rats treated with paracetamol show significant decrease in red blood cells count and hemoglobin (Adedapo *et al*., 2007; Ikpi and Nku, 2008; Juma *et al*., 2015). Similarly, paracetamol has been reported to cause significant decrease in mean values of haemoglobin, haematocrit and total erythrocyte count (Nwodo *et al*., 2010). Haematopoetic system is susceptible to xenobiotic attack since blood is involved in transportation of substances (Adeniyi *et al*., 2010). Treatment of rats with paracetamol causes non-significant changes in total white blood cells, neutrophil, eosinophil, monocyte and lymphocyte counts as well as platelet count (Oyedeji *et al*., 2013). However, another study reported that paracetamol overdose results to significant increase in mean corpuscular hemoglobin and a rise in lymphocytes (Juma *et al*., 2015). In the present study HGB and PCV in group III non-significantly increased as compared to control and group II, while RBC count in group II non significantly increased and in group III non-significant decreased as compared to control group .The non-significant change in each of the HGB , PCV and RBC count are in agreement with (Rao *et al*., 1996; Khalil , 2004; Al-Saiady *et al*., 2007). The normal value of MCV in both experimental groups when compared to control group indicates the safety evaluation of the effects of aqueous extract of fenugreek seeds on the RBC volume. There are different results obtained from investigations which performed on the effect of fenugreek seeds on haematological parameters. Rao *et al*.(1996) , showed that the effect of fenugreek seed flour in the diet of rat at three level (5%, 10% and 20%) for a period of 90 days produce non-significant changes in the HGB , PCV and total as well as differential WBC. While Effraim *et al*. (1999) reported that oral administration of aqueous extract of fenugreek seeds in various doses (300-900 mg/kg B.W) after 7 days significantly increased HGB, PCV and WBC count in the rat, meanwhile the levels of the above mentioned haematological parameters

decreased when treatment continued to 14 days. On the other hand, analysis of blood sample showed that neither RBC nor PCV levels were not affected by levels of fenugreek seeds in the diet of Ardi goat (5%,10% and 20%), meanwhile HGB level decreased with increasing the treatment levels (Al-Saiady *et al*., 2007). According the above information, it seems that the dose, experimental period, and species differences have importance in the effect of fenugreek seeds on blood parameters. The platelet count in both experimental groups II and III non-significant decrease as compared to control group. The non-significant change in WBC count of group II in comparison with control group is in agreement with (Rao *et al*., 1996; Khalil, 2004).

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